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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

B.TECH – BIOTECHNOLOGY

UNIT –I BIOMOLECULES OF COMMERCIAL IMPORTANCE - SBT1306

Importance of Downstream Processing

Numerous number of important products have been isolated and purified through downstream processing. These have helped make significant steps especially in the field of pharmaceutics.

Common Roles of downstream processing

Some of the important roles identified with downstream processing include:

- Purification and recovery of biosynthetic products, mostly pharmaceutics from sources that are natural. For example, plant and animal tissues or fermentation froth.
- Recycling of components that can be salvaged form waste.
- Proper waste disposal and treatment

Specific roles of downstream processing

Downstream processing is very important in the manufacture of antibiotics such as penicillin. The process is applied in the purification and separation of antibiotics from a number of mediums. This is not an easy task bearing in mind that antibiotics' concentration may be very low in the solutions in question.

Again, the can be applied in large scale manufacture of monoclonal antibodies(mAbs). Since antibody therapies are characterized by long-term administration of large antibody does, biopharmaceutical companies greatly appreciate down processing for facilitating indus

trial manufacture of antibodies.

A number of hormones are also resultant products of downstream processing. Some of the most commonly produced hormones include growth hormone and insulin. Follicle Stimulating Hormone is also another example.

Various research projects have also seen to the application of downstream processing in the manufacture of important vaccines. Common examples include vaccines for influenza and small pox.

Moreover, down stream process is also applied in industrial manufacture of enzymes. Enzymes are normally synthesized by living cells and are responsible for triggering chemical reactions. Industrial production of enzymes is therefore very important since they are appliedin processes such as food preservation and processing, manufacture of textiles, paper industry, scientific research, etc. A good example is a fungal amylase enzyme known as taka-diastate. This was the first enzyme to be produced in industry(U.S.A 1896) and was used pharmaceutically as an agent for treating digestive disorders.

Downstream processing indeed has so many applications. Another of its applications is the manufacture of natural fragrance and flavor substances.

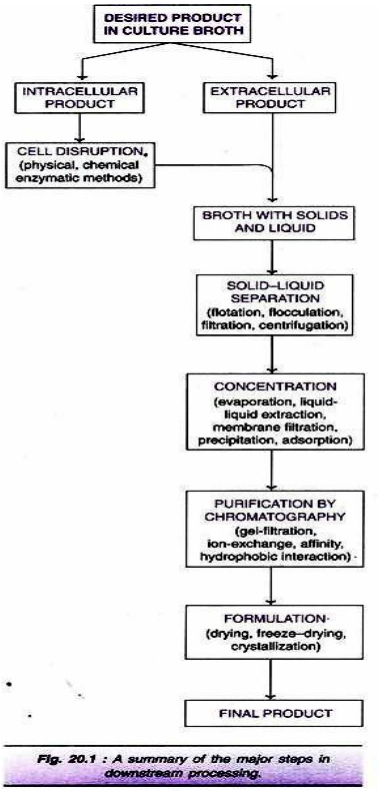

Fig. 20.1 : A summary of the major steps in downstream processing.

Solid-Liquid Separation:

The first step in product recovery is the separation of whole cells (cell biomass) and other insoluble ingredients from the culture broth (Note: If the desired product is an intracellular metabolite, it must be released from the cells before subjecting to solid-liquid separation). Some authors use the term harvesting of microbial cells for the separation of cells from the culture medium. Several methods are in use for solid-liquid separation. These include flotation, flocculation, filtration and centrifugation.

Flotation:

When a gas is introduced into the liquid broth, it forms bubbles. The cells and other solid particles get adsorbed on gas bubbles. These bubbles rise to the foam layer which can be collected and removed. The presence of certain substances, referred to as collector substances, facilitates stable foam formation e.g., long chain fatty acids, amines.

Flocculation:

In flocculation, the cells (or cell debris) form large aggregates to settle down for easy removal. The process of flocculation depends on the nature of cells and the ionic constituents of the medium. Addition of flocculating agents (inorganic salt, organic polyelectrolyte, mineral hydrocolloid) is often necessary to achieve appropriate flocculation.

Filtration:

Filtration is the most commonly used technique for separating the biomass and culture filtrate. The efficiency of filtration depends on many factors— the size of the organism, presence of other organisms, viscosity of the medium, and temperature. Several filters such as depth filters, absolute filters, rotary drum vacuum filters and membrane filters are in use.

Depth Filters:

They are composed of a filamentous matrix such as glass wool, asbestos or filter paper. The particles are trapped within the matrix and the fluid passes out. Filamentous fungi can be removed by using depth filters.

Absolute Filters:

These filters are with specific pore sizes that are smaller than the particles to be removed. Bacteria from culture medium can be removed by absolute filters.

Rotary Drum Vacuum Filters:

These filters are frequently used for separation of broth containing 10-40% solids (by volume) and particles in the size of 0.5-10µm. Rotary drum vacuum filters have been successfully used for filtration of yeast cells and filamentous fungi. The equipment is simple with low power consumption and is easy to operate. The filtration unit consists of a rotating drum partially immersed in a tank of broth. As the drum rotates, it picks up the biomass which gets deposited as a cake on the drum surface. This filter cake can be easily removed.

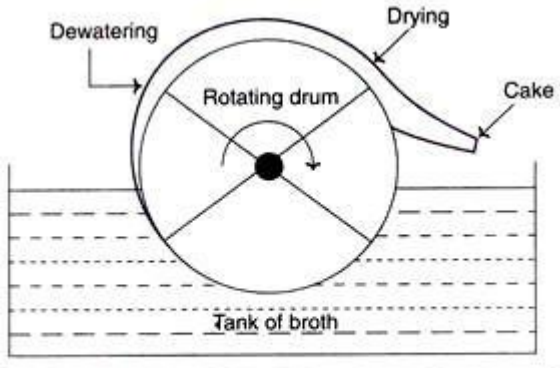

Fig. 20.2 : Diagrammatic representation of a rotary drum vacuum filter.

Membrane Filters:

In this type of filtration, membranes with specific pore sizes can be used. However, clogging of filters is a major limitation. There are two types of membrane filtrations—static filtration and cross-flow filtration (Fig. 20.3). In cross-flow filtration, the culture broth is pumped in a crosswise fashion across the membrane. This reduces the clogging process and hence better than the static filtration.

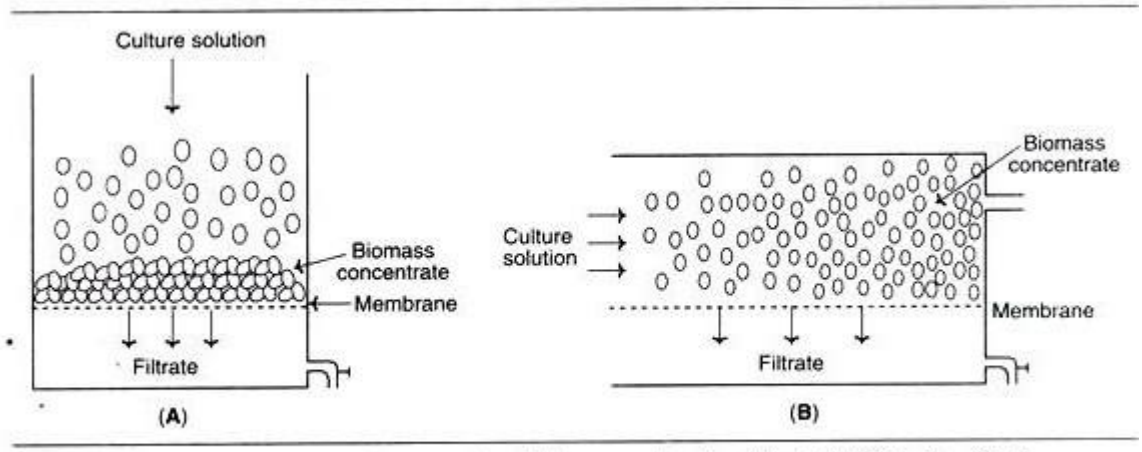

Fig. 20.3 : Filter systems for separation of biomass and culture filtrate (A) Static–flow filtration (B) Cross–flow filtration.

Types of filtration processes:

There are 3 major types of filtrations based on the particle sizes and other characters (Table 20.1). These are microfiltration, ultrafiltration and reverse osmosis.

Table 20.1 Major types of filtration processes with characteristic features

	Type	*Sizes of particles separated*	*Compound or particle separated*
1.	Microfiltration	0.1–10 μm	Cells or cell fractions, viruses.
2.	Ultrafiltration	0.001–0.1 μm	Compounds with molecular weights greater than 1000 (e.g. enzymes).
3.	Reverse osmosis (hyperfiltration)	0.0001–0.001 μm	Compounds with molecular weights less than 1000 (e.g. lactose).

Centrifugation:

The technique of centrifugation is based on the principle of density differences between the particles to be separated and the medium. Thus, centrifugation is mostly used for separating solid particles from liquid phase (fluid/particle separation). Unlike the centrifugation that is conveniently carried out in the laboratory scale, there are certain limitations for large scale industrial centrifugation.

However, in recent years, continuous flow industrial centrifuges have been developed. There is a continuous feeding of the slurry and collection of clarified fluid, while the solids deposited can be removed intermittently. The different types of centrifuges are depicted in Fig, and briefly described
hereunder.

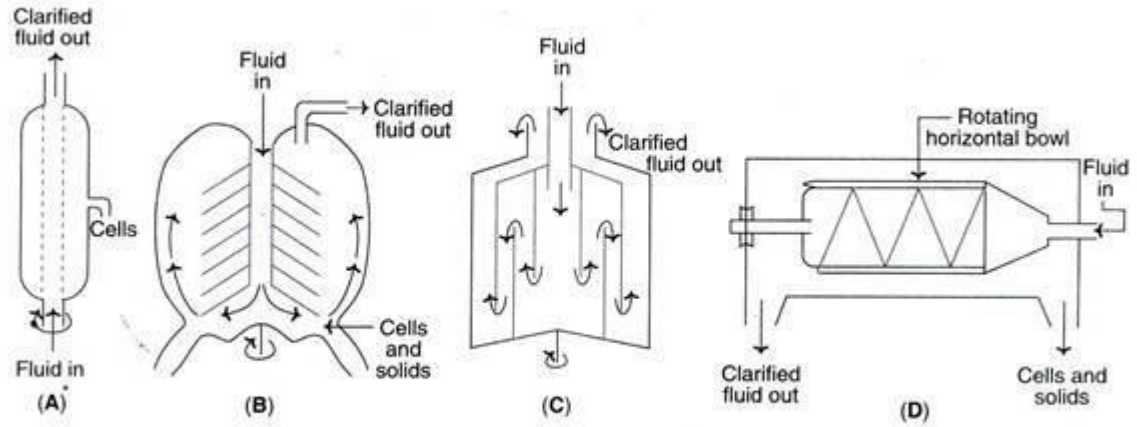

Fig. 20.4 : Centrifuges commonly used in downstream processing (A) Tubular bowl centrifuge (B) Disc centrifuge (C) Multichamber centrifuge (D) Scroll centrifuge (decanter).

Tubular bowl centrifuge :

This is a simple and a small centrifuge, commonly used in pilot plants. Tubular bowl centrifuge can be operated at a high centrifugal speed, and can be run in both batch or continuous mode. The solids are removed manually.

Disc centrifuge

It consists of several discs that separate the bowl into settling zones. The feed/slurry is fed through a central tube. The clarified fluid moves upwards while the solids settle at the lower surface.

Multi-chamber centrifuge :

This is basically a modification of tubular bowl type of centrifuge. It consists of several chambers connected in such a way that the feed flows in a zigzag fashion. There is a variation in the centrifugal force in different chambers. The force is much higher in the periphery chambers, as a result smallest particles settle down in the outermost chamber.

Scroll centrifuge or decanter :

It is composed of a rotating horizontal bowl tapered at one end. The decanter is generally used to concentrate fluids with high solid concentration (biomass content 5-80%). The solids are deposited on the wall of the bowl which can be scrapped and removed from the narrow end.

Release of Intracellular Products:

As already stated, there are several biotechnological products (vitamins, enzymes) which are located within the cells. Such compounds have to be first released (maximally and in an active form) for their further processing and final isolation. The microorganisms or other cells can be disintegrated or disrupted by physical, chemical or enzymatic methods. The outline of different techniques used for breakage of cells is given in Fig. 20.5.

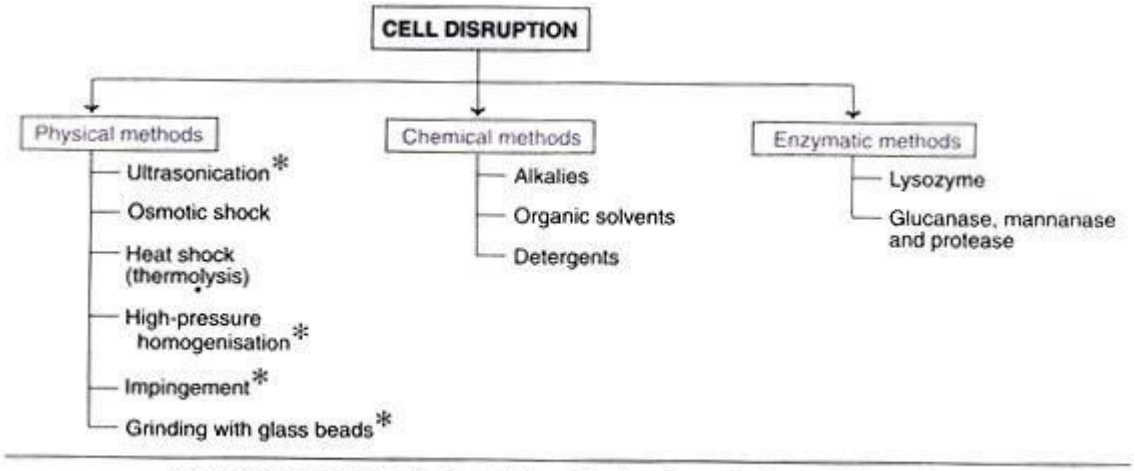

***Fig. 20.5** : Major methods for cell disruption to release the intracellular products (* indicate mechanical methods while all the remaining are non-mechanical).*

The selection of a particular method depends on the nature of the cells, since there is a wide variation in the property of cell disruption or breakage. For instance, Gram-negative bacteria and filamentous fungi can be more easily broken compared to Gram-positive bacteria arid yeasts.

Cell Disruption:

Physical methods of cell disruption:

The microorganisms or cells can be disrupted by certain physical methods to release the intracellular products.

Ultra sonication:

Ultrasonic disintegration is widely employed in the laboratory. However, due to high cost, it is not suitable for large-scale use in industries.

Osmotic shock:

This method involves the suspension of cells (free from growth medium) in 20% buffered sucrose. The cells are then transferred to water at about 4°C. Osmotic shock is used for the release of hydrolytic enzymes and binding proteins from Gram-negative bacteria.

Heat shock (thermolysis):

Breakage of cells by subjecting them to heat is relatively easy and cheap. But this technique can be used only for a very few heat-stable intracellular products.

High pressure homogenization:

This technique involves forcing of cell suspension at high pressure through a very narrow orifice to come out to atmospheric pressure. This sudden release of high pressure creates a liquid shear that can break the cells.

Impingement:

In this procedure, a stream of suspended cells at high velocity and pressure are forced to hit either a stationary surface or a second stream of suspended cells (impinge literally means to strike or hit). The cells are disrupted by the forces created at the point of contact. Micro fluidizer is a device developed based on the principle of impingement. It has been successfully used for breaking E. coli cells. The advantage with impingement technique is that it can be effectively used for disrupting cells even at a low concentration.

Grinding with glass beads:

The cells mixed with glass beads are subjected to a very high speed in a reaction vessel. The cells break as they are forced against the wall of the vessel by the beads. Several factors influence the cell breakage-size and quantity of the glass beads, concentration and age of cells, temperature and agitator speed. Under optimal conditions, one can expect a maximal breakage of about 80% of the cells.

A diagrammatic representation of a cell disrupter employing glass beeds is shown in Fig. 20.6. It contains a cylindrical body with an inlet, outlet and a central motor-driven shaft. To this shaft are fitted radial agitators. The cylinder is fitted with glass beads. The cell suspension is added through the inlet and the disrupted cells come out through the outlet. The body of the cell disrupter is kept cool while the operation is on.

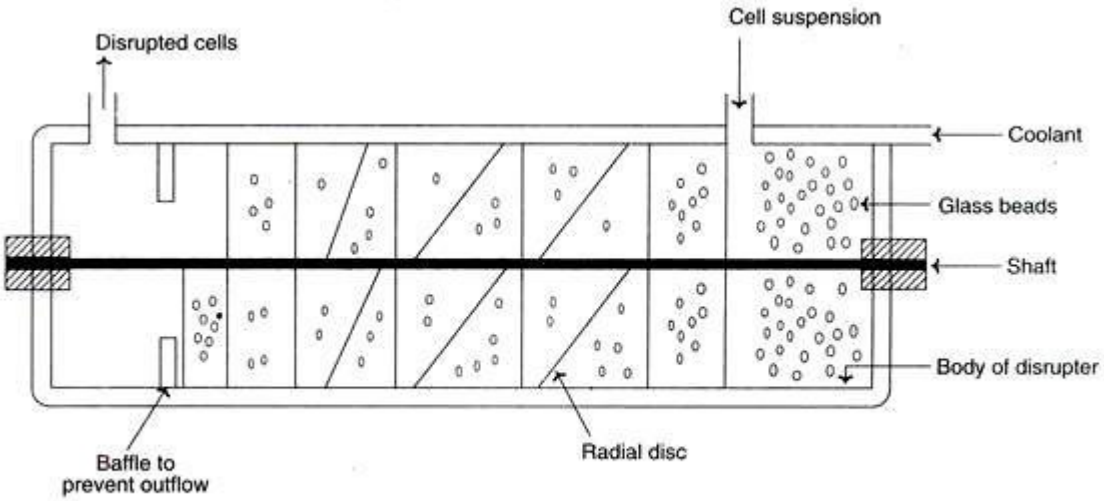

Fig. 20.6 : Diagrammatic representation of a cell disrupter.

Mechanical and non-mechanical methods:

Among the physical methods of cell disruption described above, ultra sonication, high-pressure homogenization, impingement and grinding with glass beads are mechanical while osmotic shock and heat shock are non-mechanical. The chemical and enzymatic methods (described below) are non- mechanical in nature.

Chemical methods of cell disruption:

Treatment with alkalies, organic solvents and detergents can lyse the cells to release the contents.

Alkalies:

Alkali treatment has been used for the extraction of some bacterial proteins. However, the alkali stability of the desired product is very crucial for the success of this method e.g., recombinant growth hormone can be efficiently released from E. coli by treatment with sodium hydroxide at pH 11.

Organic solvents:

Several water miscible organic solvents can be used to disrupt the cells e.g., methanol, ethanol, isopropanol, butanol. These compounds are inflammable; hence require specialised equipment for fire safety. The organic solvent toluene is frequently used. It is believed that toluene dissolves membrane phospholipids and creates membrane pores for release of intracellular contents.

Detergents:

Detergents that are ionic in nature, cationic-cetyl trimethyl ammonium bromide or anionic-sodium lauryl sulfate can denature membrane proteins and lyse the cells. Non-ionic detergents (although less reactive than ionic ones) are also used to some extent e.g., Triton X-100 or Tween. The problem with the use of detergents is that they affect purification steps, particularly the salt precipitation. This limitation can be overcome by using ultrafiltration or ion-exchange chromatography for purification.

Enzymatic methods of cell disruption:

Cell disruption by enzymatic methods has certain advantages i.e., lysis of cells occurs under mild conditions in a selective manner. This is quite advantageous for product recovery. Lysozyme is the most frequently used enzyme and is commercially available (produced from hen egg white). It hydrolyses β-1, 4-glycosidic bonds of the mucopeptide in bacterial cell walls. The Gram-positive bacteria (with high content of cell wall mucopeptides) are more susceptible for the action of lysozyme.

For Gram-negative bacteria, lysozyme in association with EDTA can break the cells. As the cell wall gets digested by lysozyme, the osmotic effects break the periplasmic membrane to release the intracellular contents. Certain other enzymes are also used, although less frequently, for cell disruption. For the lysis of yeast cell walls, glucanase and mannanase in combination with proteases are used.

Combination of methods:

In order to increase the efficiency of cell disintegration in a cost-effective manner, a combination of physical, chemical and enzymatic methods is employed.

Stage # 3. Concentration:

The filtrate that is free from suspended particles (cells, cell debris etc.) usually contains 80-98% of water. The desired product is a very minor constituent. The water has to be removed to achieve the product concentration. The commonly used techniques for concentrating biological products are evaporation, liquid-liquid extraction, membrane filtration, precipitation and adsorption. The actual procedure adopted depends on the nature of the desired product (quality and quantity to be retained as far as possible) and the cost factor.

Evaporation:

Water in the broth filtrate can be removed by a simple evaporation process. The evaporators, in general, have a heating device for supply of steam, and unit for the separation of concentrated product and vapour, a condenser for condensing vapour, accessories and control equipment. The capacity of the equipment is variable that may range from small laboratory scale to industrial scale. Some of the important types of evaporators in common use are briefly described.

Plate evaporators:

The liquid to be concentrated flows over plates. As the steam is supplied, the liquid gets concentrated and becomes viscous.

Falling film evaporators:

In this case, the liquid flows down long tubes which gets distributed as a thin film over the heating surface. Falling film evaporators are suitable for removing water from viscous products of fermentation.

Forced film evaporators:

The liquid films are mechanically driven and these devices are suitable for producing dry product concentrates.

Centrifugal forced film evaporators:

These equipment evaporate the liquid very quickly (in seconds), hence suitable for concentrating even heat-labile substances. In these evaporators, a centrifugal force is used to pass on the liquid over heated plates or conical surfaces for instantaneous evaporation.

Liquid-Liquid Extraction:

The concentration of biological products can be achieved by transferring the desired product (solute) from one liquid phase to another liquid phase, a phenomenon referred to as liquid-liquid extraction. Besides concentration, this technique is also useful for partial purification of a product.

The efficiency of extraction is dependent on the partition coefficient i.e. the relative distribution of a substance between the two liquid phases. The process of liquid-liquid extraction may be broadly categorized as extraction of low molecular weight products and extraction of high molecular weight products.

Extraction of low molecular weight products:

By using organic solvents, the lipophilic compounds can be conveniently extracted. However, it is quite difficult to extract hydrophilic compounds. Extraction of lipophilic products can be done by the following techniques.

Physical extraction:

The compound gets itself distributed between two liquid phases based on the physical properties. This technique is used for extraction of non-ionising compounds.

Dissociation extraction:

This technique is suitable for the extraction of ionisable compounds. Certain antibiotics can be extracted by this procedure.

Reactive extraction:

In this case, the desired product is made to react with a carrier molecule (e.g., phosphorus compound, aliphatic amine) and extracted into organic solvent. Reactive extraction procedure is quite useful for the extraction of certain compounds that are highly soluble in water (aqueous phase) e.g., organic acids.

Supercritical fluid (SCF) extraction:

This technique differs from the above procedures, since the materials used for extraction are supercritical fluids (SCFs). SCFs are intermediates between gases and liquids and exist as fluids above their critical temperature and pressure. Supercritical CO_2, with a low critical temperature and pressure is commonly used in the extraction. Supercritical fluid extraction is rather expensive, hence not widely used (SCF has been used for the extraction of caffeine from coffee beans, and pigments and flavor ingredients from biological materials).

Extraction of high molecular weight compounds:

Proteins are the most predominant high molecular weight products produced in fermentation industries. Organic solvents cannot be used for protein extraction, as they lose their biological activities. They are extracted by using an aqueous two-phase systems or reverse micelles formation.

Aqueous two-phase systems (ATPS):

They can be prepared by mixing a polymer (e.g., polyethylene glycol) and a salt solution (ammonium sulfate) or two different polymers. Water is the main component in ATPS, but the two phases are not miscible. Cells and other solids remain in one phase while the proteins are transferred to other phase. The distribution of the desired product is based on its surface and ionic character and the nature of phases. The separation takes much longer time by ATPS.

Reverse miceller systems:

Reverse micelles are stable aggregates of surfactant molecules and water in organic solvents. The proteins can be extracted from the aqueous medium by forming reverse micelles. In fact, the enzymes can be extracted by this procedure without loss of biological activity.

Membrane Filtration:

Membrane filtration has become a common separation technique in industrial biotechnology. It can be conveniently used for the separation of biomolecules and particles, and for the concentration of fluids. The membrane filtration technique basically involves the use of a semi-permeable membrane that selectively retains the particles/molecules that are bigger than the pore size while the smaller molecules pass through the membrane pores.

Membranes used in filtration are made up of polymeric materials such as polyethersulfone and polyvinyl di-fluoride. It is rather difficult to sterilize membrane filters. In recent years, micro-filters and ultrafiIters composed of ceramics and steel are available. Cleaning and sterilization of such filters are easy. The other types of membrane filtration techniques are described briefly.

Membrane adsorbers:

They are micro- or macro porous membranes with ion exchange groups and/or affinity ligands. Membrane adsorbers can bind to proteins and retain them. Such proteins can be eluted by employing solutions in chromatography.

Pervaporation:

This is a technique in which volatile products can be separated by a process of permeation through a membrane coupled with evaporation. Pervaporation is quite useful for the extraction, recovery and concentration of volatile products. However, this procedure has a limitation since it cannot be used for large scale separation of volatile products due to cost factor.

Perstraction:

This is an advanced technique working on the principle of membrane filtration coupled with solvent extraction. The hydrophobic compounds can be recovered/ concentrated by this method.

Precipitation:

Precipitation is the most commonly used technique in industry for the concentration of macromolecules such as proteins and polysaccharides. Further, precipitation technique can also be employed for the removal of certain unwanted byproducts e.g. nucleic acids, pigments.

Neutral salts, organic solvents, high molecular weight polymers (ionic or non-ionic), besides alteration in temperature and pH are used in precipitation. In addition to these non-specific protein precipitation reactions (i.e. the nature of the protein is unimportant), there are some protein specific precipitations e.g., affinity precipitation, ligand precipitation.

Neutral salts:

The most commonly used salt is ammonium sulfate, since it is highly soluble, nontoxic to proteins and low-priced. Ammonium sulfate increases hydrophobic interactions between protein molecules that result in their precipitation. The precipitation of proteins is dependent on several factors such as protein concentration, pH and temperature.

Organic solvents:

Ethanol, acetone and propanol are the commonly used organic solvents for protein precipitation. They reduce the dielectric constant of the medium and enhance electrostatic interaction between protein molecules that lead to precipitation. Since proteins are denatured by organic solvents, the precipitation process has to be carried out below 0°C.

Non-ionic polymers:

Polyethylene glycol (PEG) is a high molecular weight non-ionic polymer that can precipitate proteins. It reduces the quantity of water available for protein solvation and precipitates protein. PEG does not denature proteins, besides being non-toxic.

Ionic polymers:

The charged polymers such as polyacrylic acid and polyethyleneimine are used. They form complexes with oppositely charged protein molecules that causes charge neutralisation and precepitation.

Increase in temperature:

The heat sensitive proteins can be precipitated by increasing the temperature.

Change in pH:

Alterations in pH can also lead to protein precipitation.

Affinity precipitation:

The affinity interaction (e.g., between antigen and antibody) is exploited for precipitation of proteins.

Precipitation by ligands:

Ligands with specific binding sites for proteins have been successfully used for selective precipitation.

Adsorption:

The biological products of fermentation can be concentrated by using solid adsorbent particles. In the early days, activated charcoal was used as the adsorbent material. In recent years, cellulose-based adsorbents are employed for protein concentration.

And for concentration of low molecular weight compounds (vitamins, antibiotics, peptides) polystyrene, methacrylate and acrylate based matrices are used. The process of adsorption can be carried out by making a bed of adsorbent column and passing the culture broth through it. The desired product, held by the adsorbent, can be eluted.

Purification by Chromatography:

The biological products of fermentation (proteins, pharmaceuticals, diagnostic compounds and research materials) are very effectively purified by chromatography. It is basically an analytical technique dealing with the separation of closely related compounds from a mixture. Chromatography usually consists of a stationary phase and mobile phase.

The stationary phase is the porous solid matrix packed in a column (equilibrated with a suitable solvent) on to which the mixture of compounds to be separated is loaded. The compounds are eluted by a mobile phase.

A single mobile phase may be used continuously or it may be changed appropriately to facilitate the release of desired compounds. The eluate from the column can be monitored continuously (e.g. protein elution can be monitored by ultraviolet adsorption at 280 nm), and collected in fractions of definite volumes.

The different types of chromatography techniques used for separation (mainly proteins) along with the principles are given in Table 20.2. A large number of matrices are commercially available for purification of proteins e.g., agarose, cellulose, polyacrylamide, porous silica, cross-

linked dextran, polystyrene. Some of the important features of selected chromatographic techniques are briefly described.

Table 20.2 Chromatographic techniques along with the principles for separation of proteins

Chromatography	*Principle*
Gel-filtration (size exclusion)	Size and shape
Ion-exchange	Net charge
Chromatofocussing	Net charge
Affinity	Biological affinity and molecular recognition
Hydrophobic interaction	Polarity (hydrophobicity of molecules)
Immobilized metal-ion affinity	Metal ion binding

Gel-filtration chromatography:

This is also referred to as size-exclusion chromatography. In this technique, the separation of molecules is based on the size, shape and molecular weight. The sponge-like gel beads with pores serve as molecular sieves for separation of smaller and bigger molecules. A solution mixture containing molecules of different sizes (e.g. different proteins) is applied to the column and eluted.

The smaller molecules enter the gel beads through their pores and get trapped. On the other hand, the larger molecules cannot pass through the pores and therefore come out first with the mobile liquid (Fig. 20.7). At the industrial scale, gel-filtration is particularly useful to remove salts and low molecular weight compounds from high molecular weight products.

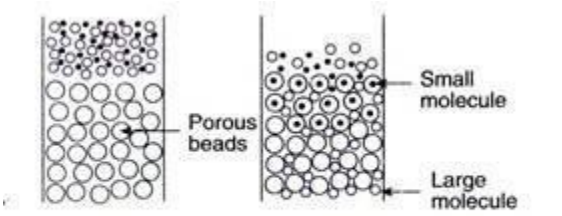

Fig. 20.7 : The principle of gel-filtration chromatography.

Ion-exchange chromatography:

It involves the separation of molecules based on their surface charges. Ion-exchangers are of two types (cation- exchangers which have negatively charged groups like carboxymethyl and sulfonate, and anion- exchangers with positively charged groups like diethylaminoethyl (DEAE). The most commonly used cation-exchangers are Dowex HCR and Amberlite IR, the anion-exchangers are Dowex SAR and Amberlite IRA.

In ion-exchange chromatography, the pH of the medium is very crucial, since the net charge varies with pH. In other words, the pH determines the effective charge on both the target molecule and the ion-exchanger. The ionic bound molecules can be eluted from the matrix by changing the pH of the eluant or by increasing the concentration of salt solution. Ion-exchange chromatography is useful for the purification of antibiotics, besides the purification of proteins.

Affinity chromatography:

This is an elegant method for the purification of proteins from a complex mixture. Affinity chromatography is based on an interaction of a protein with an immobilized ligand. The ligand can be a specific antibody, substrate, substrate analogue or an inhibitor. The immobilized ligand on a solid matrix can be effectively used to fish out complementary structures.

In Table 20.3, some examples of ligands used for the purification of proteins are given. The protein bound to the ligand can be eluted by reducing their interaction. This can be achieved by changing the pH of the buffer, altering the ionic strength or by using another free ligand molecule. The fresh ligand used has to be removed in the subsequent steps.

TABLE 20.3 Some examples of ligands used for separation of proteins by affinity chromatography

Ligand	*Type of protein*
Antibody	Antigen
Cofactor	Enzyme
Receptor	Hormone
Hapten	Antibody
Inhibitor	Enzyme
Lectins	Glycoproteins
Heparin	Coagulation factors
Metal ions	Metal ion binding proteins

Hydrophobic interaction chromatography (HIC):

This is based on the principle of weak hydrophobic interactions between the hydrophobic ligands (alkyl, aryl side chains on matrix) and hydrophobic amino acids of proteins. The differences in the composition of hydrophobic amino acids in proteins can be used for their separation. The elution of proteins can be done by lowering the salt concentration, decreasing the polarity of the medium or reducing the temperature.

Stage # 5. Formulation:

Formulation broadly refers to the maintenance of activity and stability of a biotechnological products during storage and distribution. The formulation of low molecular weight products (solvents, organic acids) can be achieved by concentrating them with removal of most of the water. For certain small molecules, (antibiotics, citric acid), formulation can be done by crystallization by adding salts.

Proteins are highly susceptible for loss of biological activity; hence their formulation requires special care. Certain stabilizing additives are added to prolong the shelf life of protein. The stabilizers of protein formulation include sugars (sucrose, lactose), salts (sodium chloride, ammonium sulfate), polymers (polyethylene glycol) and polyhydric alcohols (glycerol). Proteins may be formulated in the form of solutions, suspensions or dry powders.

Drying:

Drying is an essential component of product formulation. It basically involves the transfer of heat to a wet product for removal of moisture. Most of the biological products of fermentation are sensitive to heat, and therefore require gentle drying methods. Based on the method of heat transfer, drying devices may be categorized as contact, convection, radiation dryers. These three types of dryers are commercially available.

Spray drying:

Spray drying is used for drying large volumes of liquids. In spray drying, small droplets of liquid containing the product are passed through a nozzle directing it over a stream of hot gas. The water evaporates and the solid particles are left behind.

Freeze-drying:

Freeze-drying or lyophilization is the most preferred method for drying and formulation of a wide-range of products—pharmaceuticals, foodstuffs, diagnostics, bacteria, viruses. This is mainly because freeze-drying usually does not cause loss of biological activity of the desired product.

Lyophilization is based on the principle of sublimation of a liquid from a frozen state. In the actual technique, the liquid containing the product is frozen and then dried in a freeze-dryer under vacuum. The vacuum can now be released and the product containing vials can be sealed e.g., penicillin can be freeze dried directly in ampules.

Integration of Different Processes:

It is ideal to integrate the fermentation and downstream processing to finally get the desired product. However, this has not been practicable for various reasons. Integration of certain stages in downstream processing for purification of product has met with some success. For instance, protein concentration by extraction into two phase systems combined with clarification and purification can be done together.

Criteria For Selection Of Separation Techniques

Biomolecules	Criteria selection	Techniques
Protein	Iso electric pH, charges	2d electrophoresis,SDS PAGE
DNA	Charge	Gel electrophoresis
Protein	Ion, charges	Salting out,ion exchange
Biomass	mass	Centrifugation
Protein	Biological properties	Affinity

COAGULATION AND FLOCCULATION PROCESS FUNDAMENTALS

Coagulation and Flocculation Groundwater and surface water contain both dissolved and suspended particles. Coagulation and flocculation are used to separate the suspended solids portion from the water. Suspended particles vary in source, charge, particle size, shape, and density. Correct application of coagulation and flocculation depends upon these factors. Suspended solids in water have a negative charge and since they have the same type of surface charge, they repel each other when they come close together. Therefore, suspended solids will remain in suspension and will not clump together and settle out of the water, unless proper coagulation and flocculation is used. Coagulation and flocculation occurs in successive steps, allowing particle collision and growth of floc. This is then followed by sedimentation (see Sedimentation Chapter). If coagulation is incomplete, flocculation step will be unsuccessful, and if flocculation is incomplete, sedimentation will be unsuccessful.

COAGULATION Coagulant chemicals with charges opposite those of the suspended solids are added to the water to neutralize the negative charges on non-settlable solids (such as clay and color-producing organic substances). Once the charge is neutralized, the small suspended particles are capable of sticking together. These slightly larger particles are called microflocs, and are not visible to the naked eye. Water surrounding the newly formed microflocs should be

clear. If not, coagulation and some of the particles charge have not been neutralized. More coagulant chemicals may need to be added. A high-energy, rapid-mix to properly disperse coagulant and promote particle collisions is needed to achieve good coagulation. Over-mixing does not affect coagulation, but insufficient mixing will leave this step incomplete. Contact time in the rapid-mix chamber is typically 1 to 3 minutes.

Alum A12(SO4)3 + 3 Ca(HCO3)2 ----------> 2 Al(OH)3 + 3CaSO4 + 6 CO2 Aluminum + Calcium gives Aluminum + Calcium + Carbon Sulfate Bicarbonate Hydroxide Sulfate Dioxide (present in the water to treat) Ferric Sulfate Fe2(SO4)3 + 3 Ca(HCO3)2 ----------> 2 Fe(OH)3 + 3CaSO4 + 6 CO2 Ferric + Calcium gives Ferric + Calcium + Carbon Sulfate Bicarbonate Hydroxide Sulfate Dioxide (present in the water to treat) Ferric Chloride 2 Fe Cl3 + 3 Ca(HCO3)2---------- > 2 Fe(OH)3 + 3CaCl2 + 6CO2 Ferric + Calcium gives Ferric + Calcium + Carbon Chloride Bicarbonate Hydroxide Chloride Dioxide (present in the water to treat) Polymers Polymers (long-chained, high-molecular-weight, organic chemicals) are becoming more widely used. These can be used as coagulant aids along with the regular inorganic coagulants. Anionic (negatively charged) polymers are often used with metal coagulants. Low-to-medium weight cationic (positively charged) polymers may be used alone, or in combination with alumor ferric coagulants to attract suspended solids and neutralize their surface charge. Manufacturers can produce a wide range of polymers that meet a variety of source-water conditions by controlling the amount and type of charge and the polymers molecular weight. Polymers are effective over a wider pH range than inorganic coagulants. They can be applied at lower doses, and do not consume alkalinity. They produce smaller volumes of more concentrated, rapidly settling floc. Floc formed from use of a properly selected polymer will be more resistant to shear, resulting in less carryover and a cleaner effluent. Polymers are generally several times more expensive in price per pound than inorganic coagulants. Selection of the proper polymer requires considerable jar testing under simulated plant conditions, followed by pilot or plant-scale trials. All polymers must also be approved for potable water use by regulatory agencies.

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DEPARTMENT OF BIOTECHNOLOGY

B.TECH – BIOTECHNOLOGY

UNIT –II TECHNIQUES AND INSTRUMENTATION - SBT1306

PRIMARY SEPARATION AND RECOVERY PROCESS

Solid-Liquid Separation:

The first step in product recovery is the separation of whole cells (cell biomass) and other insoluble ingredients from the culture broth (Note: If the desired product is an intracellular metabolite, it must be released from the cells before subjecting to solid-liquid separation). Some authors use the term harvesting of microbial cells for the separation of cells from the culture

medium. Several methods are in use for solid-liquid separation. These include flotation, flocculation, filtration and centrifugation.

Flotation:

When a gas is introduced into the liquid broth, it forms bubbles. The cells and other solid particles get adsorbed on gas bubbles. These bubbles rise to the foam layer which can be collected and removed. The presence of certain substances, referred to as collector substances, facilitates stable foam formation e.g., long chain fatty acids, amines.

Flocculation:

In flocculation, the cells (or cell debris) form large aggregates to settle down for easy removal. The process of flocculation depends on the nature of cells and the ionic constituents of the medium. Addition of flocculating agents (inorganic salt, organic polyelectrolyte, mineral hydrocolloid) is often necessary to achieve appropriate flocculation.

Filtration:

Filtration is the most commonly used technique for separating the biomass and culture filtrate. The efficiency of filtration depends on many factors— the size of the organism, presence of other organisms, viscosity of the medium, and temperature. Several filters such as depth filters, absolute filters, rotary drum vacuum filters and membrane filters are in use.

Centrifugation

It may be used to separate bacteria and usually protein precipitates. But difficulties arise due to small differences in the densities of the particles and the medium. In addition, equipment cost, power consumption, temperature etc. are the other disadvantages. **Centrifugation** is the technique which involves the application of centrifugal force to separate particles from a solution according to their size, shape, density, viscosity of the medium and rotor speed.

SEDIMENTATION,

Sedimentation, or clarification, is the processes of letting suspended material settle by gravity. Suspended material may be particles, such as clay or silts, originally present in the source water. Suspended material or floc is typically created from materials in the water and chemicals used in coagulation or, in other treatment processes, such as lime softening (see Lime Softening chapter). Sedimentation is accomplished by decreasing the velocity of the water to a point which the particles will no longer remain in suspension. When the velocity no longer supports the particles, gravity will remove them from the water flow

Cell Disruption:

Physical methods of cell disruption:
The microorganisms or cells can be disrupted by certain physical methods to release the intracellular products.

Ultra sonication:
Ultrasonic disintegration is widely employed in the laboratory. However, due to high cost, it is not suitable for large-scale use in industries.

Osmotic shock:
This method involves the suspension of cells (free from growth medium) in 20% buffered sucrose. The cells are then transferred to water at about 4°C. Osmotic shock is used for the release of hydrolytic enzymes and binding proteins from Gram-negative bacteria.

Heat shock (thermolysis):

Breakage of cells by subjecting them to heat is relatively easy and cheap. But this technique can be used only for a very few heat-stable intracellular products.

High pressure homogenization:

This technique involves forcing of cell suspension at high pressure through a very narrow orifice to come out to atmospheric pressure. This sudden release of high pressure creates a liquid shear that can break the cells.

Impingement:

In this procedure, a stream of suspended cells at high velocity and pressure are forced to hit either a stationary surface or a second stream of suspended cells (impinge literally means to strike or hit). The cells are disrupted by the forces created at the point of contact. Micro fluidizer is a device developed based on the principle of impingement. It has been successfully used for breaking E. coli cells. The advantage with impingement technique is that it can be effectively used for disrupting cells even at a low concentration.

Grinding with glass beads:

The cells mixed with glass beads are subjected to a very high speed in a reaction vessel. The cells break as they are forced against the wall of the vessel by the beads. Several factors influence the cell breakage-size and quantity of the glass beads, concentration and age of cells, temperature and agitator speed. Under optimal conditions, one can expect a maximal breakage of about 80% of the cells.

A diagrammatic representation of a cell disrupter employing glass beeds is shown in Fig. 20.6. It contains a cylindrical body with an inlet, outlet and a central motor-driven shaft. To this shaft are fitted radial agitators. The cylinder is fitted with glass beads. The cell suspension is added through the inlet and the disrupted cells come out through the outlet. The body of the cell disrupter is kept cool while the operation is on.

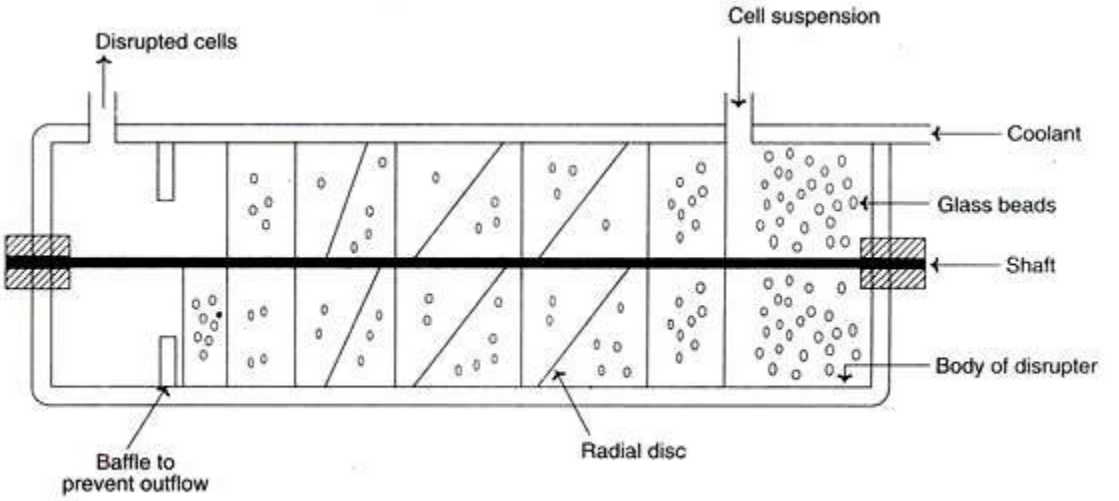


Fig. 20.6 : Diagrammatic representation of a cell disrupter.

Mechanical and non-mechanical methods:

Among the physical methods of cell disruption described above, ultra sonication, high-pressure homogenization, impingement and grinding with glass beads are mechanical while osmotic shock and heat shock are non-mechanical. The chemical and enzymatic methods (described below) are non- mechanical in nature.

Chemical methods of cell disruption:

Treatment with alkalies, organic solvents and detergents can lyse the cells to release the contents.

Alkalies:

Alkali treatment has been used for the extraction of some bacterial proteins. However, the alkali stability of the desired product is very crucial for the success of this method e.g., recombinant growth hormone can be efficiently released from E. coli by treatment with sodium hydroxide at pH 11.

Organic solvents:

Several water miscible organic solvents can be used to disrupt the cells e.g., methanol, ethanol, isopropanol, butanol. These compounds are inflammable; hence require specialised equipment for fire safety. The organic solvent toluene is frequently used. It is believed that toluene dissolves membrane phospholipids and creates membrane pores for release of intracellular contents.

Detergents:

Detergents that are ionic in nature, cationic-cetyl trimethyl ammonium bromide or anionic-sodium lauryl sulfate can denature membrane proteins and lyse the cells. Non-ionic detergents (although less reactive than ionic ones) are also used to some extent e.g., Triton X-100 or Tween. The problem with the use of detergents is that they affect purification steps, particularly the salt precipitation. This limitation can be overcome by using ultrafiltration or ion-exchange chromatography for purification.

Enzymatic methods of cell disruption:

Cell disruption by enzymatic methods has certain advantages i.e., lysis of cells occurs under mild conditions in a selective manner. This is quite advantageous for product recovery. Lysozyme is the most frequently used enzyme and is commercially available (produced from hen egg white). It hydrolyses β-1, 4-glycosidic bonds of the mucopeptide in bacterial cell walls. The Gram-positive bacteria (with high content of cell wall mucopeptides) are more susceptible for the action of lysozyme.

For Gram-negative bacteria, lysozyme in association with EDTA can break the cells. As the cell wall gets digested by lysozyme, the osmotic effects break the periplasmic membrane to release the intracellular contents. Certain other enzymes are also used, although less frequently, for cell disruption. For the lysis of yeast cell walls, glucanase and mannanase in combination with proteases are used.

Filtration:

It is used for the separation of filamentous fungi and filamentous bacteria, e.g., streptomycetes, and often for yeast flocks. Filtration uses pressure created by overpressure or vacuum, and its rate depends on filter area, fluid viscosity and the resistance generated by filter cake which increases with time.

The various techniques of filtration employed are: surface filtration, depth filtration, centrifugal filtration, cross-flow filtration, rotary drum vacuum filtration. Rotary drum vacuum filters are the most commonly used: the filter is in form of a rotating drum with a partial vacuum on the inside of the drum.

A portion of the drum rotates through the medium and the cells are sucked to form a coating on the drum; the cells are continuously scraped off to prevent blocking of the filter.

FILTRATION TECHNIQUES

Filtration, the technique used to separate solids from liquids, is the act of pouring a mixture onto a membrane (filter paper) that allows the passage of liquid (the filtrate) and results in the collection of the solid. Two filtration techniques are generally used in chemical separations in general chemistry lab: "gravity" filtration and "vacuum" filtration. Gravity Filtration Gravity filtration uses a polyethylene or glass funnel with a stem and filter paper. Filter paper can have pore sizes ranging from small to large to permit slow to fast filtering. The paper is folded in half , then folded in quarters, and the tip of one corner is torn off to allow for a snug fit in the funnel cone. (If the paper has been pre-weighed, the torn corner piece must be saved to add to the post-filter weighing to avoid any errors.) The paper cone is fitted to the funnel so three thicknesses of the paper line one-half of the cone and one thickness lines the opposite half .Now place the funnel into a beaker and wet the filter paper completely Open filter paper cone and line funnel Tear off corner. Save corner if filter paper has been weighed. Fold paper in half Fold paper in quarters.How to Fold Filter Paper with the dominate solvent or solvents in the mixture to be filtered. This step adheres the filter paper to the funnel walls preventing solid from escaping. Then, support the funnel with a clamp Filtration Techniques Revised 8/6/12 2 or ring (if necessary) and place a clean beaker beneath the funnel so the stem rests against the side of the beaker (this prevents splattering). Before filtering, allow most of the solid in the mixture to settle. Now pour the supernatant liquid (the liquid standing over the solid in a mixture) through the filter first. This will allow the initial part of the filtration to proceed faster and may prevent clogging of the filter by the solid. To prevent splattering pour the liquid down a glass rod .

Gravity Filtration Scrape the solid onto the filter with a rubber policeman or spatula. Rinse the spatula, glass rod and beaker and pour the washings into the filter funnel. If the remaining solid residue is to be washed, rinse with three small portions (a few milliliters each) of an appropriate

solvent. If the solid is to be saved, remove the filter paper carefully and place it on a watch glass to dry. Caution: Wet filter paper tears easily. Supernatant liquid containing settled solids. Allow liquid to travel along a glass stir rod to prevent splashing & control flow. Collection Beaker Iron Support Ring Line funnel with filter paper

Sometimes the filtrate is tested to determine if a product or reactant has or has not passed through the filter. The test depends on what is being separated. For example: All barium ions (Ba2+) should have removed from solution by the formation of the precipitate BaSO4. To check this a few drops of Na2SO4 solution can be added to a small portion of the filtrate. If no BaSO4 precipitate forms, the filtration was successful. If a precipitate forms, additional precipitating (sulfate ion containing) reagents must be added to the filtrate and the resulting mixture must be filtered again. Vacuum (or Suction) Filtration Vacuum filtration uses a Buchner (pronounced "byook-ner") funnel and a water aspirator assembly. A Buchner funnel is a flat bottomed, porous, circular porcelain bowl with a short stem. The stem is fitted with a rubber stopper and inserted in the mouth of a side arm filter flask. Circular filter paper, the same diameter as the bowl, is placed on the flat bottom and wetted with the appropriate solvent to create a seal before starting the filtration. Buchner Funnel on a side arm filter flask. The hose in the above figure is attaches the side arm of the filter flask to a vacuum aspirator in the hood or at the lab bench. This vacuum aspirator creates the suction that pulls liquid through the filter and filter paper.

Vacuum Aspirator (front & rear views) Figure 5 shows the entire set-up consisting of a Buchner funnel, the side arm flask, and the vacuum aspirator. When the water aspirator is turned on the flow of water creates suction. The Buchner Funnel Side Arm Filter Flask Hose attachment to the vacuum aspirator hose is part of the filtration system and should be heavy enough to prevent pinching or collapse under external atmospheric pressure. To filter a sample, turn on the aspirator and carry out the filtration in the same manner described for gravity filtration. (Note: NEVER pry off the funnel if the system is under vacuum! Water can flash back into the collection flask or the filter paper can be damaged resulting in the loss of filtered solid.) Turn off the water aspirator before carefully removing the wet filter paper without tearing. Reaction

byproducts (either the solid or filtrate) should be placed into appropriate labeled containers in the hood.

Centrifugation

It may be used to separate bacteria and usually protein precipitates. But difficulties arise due to small differences in the densities of the particles and the medium. In addition, equipment cost, power consumption, temperature etc. are the other disadvantages. **Centrifugation** is the technique which involves the application of centrifugal force to separate particles from a solution according to their size, shape, density, viscosity of the medium and rotor speed. Not only is this process 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 (move to the outside), while less-dense components of the mixture migrate towards the axis, i. e., move to the center. 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 of protein solution, for example, allows elimination of impurities into the supernatant.

There is a correlation between the size and density of a particle and the rate that the particle separates from a heterogeneous mixture, when the only force applied is that of gravity. The larger the size and the larger the density of the particles, the faster they separate from the mixture. By applying a larger effective gravitational force to the mixture, like a centrifuge does, the separation of the particles is accelerated. This is ideal in industrial and lab settings because particles that would naturally separate over a long period of time can be separated in much less time.

The rate of centrifugation is specified by the angular velocity usually expressed as revolutions per minute (RPM), or acceleration expressed as *g*. The conversion factor between RPM

and *g* depends on the radius of the centrifuge rotor. The particles' settling velocity in centrifugation is a function of their size and shape, centrifugal acceleration, the volume fraction of solids present, the density difference between the particle and the liquid, and the viscosity. The most common application is the separation of solid from highly concentrated suspensions, which is used in the treatment of sewage sludges for dewatering where less consistent sediment is produced.

In the chemical and food industries, special centrifuges can process a continuous stream of particle-laden liquid.

Centrifugation is the most common method used for uranium enrichment, relying on the slight mass difference between atoms of U238 and U235 in uranium hexafluoride gas.

Microcentrifuges

Microcentrifuges are used to process small volumes of biological molecules, cells, or nuclei. Microcentrifuge tubes generally hold 0.5 - 2.0 mL of liquid, and are spun at maximum angular speeds of 12,000–13,000 rpm. Microcentrifuges are small enough to fit on a table-top and have rotors that can quickly change speeds. They may or may not have a refrigeration function.

High-speed centrifuges

High-speed or superspeed centrifuges can handle larger sample volumes, from a few tens of millilitres to several litres. Additionally, larger centrifuges can also reach higher angular velocities (around 30,000 rpm). The rotors may come with different adapters to hold various sizes of test tubes, bottles, or microtiter plates.

Fractionation process

General method of fractionation: Cell sample is stored in a suspension which is:

1. Buffered - neutral pH, preventing damage to the structure of proteins including enzymes (which could affect ionic bonds)
2. Isotonic (of equal water potential) - this prevents water gain or loss by the organelles
3. Cool - reducing the overall activity of enzyme released later in the procedure

- Cells are homogenised in a blender and filtered to remove debris
- The homogenised sample is placed in an ultracentrifuge and spun in low speed - nuclei settle out, forming a pellet
- The supernatant (suspension containing remaining organelles) is spun at a higher speed - chloroplasts settle out
- The supernatant is spun at a higher speed still - mitochondria and lysosomes settle out
- The supernatant is spun at an even higher speed - ribosomes, membranes settle out
- The ribosomes, membranes and Golgi complexes can be separated by another technique called density gradient centrifugation.

Ultracentrifugations

Differential centrifugation, Isopycnic centrifugation, and ultracentrifugation

Ultracentrifugation makes use of high centrifugal force for studying properties of biological particles. Compared to microcentrifuges or high-speed centrifuges, ultracentrifuges can isolate much smaller particles, including ribosomes, proteins, and viruses. Ultracentrifuges can also be used in the study of membrane fractionation. This occurs because ultracentrifuges can reach maximum angular velocities in excess of 70,000 rpm. Additionally, while microcentrifuges and supercentrifuges separate particles in batches (limited volumes of samples must be handled manually in test tubes or bottles), ultracentrifuges can separate molecules in batch or continuous flow systems.

In addition to purification, analytical ultracentrifugation (AUC) can be used for determination of the properties of macromolecules such as shape, mass, composition, and conformation. Samples are centrifuged with a high-density solution such as sucrose, caesium chloride, or iodixanol. The high-density solution may be at a uniform concentration throughout the test tube ("cushion") or a varying concentration ("gradient"). Molecular properties can be modeled through sedimentation velocity analysis or sedimentation equilibrium analysis. During the run, the particle or molecules will migrate through the test tube at different speeds depending on their physical properties and the properties of the solution, and eventually form a pellet at the bottom of the tube, or bands at various heights.

Density Gradient Centrifugation

Density gradient centrifugation is considered one of the more efficient methods of separating suspended particles. Density gradient centrifugation can be used both as a separation technique and as a method of measuring the densities of particles or molecules in a mixture. A tube, after being centrifuged by this method, has particles in order of density based on height. The object or particle of interest will reside in the position within the tube corresponding to its density.

Linderstorm-Lang, in 1937, discovered that density gradient tubes could be used for density measurements. He discovered this when working with potato yellow-dwarf virus.

This method was also used in Meselson and Stahl's famous experiment in which they proved that DNA replication is semi-conservative by using different isotopes of nitrogen. They used density gradient centrifugation to determine which isotope or isotopes of nitrogen were present in the DNA after cycles of replication.

Nevertheless, some non-ideal sedimentations are still possible when using this method. The first potential issue is the unwanted aggregation of particles, but this can occur in any centrifugation. The second possibility occurs when droplets of solution that contain particles sediment. This is more likely to occur when working with a solution that has a layer of suspension floating on a dense liquid, which in fact have little to no density gradient.

Differential Centrifugation is a type of centrifugation in which one selectively spins down components of a mixture by a series of increasing centrifugation forces. This method is commonly used to separate organelles and membranes found in cells. Organelles generally differ from each other in density in size, making the use of differential centrifugation, and centrifugation in general, possible. The organelles can then be identified by testing for indicators that are unique to the specific organelles.

Other applications

- Separating chalk powder from water

- Removing fat from milk to produce skimmed milk
- Separating particles from an air-flow using cyclonic separation
- The clarification and stabilization of wine
- Separation of urine components and blood components in forensic and research laboratories
- Aids in separation of proteins using purification techniques such as salting out, e.g. ammonium sulfate precipitation.

SEDIMENTATION

Sedimentation, or clarification, is the processes of letting suspended material settle by gravity. Suspended material may be particles, such as clay or silts, originally present in the source water. Suspended material or floc is typically created from materials in the water and chemicals used in coagulation or, in other treatment processes, such as lime softening (see Lime Softening chapter). Sedimentation is accomplished by decreasing the velocity of the water to a point which the particles will no longer remain in suspension. When the velocity no longer supports the particles, gravity will remove them from the water flow.

PARTICLE SIZE

The size and type of particles to be removed have a significant effect on the operation of the sedimentation tank. Sand or silt can be removed very easily because of their density. The velocity of the water-flow channel can be slowed to less than one foot per second and most of the sand and silt will be removed by simple gravitational forces. In contrast, colloidal material (small particles that stay in suspension and make the water seem cloudy) will not settle until the material is coagulated and flocculated by adding a chemical, such as iron salt or aluminum sulfate. The shape of the particle also affects its settling characteristics. A round particle, for example, will settle much more readily than a particle that has ragged or irregular edges. All particles also tend to have a slight electrical charge. Particles with the

same charge tend to repel each other. This repelling action keeps the particles from congregating into flocs and settling.

WATER TEMPERATURE

When water temperature decreases, the rate of settling becomes slower. The result is that, as the water cools, detention time in the sedimentation tank must increase and the operator must make changes to the coagulant dosage to compensate for the decreased settling rate. In most cases, temperature does not have a significant effect on treatment. A water treatment plant has the highest flow demand in the summer when the temperatures are highest and settling rates are the best. When water is colder, the flow in the plant is at its lowest and, in most cases; detention time in the plant is increased so floc has time to settle in the sedimentation basin.

CURRENTS

Several types of water currents may occur in the sedimentation basin. Density currents are caused by the weight of solids, the concentration of solids, and the temperature of the water. Eddy currents are produced by the velocity and flow of the water coming into the basin and leaving the basin. Currents can be beneficial in that they promote sedimentation of the particles. However, currents also tend to distribute floc unevenly throughout the basin; as a result, do settle at an even rate. Current problems can be reduced by proper design of the basin and installation of baffles can help prevent currents from short circuiting the basin.

SEDIMENTATION BASIN ZONES

Most sedimentation tanks are divided into these separate zones: Inlet Zone The inlet or influent zone should distribute flow uniformly across the inlet to the tank. The normal design includes baffles that gently spread the flow across the total inlet of the tank and prevent short circuiting in the tank. (Short circuiting is the term used for a situation in which part of the

influent water exits the tank too quickly, by flowing across the top or along the bottom of the tank.) The baffle is sometimes designed as a wall across the inlet, with holes perforated across the width of the tank. Settling Zone The settling zone is the largest portion of the sedimentation basin. This zone provides the calm area necessary for the suspended particles to settle. Sludge Zone The sludge zone, located at the bottom of the tank, provides a storage area for the sludge before it is removed for additional treatment or disposal. Basin inlets should be designed to minimize high flow velocities near the bottom of the tank. If high flow velocities are allowed to enter the sludge zone, the sludge could be swept up and out of the tank. Sludge is removed for further treatment from the sludge zone by scraper or vacuum devices which move along the bottom. Outlet Zone The basin outlet zone (or launder) should provide a smooth transition from the sedimentation zone to the outlet from the tank. This area of the tank also controls the depth of water in the basin. Weirs set at the end of the tank control the overflow rate and prevent the solids from rising to the weirs and leaving the tank before they settle out. The tank needs enough weir length to control the overflow rate, which should not exceed 20,000 gallons per day per foot of weir.

SELECTION OF BASIN

There are many sedimentation basin shapes including rectangular, circular, and square. Rectangular Basins Rectangular basins are commonly found in large-scale water treatment plants. Rectangular tanks are popular as they tend to have: • High tolerance to shock overload • Predictable performance • Cost effectiveness due to lower construction cost • Lower maintenance • Minimal short circuiting Circular and Square Basins Circular basins are often referred to as clarifiers. These basins share some of the performance advantages of the rectangular basins, but are generally more prone to short circuiting and particle removal problems. For square tanks the design engineer must be certain that some type of sludge removal equipment for the corners is installed.

HIGH-RATE TUBE SETTLERS

High rate tube settlers are designed to improve the characteristics of the rectangular basin and to increase flow through the tank. The tube settlers consist of a series of tubes that are installed at a 60 degree angle to the surface of the tank. The flow is directed up through the settlers. Particles have a tendency to flow at an angle different than the water and to contact the tube at some point before reaching the top of the tube. After particles have been removed from the flow and collected on the tubes, they tend to slide down the tube and back into the sludge zone.

SOLIDS CONTACT UNIT

A solids contact unit combines coagulation, flocculation, and sedimentation in one unit. These units are also called upflow clarifiers or sludge-blanket clarifiers. These units are used primarily with lime-soda ash to settle floc formed during water softening. Flow is usually in an upward direction through a sludge blanket or slurry of flocculated suspended solids.

Disintegration of Cells:

Disruption of microbial cells is usually difficult due to their small size, strong cell wall and high osmotic pressure inside cells. Generally, cell disruption is achieved by mechanical means, lysis or drying.

Mechanical Cell Disruption:

This approach uses shear, e.g., grinding in a ball mill, colloid mill etc., pressure and pressure release, e.g., homogenizer and ultrasound. A widely used method is as follows; the cell suspension is forced through a fine nozzle; the cells disintegrate due to hydrodyanic shear and cavitation.

Drying:

The cells may be dried, e.g., by adding the cells into a large excess of cold acetone and subsequently extracted using buffer or salt solutions. Drying induces changes in cell wall structure which facilitates extraction. This method is widely used.

Lysis:

Microbial cells may be lysed by chemical means, e.g., salts or surfactants, osmotic shock, freezing, or by lytic enzymes, e.g., lysozyme etc.In general, recovery of enzyme activity is the best following cell disruption using enzymes or ultrasound, followed by thermal and osmotic methods, while mechanical methods are the least desirable. However, ultrasound method is confined to laboratory mainly due to difficulties in heat removal on a large scale.

Flocculation and Floatation:

Small bacterial cells, which are difficult to separate even by centrifugation, can be recovered as follows. Flocculation, i.e., sticking together of cells, can be induced by inorganic salts, mineral hydro- colloids and organic polyelectrolytes. Since sedimentation rate of a particle increases with size, flocculated cells can be recovered by centrifugation.

In cases, where flocculation is not effective, very fine gas bubbles can be created by sparging, release of overpressure or electrolysis. In any case the gas bubbles absorb to and surround the cells, raising them to the surface of medium in form of foam (floatation); long chain fatty acids or amines promote stable foam formation.

The cells collected in the foam are readily recovered. Flocculation and floatation are used for the most efficient recovery of microbial biomass in some single cell protein production systems.

Extraction:

The process of recovering a compound or a group of compounds from a mixture or from cells into a solvent phase is called extraction. Extraction usually achieves both separation as well as concentration of the product. It is especially useful for the recovery of lipophilic substances, and in antibiotic recovery; it is often an early step after cell separation.

Liquid-Liquid Extraction:

It employs two immiscible liquids into which the product is differentially soluble. Usually successively smaller volumes of the solvent are used for repeated extraction of a given sample; back- extraction also tends to increase the selectivity of extraction. The extraction may be performed in a single step, by multi-stage parallel-flow extraction, or by counter-current extraction (most complex but mosteffective).

Whole Broth (Medium + Cells) Extraction:

It should be used wherever possible since it reduces the number of steps as well as product loss. The effectiveness of extraction may, however, be reduced due to the presence of cells.

Aqueous Multiphase Extraction:

It is used for separation of enzymes from cells/cell debris. The enzymes are extracted in an aqueous polyethylene glycol-dextran mixture which form separate phases. Recovery of enzymes from these phases is rather easy and free from some of the difficulties encountered in centrifugation.

Liquid-Liquid Extraction:

The concentration of biological products can be achieved by transferring the desired product (solute) from one liquid phase to another liquid phase, a phenomenon referred to as liquid-liquid extraction. Besides concentration, this technique is also useful for partial purification of a product.

The efficiency of extraction is dependent on the partition coefficient i.e. the relative distribution of a substance between the two liquid phases. The process of liquid-liquid extraction may be broadly categorized as extraction of low molecular weight products and extraction of high molecular weight products.

Extraction of low molecular weight products:

By using organic solvents, the lipophilic compounds can be conveniently extracted. However, it is quite difficult to extract hydrophilic compounds. Extraction of lipophilic products can be done by the following techniques.

Physical extraction:

The compound gets itself distributed between two liquid phases based on the physical properties. This technique is used for extraction of non-ionising compounds.

Dissociation extraction:

This technique is suitable for the extraction of ionisable compounds. Certain antibiotics can be extracted by this procedure.

Reactive extraction:

In this case, the desired product is made to react with a carrier molecule (e.g., phosphorus compound, aliphatic amine) and extracted into organic solvent. Reactive extraction procedure is quite useful for the extraction of certain compounds that are highly soluble in water (aqueous phase) e.g., organic acids.

Supercritical fluid (SCF) extraction:

This technique differs from the above procedures, since the materials used for extraction are supercritical fluids (SCFs). SCFs are intermediates between gases and liquids and exist as fluids above their critical temperature and pressure. Supercritical CO_2, with a low critical temperature and pressure is commonly used in the extraction. Supercritical fluid extraction is rather expensive, hence not widely used (SCF has been used for the extraction of caffeine from coffee beans, and pigments and flavor ingredients from biological materials).

Extraction of high molecular weight compounds:

Proteins are the most predominant high molecular weight products produced in fermentation industries. Organic solvents cannot be used for protein extraction, as they lose their biological activities. They are extracted by using an aqueous two-phase systems or reverse micelles formation.

Aqueous two-phase systems (ATPS):

They can be prepared by mixing a polymer (e.g., polyethylene glycol) and a salt solution (ammonium sulfate) or two different polymers. Water is the main component in ATPS, but the two phases are not miscible. Cells and other solids remain in one phase while the proteins are transferred to other phase. The distribution of the desired product is based on its surface and ionic character and the nature of phases. The separation takes much longer time by ATPS.

Reverse miceller systems:

Reverse micelles are stable aggregates of surfactant molecules and water in organic solvents. The proteins can be extracted from the aqueous medium by forming reverse micelles. In fact, the enzymes can be extracted by this procedure without loss of biological activity.

Concentration:

Some concentration of the product may occur during the extraction step.

Further concentration may be achieved by:

(i) Evaporation,
(ii) Membrane filtration,
(iii) Ion exchange methods and
(iv) Adsorption methods.

Evaporation:

It is generally used in cases of solvent extraction using various devices, e.g., continuous flow evaporators, falling film evaporators, thin film evaporators, centrifugal thin film evaporators and spray- dryers. Efficient arrangements must be made for recovery of the evaporated solvent to reduce costs. For low grade products, often evaporation of the whole broth is undertaken using a spray-drier.

Membrane Filtration:

It generally achieves both concentration and separation of the products usually based on the size of molecules. The different processes of membrane filtration are: microfiltration, ultrafiltration, reverse osmosis and electro-dialysis.Micro- and ultrafiltration work as sieves and separate molecules of different sizes, but reverse osmosis can separate molecules of similar size. Microfiltration can be used for cell separation as well.

Ion Exchange Resins:

These are polymers having firmly attached ionizable groups (anions or cations) which ionize under a suitable environment. These may be solid, e.g., dextran, cellulose, polyamine, acrylate etc., or liquid, e.g., a solvent carrying a functional group like phosphoric acid mono- or diester etc.

Solid ion exchangers may be used in two ways:

(i) They may be packed in columns or

(ii) They may be added to the extract and removed by decantation. Liquid ion exchangers dissolve only in non-aqueous solvent carrier and the separation is similar to liquid-liquid extraction.Some antibiotics are recovered directly from the whole broth using ion exchange resins. The product is recovered from the ion exchangers by ion displacement; this also regenerates the ion exchanger.

Absorption Resins:

These are porous polymers without ionization. Most compounds are adsorbed to the resins in non-nonized state. The porosity of the resin determines the surface available for adsorption. These resins may be apolar (e.g., styrene-divinyl bcneze), polar (e.g., sulfoxide, amide etc.), or semipolar (e.g., acrylic ester). The products are recovered from such resins by solvent (organic) extraction, changed pH etc.

Purification:

The final step in the recovery of a product is purification which aims at obtaining the product in highly purified state. The earlier steps will have achieved variable

degrees of purification which may determine the degree of resolution necessary during the purification step.

The degree of resolution will mainly depend on the similarities to the metabolite of other molecules present in the concentrate, and the degree of purity required in the final product.

Purification is achieved by:

(i) Crystallization and

(ii) Chromatographic procedures.

Crystallization:

It is mainly used for purification of low molecular weight compounds like antibiotics, e.g., penicillin G is usually extracted from fermentation broth in butyl acetate and cystallized by the addition of potassium acetate in ethanolic solution. Crystallization is the final stage in purification of products like citric- acid, sodium glutamate etc.

Chromatographic Methods:

These are used for purification of low molecular weight compounds from mixtures of similar molecules, e.g., homologous antibiotics, and of macromolecules, especially enzymes, which are similar in properties. The materials used for chromatography are generally coated on particulate carriers which are packed in columns through which the liquid containing the product is pumped either upward or downward.The separated product is recovered in some sort of fraction collector. On a large, scale organic solvents are used for collection; therefore, the whole system has to be installed in a flame-proof and explosion- proof room.

The different chromatographic procedures are:

(i) Adsorption,(ii) Ion exchange, (iii) Gel filtration, (iv) Hydrophobic, (v)Affinity,(vi) Covalent and (vii) Partition chromatography.

Adsorption chromatography separates molecules due to their differential affinities for the surface of a solid matrix, e.g., silica gel, alumina, hydroxyapatite (all

inorganic) or an organic polymer. In case of ion exchange chromatography, resins or polysaccharides, e.g., cellulose, sepharose, having attached ionized functional groups are used for a high resolution separation of macromolecules, e.g.,proteins.

Gel filtration uses molecular sieves, composed of neutral cross-linked carriers (e.g., polymers like agarose, dextran's), of different pore sizes. Molecules smaller than the pore size enter the carrier and are retained; they are later eluted (in order of molecule size) and collected. Gel filtration is used in aqueous systems. Hydrophobic carriers are used for purification of hydrophobic molecules, e.g., many enzymes and other proteins.

Affinity chromatography uses molecules, called effectors (Table 39.7), to which the product has high and specific affinity, e.g., using an antibody (effector) for the purification of the antigen to which it is specific. The effector is immobilized on a water insoluble carrier which is packed in a column through which the mixture is passed.The effector binds only to the molecules for which it is specific and retains it in the column; it is later recovered by elution using a buffer solution of a specified pH (Fig. 39.4). For example, human leucocyte interferon is recovered in high yield and in high purity by affinity chromatography using monoclonal antibody immobilized on a sepharose column.Group specific affinity chromatography and covalent chromatography are based on chemical interactions between the carrier and the product molecules.

Drying:

Drying makes the products suitable for handling and storage. It should be accomplished with a minimum rise in temperature due to heat sensitivity of most products. Addition of sugars or other stabilizers improves the heat tolerance of some products like enzymes and pharmaceutical preparations.

The most common approaches to drying are as follows:

(i) Vacuum drying, (ii) Spray drying and (iii) Freeze drying.

In spray drying, the solution or slurry to be dried is atomized by a nozzle or a rotating disc. A current of hot (150-250°C) air is passed; the drying is so rapid that the temperature of particles remains very low.

Spray drying is used for enzymes, antibiotics, and food products. Vacuum drying uses both heat and vacuum for drying; it can be applied both in batch mode (e.g., chamber dryers) or in continuous mode (e.g., rotating drum vacuum dryers).

In freeze drying, the liquor to be dried is first frozen and the water is sublimed from the frozen mass. A very low pressure (partial vacuum) is maintained to promote sublimation of water. The energy needed for sublimation is provided by heated plates and radiation on to the surface.

The temperature of solid is regulated by regulating the pressure in the drying chamber. This is the most gentle method of drying, and is used for many pharmaceutical products, e.g., viruses, vaccines, plasma fractions, enzymes etc., and in food industries.

Circular dichroism spectroscopy

The phenomenon of circular dichroism is very sensitive to the secondary structure of polypeptides and proteins Circular dichroism (CD) spectroscopy is a form of light absorption spectroscopy that measures the difference in absorbance of right- and left-circularly polarized light (rather than the commonly used absorbance of isotropic light) by a substance. It has been shown that CD spectra between 260 and approximately 180 nm can be analyzed for the different secondary structural types: alpha helix, parallel and antiparallel beta sheet, turn, and other. A number of excellent review articles are available describing the technique and its application (Woody, 1985;Johnson, 1990). In fact, optical rotary dispersion (ORD, see below) data suggested a right-handed helical conformation as a major protein structural element before the Pauling and Corey model (Pauling & Corey, 1951) and Kendrew's structure of myoglobin.

Modern secondary structure determination by CD are reported to achieve accuracies of 0.97 for helices, 0.75 for beta sheet, 0.50 for turns, and 0.89 for other structure types (Manavalan & Johnson, 1987).

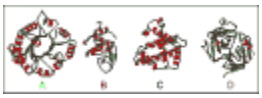


Figure 21. *Cartoon drawings of: A) triosephosphate isomerase (H:0.52, S:0.14, T:0.11, O:0.23); B) hen egg lysozyme (H:0.36, S:0.09, T:0.32, O:0.23); C) myoglobin (H:0.78, S:0.0, T:0.12, O:0.10); and D) chymotrypsin (H:0.10, S:0.34, T:0.20, O:0.36). Secondary structures are color coded red:helix. green:strand, and yellow:other.*

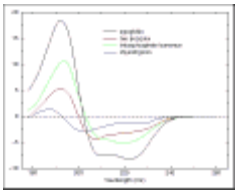


Figure 22. *Circular dichroism spectra of triosephosphate isomerase (H:0.52, S:0.14, T:0.11, O:0.23), hen egg lysozyme (H:0.36, S:0.09, T:0.32, O:0.23), myoglobin (H:0.78, S:0.0, T:0.12, O:0.10), and chymotrypsin (H:0.10, S:0.34, T:0.20, O:0.36).*

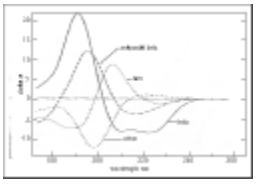


Figure 23. *Circular dichroism spectra of "pure" secondary structures. Redrawn from Brahms & Brahms, 1980.*

Physical principles of CD

Inherently asymmetric chromophores (uncommon) or symmetric chromophores in asymmetric environments will interact differently with right- and left-circularly polarized light resulting in two related phenomena. Circularly-polarized light rays will travel through an optically active medium with different velocities due to the different indices of refraction for right- and left-circularly polarized light called **optical rotation** or **circular birefringence**. The variation of optical rotation as a function of wavelength is called **optical rotary dispersion (ORD)**. Right- and left-circularly polarized light will also be absorbed to different extents at some wavelengths due to differences in extinction coefficients for the two polarized rays called **circular dichroism (CD)**. Optical rotary dispersion enables a chiral molecule to rotate the plane of polarized light. ORD spectra are dispersive (called a **Cotton effect** for a single band) whereas circular dichroism spectra are absorptive. The two phenomena are related by the so-called König-Kramers transforms.

It can be shown that if right- and left-circularly polarized light is absorbed to different extents at any wavelength, there will be a difference in refractive indices at virtually all wavelengths. This accounts for the ability of small saturated chiral molecules to rotate the plane of polarized light of the D-line of sodium (589 nm), far away from absorptive bands. The dispersive nature of ORD is also the reason CD is a more sensitive analytical technique. Unlike the dispersive ORD phenomenon, circular dichroism can only occur within a normal absorption band and thus requires either an inherently asymmetric chromophore (uncommon) or a symmetric one in an asymmetric environment.

Most people are familiar with the concept of linearly-polarized light due to commonly-used Polarized sunglasses. However, circularly-polarized light may not be so easily conceived. Recall that electromagnetic radiation is a complex wave form that can be considered to behave as two simple wave motions at right angles to each other. One of these waves is **magnetic** (M) and the other is **electric** (E). Electromagnetic waves are generated by oscillating electric or magnetic dipoles and are propagated at the speed of light (c). Since the E- and the M-components are

always perpendicular to each other, it is sufficient, in many cases, to consider only the E-component in describing the wave.

Figure 24. *Schematic diagram showing the orthogonal electronic (E) and magnetic (B) components of linearly polarized light.*

Although the amplitude of the E-wave oscillates in the zx-plane in the figure above, it could oscillate in any direction perpendicular to the direction of propagation (z). **Unpolarized light**, the type we get from the sun or a light bulb contains oscillations of the E-components in all directions perpendicular to the direction of propagation. **Linearly polarized light** results when the direction of the E-component is restricted to a plane perpendicular to the direction of propagation while its magnitude oscillates. **Circularly polarized light** is another form of polarization - in this case, the magnitude of the oscillation is constant and the direction oscillates.

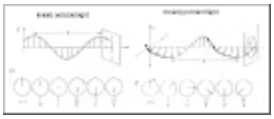


Figure 25. *Schematic diagram showing the electronic component of linearly polarized light (left) and right-handed circularly polarized light (right). Below each are the electronic component vectors as viewed along the axis of propagation from left of the diagram above.*

The differential absorption of radiation polarized in two directions as function of frequency is called dichroism. When applied to plane polarized light, this is called linear dichroism; for circularly polarized light, circular dichroism. We can think of linear polarized light as the result of two equal amplitudes of opposite circular polarization. After passing through an optically active sample, circularly polarized light will be changed in two aspects. The two components are

still circularly-polarized, but the magnitudes of the counter-rotating E-components will no longer be equal as the molar extinction coefficients for right- and left-polarized light are unequal. The direction of the E-vector no longer traces a circle - instead it traces an ellipse (actually an elliptical screw if we do not confine ourselves to the projection) There will also be a rotation of the major axis of the ellipse due to differences in refractive indices.

Historically, **ellipticity** is the unit of circular dichroism and is defined as the tangent of the ratio of the minor to major elliptical axis. Axial ratios of 1:100 will then result in an ellipticity of 0.57 degrees. Modern CD instruments are capable of millidegree precision. The unit ellipticity persists despite the fact that CD is now measured as the difference in absorbance of right- and left- circularly polarized light as a function of wavelength. Each type obeys the Lambert-Beer law so that the difference in extinction coefficients (left - right) at a given wavelength is equal to the difference in absorbance divided by the product of the pathlength and the concentration (Sorry, but I cannot format equations in html).

There exists a number of ways the CD of a sample has been reported in the literature. The most commonly used units in current literature are **mean residue ellipticity** (degree cm2 dmol -1) and the difference in molar extinction coefficients called the **molar circular dichroism** or **delta epsilon** (liter mol-1 cm-1). The molar ellipticity [theta] is related to the difference in extinction coefficients by [theta] = 3298(delta epsilon). Here [theta] has the standard units of degrees cm2 dmol -1 and the molar ellipticity has the units degrees deciliters mol-1 decimeter-1.

For proteins we will be mainly concerned with absorption in the ultraviolet region of the spectrum from the peptide bonds (symmetric chromophores) and amino acid sidechains in proteins in this exercise. Protein chromaphores can be divided into three classes: the peptide bond; the amino acid sidechains, and any prosthetic groups. The lowest energy transition in the peptide chromophore is an n --> p* transition observed at 210-220 nm with very weak intensity (emax xbb 100). Compare the UV and CD spectra of poly-L-lysine which can adopt three different secondary structure forms as a function of pH. The n --> p* transition appears in the a-

helical form of the polymer as a small shoulder near 220 nm on the tail of a much stronger absorption band centered at 190 nm. This intense band, responsible for the majority of the peptide bond absorbance is a p --> p* transition (emax xbb 7000).

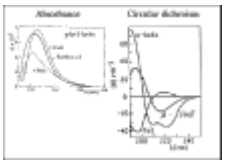


Figure 26. *Comparison of the UV absorbance and the circular dichroism of poly-L-lysine in different secondary structure conformations. The figure on the left was taken from Cantor & Schimmel, 1980 and the figure on the right was taken from Campbell & Dwek, 1984*.

Using CD, these different transitions are more clearly evident (no one has had success predicting secondary structure content from simple UV spectra). Exciton splitting of the p --> p* transition results in the negative band at 208 and positive band at 192.

The three aromatic side chains that occur in proteins (phenyl group of Phe, phenolic group of Tyr, and indole group of Trp) also have absorption bands in the ultraviolet spectrum. However in proteins, the contributions to the CD spectra in the near UV (where secondary structural information is located) is usually negligible. The disulfide group is an inherently asymmetric chromophore as it prefers a gauche conformation with css = xb1 90xb0 , corresponding to a right- or left-handed helical sense and can lead to a broad CD absorption around 250 nm. Aromatic residues, if unusually abundant, can have significant effects on the CD spectra in the region < 230 nm complicating analysis.

Like the other forms of absorption spectroscopy (UV/Vis, IR, etc.), CD is particularly powerful in monitoring **conformational changes**. In the region of 230-178 nm, one expects to observe effects of backbone conformational changes while CD effects at longer wavelengths (>230 nm) should isolate aromatic chromophore contributions and being environment dependent should reflect more global, three-dimensional properties of the protein.

If one is considering using CD spectroscopy, you should definitely read one of the reviews by the preeminent experimental CD spectroscopist W. Curtis Johnson jr (e.g., Johnson 1988 or Johnson, 1990; Johnson, 1992). To give you an idea what you will need, I offer the following brief **summary of experimental conditions**.

The concentration of peptide needed for CD studies will depend on exactly what information is expected from the spectra. As a general rule of thumb, one requires that the total absorbance of the cell, buffer, and protein be between 0.4 and 1.0 (theoretically, 0.87 is optimal). This means for a 0.01 cm cell, 20-50 ul of a **protein concentration** of 0.2-0.5 mg/ml is needed to record spectra to 178 nm. To achieve adequate signal-to-noise (S/N is proportional to the square-root of the amount of time measuring the spectrum), a spectra for secondary structure determination (260-178 nm) will require **30-60 minutes** to record (plus an equivalent amount of time for a baseline as every CD spectrometer I have seen is a single beam instrument). Another consideration is that oxygen absorbs strongly below about 200 nm so very extensive purging with pure (oxygen-free) nitrogen (>16 liter/min) is necessary for these measurements. A typical **buffer** used in CD experiments is 10mM phosphate, although low concentrations of Tris, perchlorate or borate is also acceptable. Potassium fluoride is preferred to NaCl for increasing the ionic strength as chloride ion has a strong UV absorbance at low wavelengths.

Secondary structure from CD spectra

The simplest method of extracting secondary structure content from CD data is to assume that a spectrum is a linear combination of CD spectra of each contributing secondary structure type (e.g., "pure" alpha helix, "pure" beta strand etc.) weighted by its abundance in the polypeptide conformation. The major drawback of this approach that there is no standard reference CD spectra for "pure" secondary structures. Synthetic homopolypeptides used to obtain reference spectra are in general, poor models for the secondary structures found in proteins. For example, the CD of an alpha helix has been shown to be length dependent and no homopolypeptide system has been found that is a good example of the beta sheet structure found in proteins.

In response to these shortcomings, several methods have been developed which analyze the experimental CD spectra using a database of reference protein CD spectra containing known amounts of secondary structure (Provencher & Glöckner, 1981; Hennesey & Johnson, 1981; Manalavan & Johnson, 1987; Sreerama & Woody, 1994). These methods are in general more accurate and reliable than the novel approach outlined above.

In one method (Manalavan & Johnson, 1987), single value decomposition is used to create orthogonal CD basis vectors from CD spectra of proteins with known secondary structure. Using the statistical technique of variable selection, unimportant variables are removed from an underdetermined system of equations allowing the solution for the important ones. The great advantage of this technique (and others like it) is that one makes no assumptions on the form of the CD from the individual secondary structural elements. One needs only to be sure that the overall structural characteristics of the analyzed protein are represented in the set of reference spectra. In this way irregularities of secondary structure and length dependencies should be taken into account

Two very important requirements for successful secondary structure analysis using these techniques deserves further comment. One is that the CD spectra need to be recorded from about 260 nm to at least 184nm (and preferable 178 or below; Johnson, 1990) and the other is that an accurate protein concentration (< 10% error) is essential. The assumptions and limitations of these techniques are discussed in detail by Manning (1989).

Circular dichroism spectroscpy has also been used to determine the tertiary structure class of globular proteins. The method proposed by Venyaminov & Vassilenko (1994) claim 100% accuracy for predicting all alpha, alpha/beta, and denatured proteins; 85% for alpha + beta; and 75% for all beta proteins.

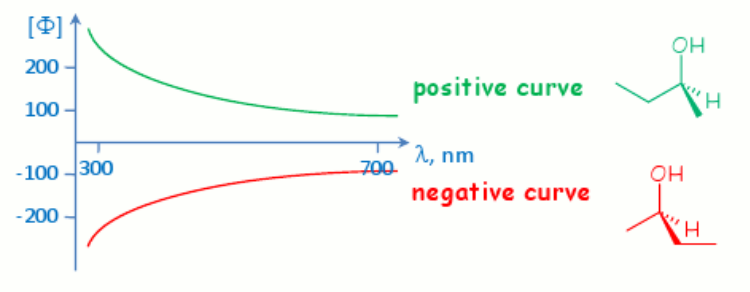
CD of peptides

Circular dichroism spectroscopy has been extensively applied to the structural characterization of peptides. The application of CD for conformational studies in peptides (like proteins) can be largely grouped into 1.) monitoring conformational changes (e.g., monomer-oligomer, substrate binding, denaturation, etc.) and 2.) estimation of secondary structural content (e.g., this peptide is 25% helical under these conditions). As already mentioned, CD is particularly well-suited to determine structural changes in both proteins and peptides. However, absolute structural content is more difficult and is prone to over-interpretation. Length-dependencies of the CD spectra of "pure" secondary structures are only one potential caveat.

The CD spectra of peptides have been reported in a number of solvent systems. The helix-promoting characteristics of trifluoroethanol (TFE) and hexafluoroisopropanol (HFIP) are well-known. A number of authors have argued that these solvents mimic a particular characteristic of the in vivo system and therefore attach relevance to structural studies performed in these solvents. Of particular importance to this matter is a study by Waterhous & Johnson (1994) who demonstrated that peptide sequences could be induced to form alpha helices or beta strands in TFE and non-micellar SDS solutions, respectively, regardless of their secondary structure in the native protein. This result underscores the difficulties in assigning relevance to structural studies in peptides using CD spectroscopy.

ORD spectra

Plain ORD curve These curves are observed for transparent compounds in the UV range. A plain curve results showing a steady increase (or decrease) of optical rotation with decrease in wavelength. These curves does not cross the zero-rotation axis and are devoid of maxima or minima within measurable range. So-called plain curve is the ORD for a chiral compound that lacks a chromophore. For Example, alcohols and hydrocarbons exhibit such kind of ORD curves.



Another Property, Absorbance : Anomalous ORD Curve

We have mentioned the term chromophore. It is the part of a molecule where absorbance of light takes place. This occurs because at this part energy of electronic transition between two molecular orbitals with energy of the photons of the incident light. Incident light that hits the chromophore can thus be absorbed by exciting an electron from its ground state into an excited state. So there is a range of wavelength, called the absorption band, where the light is absorved. This absorption band has a maxima at a particular wavelength λmax. As a result there comes an anomaly in the ORD curve at the neighborhood of the chromophore. The absolute magnitude of the optical rotation at first varies rapidly with wavelength, crosses zero at absorption maxima and then again varies rapidly with wavelength but in opposite direction. Such anomaly is known as Cotton effect. As opposed to plain curves, these curves exhibit a number of extreme peaks and troughs depending on the number of absorbing groups. The Cotton effect is called positive if the optical rotation first increases as the wavelength decreases (as first observed by Cotton), and negative if the rotation first decreases. The following figure exhibits both types of Cotton effects.

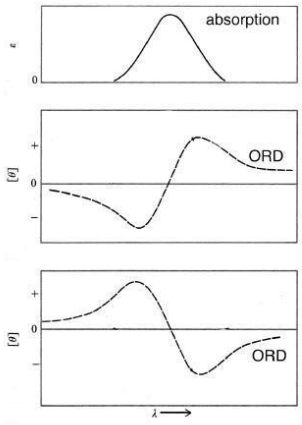
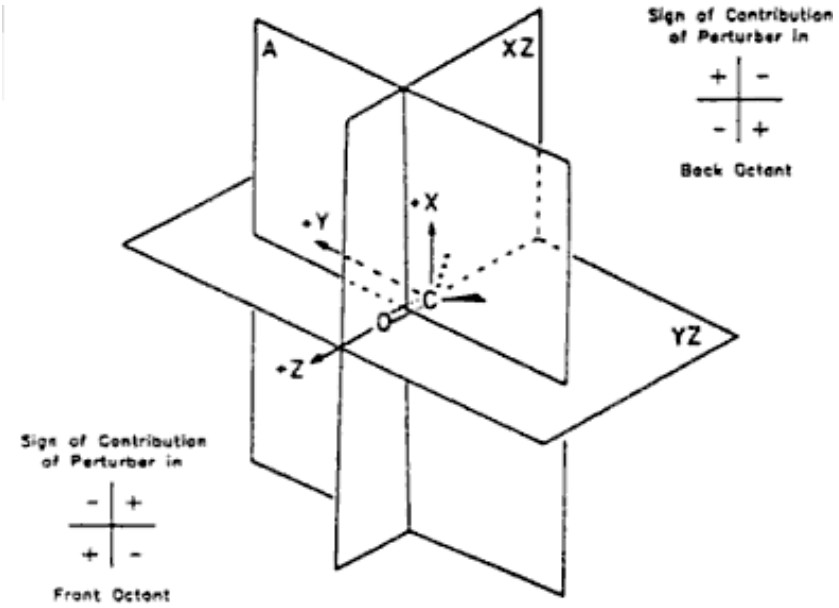


Figure shows absorption band and negative and positive cotton effect near the absorption maxima

Octant Rule: an empirical method to determine type of Cotton effect in Ketones

In case of saturated alkyl ketones there is a way, known as octant rule, to predict the sign of cotton effect (+ve or -ve) directly from the understanding of the structure. We can divide the space around the ketone into eight octants by looking down the carbonyl as shown below.



source: Circular Dichroism Principles and Applications Second Edition edited by Nina Berova,Koji Nakanishi,Robert W Woody Page 233

Four octants in the front of the oxygen (toward the observer) and four behind. The front four can be ignored but the rear four usually contain all the atoms in the molecule. Each of these four rear octants are assigned a sign(+ ve or -ve) and all the atoms of the molecule are distributed in these four octants. Now depending on the occupancy of these four octants the sign of the cotton effect may be determined. The sign of the octant having highest occupancy determines the sign of the cotton effect.

This experiment has got two parts. In the first part (experiment 2a) we will examine how the specific rotation changes with wavelength by actually handling example EM wave functions using a programme called emanim_ord.py . And in the second part (experiment 2b) we will try to determine the ORD spectra of some sample compounds exhibiting plain as well as anomalous ORD spectra.

This experiment also has got two parts. In the first part (experiment 3a) we will examine how the molar ellipticity changes with wavelength by actually handling example EM wave functions using a programme called emanim_cd.py . And in the second part (experiment 3b) we will try to determine the CD spectra of some sample compounds exhibiting different CD spectra.

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UNIT –III MEMBRANE SEPARATION PROCESS- SBT1306

MEMBRANE SEPARATION PROCESS

Introduction :

Membranes are the selective barriers normally used to separate two phases and restrict the transport of various chemicals in a selective manner. Membranes can be homogenous or heterogeneous, symmetric or asymmetric in structure, solid or liquid, porous or non porous. In case of porous membrane pore, size is the key parameters which determine the effective ness and efficiency of the membrane. It can carry a positive or negative charge or be neutral or bipolar. Transport through a membrane can be affected by convection or by diffusion of individual molecules, induced by an electric field or concentration, pressure or temperature gradient. Normally, separation occurs under a pressure gradient or sometimes under an electrical potential gradient, associate with or without a catalytic reaction. System which can be separated, are solid particles suspended in a fluid medium and mixture of two different liquids or gases. Separation through a membrane is schematically shown in Fig 1.1. Porous membranes are typically classified according to their pore sizes in the following manner:

Membrane Separation technology:

A membrane separation system separates an influent stream into two effluent streams known as permeate and the concentrate. Permeate is the portion of the fluid that has passed through the semi-permeable membrane. Whereas the concentrate stream contains the constituents that have been

rejected by the membrane. Membrane separation process enjoys numerous industrial applications with the following advantages:

1. Appreciable energy savings
2. Environmentally benign
3. Clean technology with operational ease
4. It replaces the conventional processes like filtration, distillation, ion-exchange and chemical treatment systems.
5. It produces high, quality products.

6. Greater flexibility in designing systems.

Membrane Structure and morphology:

From a structural point of view membranes are broadly divided into two types as shown in Fig. 1.2:

a. Symmetrical, and
b. Asymmetrical (or anisotropic)

Symmetrical membrane has similar structural morphology at all positions within it. An anisotropic membrane is constituted of two or more structural planes of non-identical morphologies. From a morphological point of view, membranes can be porous or dense. Porous membranes have been tiny pores or pore networks within themselves (see Fig. 1.3). On the other hand, dense membranes do not have any pores and solute or solvent transport through these takes place by a solubilization mechanism.

Driving force in membrane separation

In order to drive the solutes and solvents through a membrane driving force is necessary. These include:

1. Transmembrane (hydrostatic) pressure (TMP)
2. Concentration or electrochemical gradient
3. Osmotic pressure
4. Electrical field
5. Partial pressure

Membrane processes that separate primarily based on size:

Membrane processes are divided into four types based upon the size of component in the feed solution that is allowed to pass. With some overlap, the categorization, from largest to smallest permeable species, is microfiltration, ultrafiltration, nanofiltration and reverse osmosis (see Fig.). The different applications are listed in Table 1.1. A fifth type of size based membrane separation process called dialysis allows solutes similar those in reverse osmosis to pass

through. However, unlike reverse osmosis, which is a pressure driven process, dialysis is a concentration gradient driven process.

Microfiltration (MF)

Micro filtration (MF) is the process of removing particles or biological entities in the 0.025 µm to 10.0µm range from fluids by passage through a microporous medium such as a membrane filter. Transmembrane pressures ranging from 1 to 50 psi are used as the driving force. If the pore sizes of the membrane are smaller than the particles in the solution, surface filtration results. Although micron-sized particles can be removed by use of non-membrane or depth materials such as those found in fibrous media, only a membrane filter having a precisely defined pore size can ensure quantitative retention. Membrane filters can be used for final filtration or prefiltration, whereas a depth filter is generally used in clarifying applications where quantitative retention is not required or as a prefilter to prolong the life of a downstream membrane. Membrane and depth filters offer certain advantages and limitations. They can complement each other when used together in a microfiltration process system or fabricated device. The retention boundary defined by a membrane filter can also be used as an analytical tool to validate the integrity and efficiency of a system. For example, in addition to clarifying or sterilizing filtration, fluids containing bacteria can be filtered to trap the microorganisms on the membrane surface for subsequent culture and analysis. Microfiltration can also be used in sample preparation to remove intact cells and some cell debris from the lysate. Membrane pore size cut- offs used for these types of separation are typically in the range of 0.05 µm to 1.0 µm.[3]

Ultrafiltration (UF)

Ultrafiltration (UF) is the process of separating extremely small particles and dissolved molecules from fluids. The primary basis for separation is the molecular size, although in all filtration applications, the permeability of a filter medium can be affected by the chemical, molecular or electrostatic properties for the sample. Ultra filtration can only separate molecules,

which differ by at least an order of magnitude in size. Molecules of similar size cannot be separated by ultra filtration. Normal transmembrane pressure ranges from 10 to 100 psi. The product can be the permeate, the retentate, or both Materials ranging in size from 1K to 1000K molecular weights (MW) are retained by certain ultrafiltration membranes, while salts and water will pass through. Colloidal and particulate matter can also be retained. Ultrafiltration membranes can be used both to purify material passing through the filter and also to collect material retained by the filter. Materials significantly smaller than the pore size rating pass through the filter and can be dehydrogenated, clarified and separated from high molecular weight contaminants. Materials larger than the pore size rating are retained by the filter and can be concentrated or separated from low molecular weight contaminants. Ultrafiltration is typically used to separate proteins from buffer components for buffer exchange, desalting, or concentration. Ultrafilters are also ideal for removal or exchange of sugars, non-aqueous solvents, the separation of free from protein-bound ligands, the removal of materials of low molecular weight, or the rapid change of ionic and/or pH environment (see Figure 1.1). Depending on the protein to be retained, the most frequently used membranes have a nominal molecular weight limit (NMWL) of 3 kDa to 100 kDa. Ultrafiltration is far gentler to solutes than processes such as precipitation. UF is more efficient because it can simultaneously concentrate and desalt solutes. It does not require a phase change, which often denatures labile species, and UF can be performed either at room temperature or in a cold room [3,4].

Nanofiltration (NF)

Nanofiltration is a liquid separation membrane technology positioned between reverse osmosis (RO) and ultrafiltration. While RO can remove the smallest of solute molecules, in the range of 0.0001 micron in diameter and smaller, nanofiltration (NF) removes molecules in the range 0.001 micron. NF refers to a membrane process that rejects solutes approximately 1 nanometer (10 angstroms) in size with molecular weights above 200. Because they feature pore sizes larger than RO membranes, NF membranes remove organic compounds and selected salts at the lower pressure than RO system. It separates at the molecular level, removing all suspended solids and most dissolved solids. Transmembrane pressures range from 40 to 200 psi (2.0 kg/cm$_2$ to 14 kg/cm$_2$. NF essentially is a lower-pressure version of RO where the purity of product water is not as critical as with pharmaceutical grade water, or the level of dissolved solids to be removed is less than what typically is encountered in brackish water or seawater. [3, 4]

Reverse Osmosis (RO)

It is used to remove dissolved solids from solvents. By applying transmembrane pressure to concentrated solutions, it is possible to force the solvent through the RO membrane towards the lower concentration. Hence the terms reverse osmosis. It is a separation process of small (monovalent) ions and molecules ($M < 300$ Da) on so called "dense" membranes. The range of the sizes of molecules that are separated with RO is 1–10 Å. The range of the transmembrane pressure applied is 10–100 bars depending upon the concentration difference of the separated species on both sides of the solution. Reverse osmosis (RO) is increasingly used in chemical, textile, petrochemical, electrochemical, food, paper and tanning industries, as well as in the treatment of tap water and wastewaters. Reverse osmosis is mainly used for water purification, including ultrapure water production, desalination, water treatment, wastewater treatment and landfill leachates treatment. After RO purification, pure water may be easily recovered from wastewater and subsequently reused in various production steps. RO is used for industrial effluents treatment, for water reuse, and for concentration of valuable products, for TDS and COD removal from wastewater [2,3,5].

Membrane processes that separate based on principles other than size

Pervaporation (PV)

It is a process to separate a volatile or low-boiling-point liquid from a non-volatile liquid. The driving force is a vacuum on the gaseous side of the membrane. It is a tool for separation of liquid mixtures, especially dehydration of liquid hydrocarbons.

Dialysis

It is a membrane separation process in which one or more dissolved species flow across a selective barrier in response to a difference in concentration. It is the earliest membrane based molecular process to be developed. The mode of transport is diffusion, and separation occurs because small molecules diffuse more rapidly than larger ones, and also because the degree to which the membrane restricts the transport of molecules usually increases with solute size.

Electrodialysis (ED)

It is an electrochemical process used to separate charged particles from an aqueous solution or from other neutral solutes. A stack of membranes is used, half of them passing positively charged particles and rejecting negatively charged ones; the other half doing the opposite. An electrical potential is imposed across the membranes, and a solution with charged particles is pumped through the system. Positively charged particles migrate toward the negative electrode, but are stopped by a positive-particle-rejecting membrane. Negatively charged particles migrate in the opposite direction with similar results. Both types migrate in opposite directions out of one set of cells and collect in the remaining cells. The result is a concentrated solution of both positively and negatively charged particles in every other cell and a low concentration (the product) in the remaining cells [2,3,5].

Various configurations of operating a filtration process:

Dead-end Filtration

The most basic form of filtration is dead-end filtration. The complete feed flow is forced through the membrane, and the filtered matter is accumulated at the surface of the membrane. The dead-end filtration is a batch process as the accumulated matter in the filter decreases the filtration capacity, due to clogging. A next process step to remove the accumulated matter is required. Dead-end filtration can be a very useful technique for concentrating compounds.

Table 1.1: Represents the characteristics of membranes used in different membrane separation processes, process driving forces and applications of such processes [3]

Process	**Membrane Type and Pore**	**Membrane Material**	**Process Diving Force**	**Applications**
Microfiltration	Symmetric microporous, 0.1-10 microns	Cellulosenitrate or acetate, Polyvinylidene difluoride (PVDF), Polyamides, Polysulfone, PTFE, Metal Oxides etc.	Hydro-static pressure difference at approx. 10-500 kPa	Sterile filtration, Clarification
Ultrafiltration	Asymmetric microporous, 1- 10 nm	Polysulfone, Polypropylene, Nylon 6, PTFE, PVC, Acrylic Copolymer	Hydrostatic pressure difference at approx. 0.1-1.0 Mpa	Separation of macromolecular solutions
Reverse Osmosis	Asymmetric skin-type, 0.5-1.5 nm	Polymers, Cellulosic acetate, Aromatic Polyamide	Hydrostatic pressure difference at approx. 2-10 Mpa	Separation of salts and microsolutes from solutions
Electrodialysis	Cationand anion exchange membrane	Sulfonated cross-linked polystyrene	Electrical potential gradient	Desalting of ionic solutions

Gas Separation	Asymmetric homogeneous polymer	Polymers & copolymers	Hydrostatic pressure and concentration gradients	Separation of gas mixtures
Pervaporation	Asymmetric homogenous polymer (A non-porous membrane)	Polyacrylonitrile, Polymers	Vapourpressure gradient	Separation of azeotropic mixtures
Nanofiltration	Thin-film membranes	Cellulosic Acetate and Aromatic	9.3-15.9 bar	Removal of hardness and desalting

Cross-flow Filtration

With cross-flow filtration, a constant turbulent flow along the membrane surface prevents the accumulation of matter in the membrane surface. The membranes used in this process are commonly tubes with a membrane layer on the inside wall of the tube. The feed flow through the membrane tube has an elevated pressure as the driving force for the filtration process and a high- flow speed to create turbulent conditions. The process is referred to as "cross-flow", because the feed flow and filtration flow direction have a 90 degrees angle. Cross-flow filtration is an excellent way to filter liquids with a high concentration of filterable matter.

Hybrid-flow Filtration

The hybrid flow process combines the dead-end and the cross-flow principle. As in the cross-flow filtration tubular membranes are with the filtration layer on the inside wall are used. The filtration process has two phases: the production phase and the flushing phase. During the production phase, the tubes are closed at one side, and a dead-end filtration is performed. During the flushing phase, the tube is open to both sides and the fraction that did not pass through the membranes is removed in order to clean the membrane surface as in cross-flow filtration. This filtration technique is especially suitable for treating water streams containing suspended solids in low concentrations (polishing).

Submerged Filtration

With submerged membrane filtration, the membranes are submerged in the liquid that has to be filtered. The filtration is performed from the outside to the inside of the membrane (filtering layer is on the outer side of the tube or plate). Sheer forces along the membrane surface are created by a flow of air bubbles on the surface. In some cases, the airflow also results in a liquid flow created by the airlift principle. The driving force is a vacuum applied on the inner side of the membrane [2,3,5].

Membrane modules

A key characteristic of membrane processes is the membrane geometry in the actual equipment, which provides the membrane housing and the desired hydrodynamic conditions.Membrane processes can be classified upon the basis of the flow pattern within the membrane module. These are shown in Fig 1.5

Membrane modules can be of different types (see Table1.2 for comparison). Some of these are:

1. Stirred cell module
2. Flat sheet tangential flow (TF) module
3. Tubular membrane module
4. Spiral wound membrane module
5. Hollow fibre membrane module

Stirred cell modules

Stirred cell modules are useful for small scale and research applications. These are more commonly used for UF and MF. Stirred cell modules provide uniform transmembrane pressure and hydrodynamic conditions at all points of the membrane surface. The effects of process parameters on process efficiency can be very easily determined using stirred cell units. These are therefore very useful for small-scale process development work. However, these are not of much use in intermediate and large-scale operations.

The flat sheet TF design is similar to that of a plate and frame filter press. It consists of alternate layers of membranes, support screens (corrugated structural sheets) and distribution chambers for feed and permeates. The units may have square, rectangular or oval cross-section. These units can be easily disassembled for cleaning and for replacement of defective membranes. Other advantages include the ability to accommodate low levels of suspended solids and viscous fluids. Disadvantages include relatively low packing density. TF designs are commonly used for UF, MF and NF. Electrodialysis and electrochemical membranes use only this configuration. The liquid flow pattern in a flat sheet TF unit can be quite complex. The pressure drop distribution is also quite difficult to characterize. Consequently, design and analysis of such devices are largely based on empirical methods.

Spiral wound membrane module

The spiral wound membrane module is like a huge envelope made of membrane and containing a feed spacer. Feed flowing around the envelope at high pressure goes across the membrane and is collected inside the envelope. Permeate flows inside the envelope into the roll and then runs out the end of the module. The feed and permeate flow patterns are usually quite complex. Design and analysis is therefore largely empirical. Advantages include high packing density and relatively low cost. Disadvantages include problems handling suspended solids, difficulty in cleaning and, in high-temperature applications (plastic components may deform). Another major problem is that these devices cannot usually handle high transmembrane pressure.

Tubular membrane module

The tubular membrane module has a cylindrical geometry with the wall acting as the membrane. The tubes are generally more than 3 mm in diameter. Normally, a tubular membrane module is made up of several tubes arranged as in a shell and tube type heat exchanger. The feed stream enters the lumen of the tubes, the permeate passes through the walls and is collected in the shell while the retentate passes out the other end of the tubes. Advantages include turbulent flow (providing good membrane/solution contact and removing retentate film build-up),

relatively easy cleaning, easy handling of suspended solids and viscous fluids and ability to replace or plug a failed tube while the rest of the system runs. The feed flow pattern is easy to characterize and therefore design and analysis based on fluid dynamic principles is possible. Tubular membrane modules can handle reasonably high transmembrane pressure. Disadvantages include high capital cost, low packing density, high pumping costs, and limited achievable concentrations.

Hollow fibre membrane module

The hollow fibre membrane module is similar in design to the tubular membrane module except in terms of scale and number of tubes. The tubes or rather fibres are typically 0.25 to 2.5 mm in diameter. A hollow fibre membrane module usually consists of a bundle of several hundred fibres. The hollow fibre membranes are spun separately, bundled, and potted into

cartridge housings. The fibres may be bundled together into either a U-shape or have a straight-through configuration. Tube bundles are inside a pressure vessel and feed material normally flows inside the tubes. Fibres in the straight-through design are somewhat larger and allow low levels of suspended solids. The finer strands in the U-tube cannot tolerate suspended solids. U-tubes tend to be used for reverse osmosis, and the straight-through design for ultrafiltration. Advantages include low pumping power, very high packing density; cleaning can be accomplished with back-flushing, and ability to achieve high concentrations in the retentate. Disadvantages include the fragility of the fibres, inability to handle suspended solids well, difficult cleaning and, in the straight-through design, damage of one fibre requires replacement ofthe entire module .

Table 1.2: Comparison of different membrane modules

Type	Fluid flow regime	Membrane area/module volume	Masstransfer coefficient	Hold-up volume	Special remarks
TF flat sheet	Laminar-turbulent	Low	Lowto moderate	Moderate	Canbe dismantled and cleaned easily.
Spiral wound	Laminar	Moderate	Low	Low	Highpressures cannot be used.
Hollow fibre	Laminar-turbulent	High	Lowto moderate	Low	Susceptibleto fibre blocking.
Tubular	Turbulent	Low	Moderateto high	Moderate to high	Flow easy to characterize. Excellently suited forbasic membrane studies.

1.9 Membrane characterization

The performance of a membrane process depends on the properties of the membrane. Thus membrane characterization is an important exercise form membrane developers and membrane users. Some of these include:

1. Mechanical strength e.g. tensile strength, bursting pressure
2. Chemical resistance e.g. pH range, compatibility with solvents
3. Permeability to different species e.g. pure water permeability, gas permeability
4. Average porosity and pore size distribution
5. Sieving properties e.g. Nominal molecular weight cut-off
6. Electrical properties e.g. membrane zeta potential

Fundamental of membrane permeation:

When two multi component mixtures containing atleast one fluid are separated by a permeable membranous barrier (e.g. a solid porous film) such that the partial molar free energy of one or more components common to both the mixtures differs across the barrier, there will generally be a selective transfer of components through the barrier in the derection of the declining chemical potential for each. A few different situations may arise: (i) If the upstream and down stream fluids are gas or vapour mixtures at differing hydrostatic pressures, for example, components pass through the barrier in the direction of declining partial pressure by a process termed as "gas permeation"; (ii) If the upstream fluid is liquid mixture, and the downstream fluid is a gas in which the partial pressure of the permeating component is lower than the vapour pressure over the component of the upstream liquid, transmembrane permeation occurs by a process usually termed as "pervaporation". (iii) If the liquid phases in contact of the membrane are in the same hydrostatic pressure, component can transfer across the barrier under the action of concentration difference between the contacting liquids by the process of "dialysis". (iv) If a liquid mixture is confined at a higher hydrostatic pressure from one side of the barrier compared to that of the other, certain component of the upstream liquid will permeate through the membrane by the process of "ultrafiltration or reverse osmosis"[2,3,5,7].

All these membrane transport process have the common features of (a) transport the mass across the membrane under a specific driving force e.g. pressure difference, concentration diference, applied e.m.f. etc. (b) the capacity to alter mixture composition by virtue of the ability of the membrane barrier to pass one component more rapidly than another despite equality of driving potential. It is this unique characteristic of membrane separation which differentiates the process from the common separation techniques. Various driving forces involved in different separation processes (including membrane separation) and their useful size ranges are presented.

Applications of Membrane

The membrane processes are used for separation of chemical substances and are of immense interest for a wide range of commercial applications. The analytical applications of membranes are grossly over shadowed by the industrial applications of membranes. A complete and comprehensive discussion of all membrane based applications would be very exhaustive and is beyond the scope of this unit. However, a few major large scale applications of membranes will be discussed here.

Desalination and Water Treatment

Desalination of sea water (containing approximately 35000 to 40 000 milligrams of dissolved salts per litre) and brackish waters (containing approximately 5000 to 10000 milligram of dissolved salts per litre) to produce potable water is one of the widely known industrial applications of membrane processes. Reverse osmosis processes to a large extent has been successfully used for the last two decades in many parts of the world. In addition to desalination, reverse osmosis process is also used for the treatment of municipal waste waters and effluents from the various Separation chemical industries.

Protein Recovery

The most prominent use of ultrafiltration process is in the food industry, where it is used ,for example to recover proteins from cheese whey, to concentrate milk before cheese making and

for fruit juice clarification.Other important applications of UF process is in the removal of colour , odour and bacteria from the surface waters for drinking water needs. The pore size of most of the UF membranes used for such applications are such that they remove virtually all the microorganisms present in the water and viruses to a large extent.

Production of Table Salt

Electrdialysis was first developed for the desalination of saline waters, in particular brackish water. The production of potable water is currently the most important industrial application of electrodialysis. One significant feature of electrodialysis is that the salts can be concentrated to comparatively high values (in excess of 18 to 20 %) . The production of table salt from sea water by the use of electrodialysis to concentrate sodium chloride up to 200 grams per litre prior to evaporation is a technique developed extensively and used in Japan.

Hemodialysis

The most important application of dialysis process is in the artificial purification of blood using a dialysis membrane and this process is called hemodialysis. In hemodialysis, the impure blood from the patients flows across a dialysis membrane and a physiological saline solution flows along the other side of membrane. This process replaces kidney function in three principal areas, namely, removal of waste metabolites, removal of excess body water and restoration of acid-base and electrolyte balances. The waste metabolites include urea, uric acid, the end product of protein metabolism and creatinine, the end product of muscle metabolism.

Ion Selective Membrane Electrode

The analytical applications of membranes are largely in the area of ion selective membrane electrodes and particulate analysis. For most of the ion selective membrane electrodes, the role of membranes is not to transport specific ions but to selectively adsorb on either side giving rise to measurable electrical potential difference. A particular membrane permits only a particular kind of ion to penetrate and adsorb. Ion selective membrane electrodes have many applications in

water analysis and environmental monitoring.

Specific Gas Probes

Membranes are also used in specific gas probes for measuring dissolved gases and gas phase partial pressures. The specific gas analysis is based on the electrochemical oxidation or reduction of the gas at the appropriate electrode of an electrochemical cell giving rise to a current whose magnitude depends on the concentration of the gas consumed in the electrochemical reaction. The whole detection system is protected by the membrane which is permeable to the particular gas component of interest. The membrane can be polytetrafluoro ethylene, polyethylene, polypropylene, nylon or cellophane. The membranes typically have pore sizes of severalmicrons. Portable devices for specific gas analysis are available for detection of gaseous oxygen, hydrogen sulphide, carbon dioxide, sulphur dioxide, nitrogen dioxide and hydrogen cyanide.

Detection and Analysis of Particulate Contamination

Microfiltration membranes offer a general way of removing particulate material from fluid streams and are routinely used in a range of analytical procedures to determine particulate contamination in gases and liquids. In general, these methods involve passing a representative sample of liquid or gas through a suitable membrane. All particulate matter which exceeds the membrane pore sizes are retained on the surface where the contaminants may then be analysed.

Microbiological Analysis

Microporous membranes are important for the detection of microorganisms in foods, beverages pharmaceutical products and potable water sources. The technique involves the filtration of the samples through a microfiltration membranes to trap the microorganism, then culturing the microorganism on the membrane and then counting the grown colonies .

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What is Chromatography?

Chromatography is the technique for the separation, purification, and testing of compounds.

The term ***"chromatography"*** is derived from Greek, chroma meaning, ***"colour,"*** and graphein meaning ***–to write."***

In this process, we apply the mixture to be separated on a stationary phase (solid or liquid) and a pure solvent such as water or any gas is allowed to move slowly over the stationary phase, carrying the components separately as per their solubility in the pure solvent.

Types of Chromatography

The four main types of chromatography are

1. Adsorption Chromatography

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In the process of adsorption chromatography, different compounds are adsorbed on the adsorbent to different degrees based on the absorptivity of the component. Here also, a mobile phase is made to move over a stationary phase, thus carrying the components with higher absorptivity to a lower distance than that with lower absorptivity. The main types of chromatographic techniques that are used in industries are given as under.

2. Thin Layer Chromatography

In the process of thin-layer chromatography (TLC), the mixture of substances is separated into its components with the help of a glass plate coated with a very thin layer of adsorbent, such as silica gel and alumina, as shown in the figure below.

The plate used for this process is known as chrome plate. The solution of the mixture to be separated is applied as a small spot at a distance of 2 cm above one end of the plate. The plate is then placed in a closed jar containing a fluid termed as an eluant, which then rises up the plate carrying different components of the mixture to different heights.

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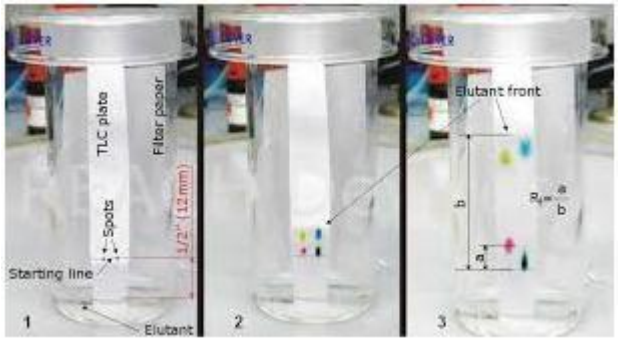

Thin Layer Chromatography

3. Column Chromatography

Column chromatography is the technique used to separate the components of a mixture using a column of suitable adsorbent packed in a glass tube, as shown in the figure below. The mixture is placed on the top of the column, and an appropriate eluant is made to flow down the column slowly.

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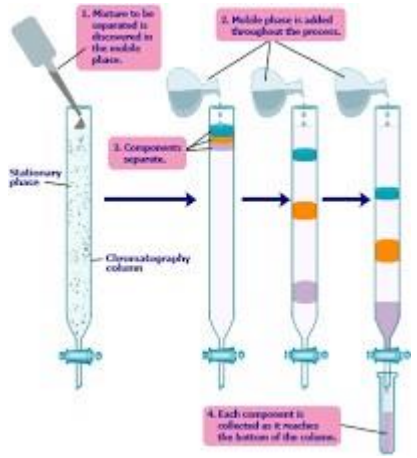
Depending upon the degree of adsorption of the components on the wall adsorbent column, the separation of the components takes place. The component with the highest absorptivity is retained at the top, while the other flow down to different heights accordingly.



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Column Chromatography

4. Partition chromatography

In this process, a continuous differential partitioning of components of a mixture into a stationary phase and mobile phase takes place. The example of partition chromatography can be seen in paper chromatography. In this process, chromatography paper is used as a stationary phase which is suspended in a mixture of solvents that act as a mobile phase.

Here, we put a spot at the base of the chromatographic paper with the mixture to be separated and as the solvent rises up this paper, the components are carried to different degrees depending upon their retention on the paper. The components are thus separated at different heights.

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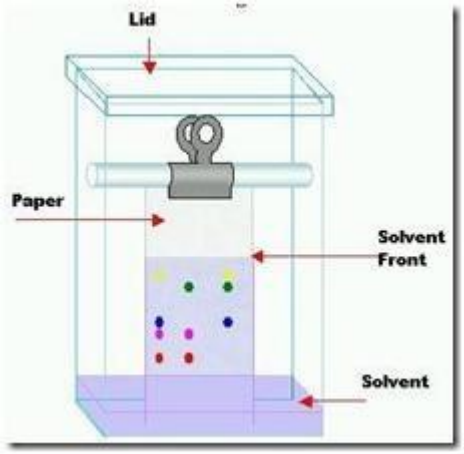

Partition chromatography

What is Differential Extraction?

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Differential extraction is the method of separation of any organic component present in an aqueous solution. In this process, we use an organic solvent for which the solubility of the desired compound is more as compared to that in water. Also, the organic solvent is chosen such that it is immiscible with the aqueous solution so that it can form layers and can be separated easily using a separating funnel.

The organic compound is later recovered by the process of distillation or evaporation. The process of continuous extraction is used in cases when the solubility of the compound is less in the organic solvent.

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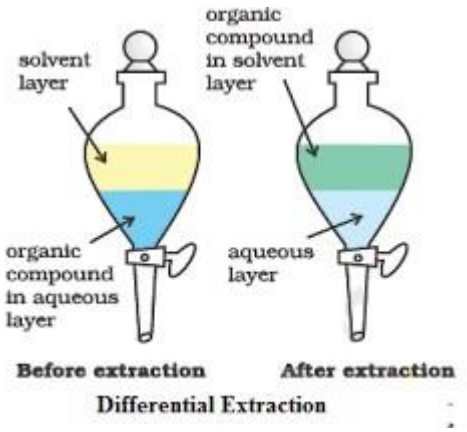

Differential Extraction

1. What is the basic principle of chromatography?

Ans: Chromatography is based on the concept of separating molecules in a mixture added to the ground or solid and liquid stationary state (stable phase) when traveling with the aid of a mobile phase.

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2. What is the Rf value in chromatography?

Ans: RF stands for retention factor in paper chromatography, or the distance a fluid compound moves up a plate of chromatography. For each particular solvent, all compounds have a common RF value, and RF values are used to equate unknown samples with known compounds.

3. How is RF value useful?

Ans: The distance traveled by an element divided by the distance traveled on the front of the solvent. It is a component characteristic and can be used to classify components for a given system at a known temperature.

4. Where is chromatography used?

Ans: Chromatography is used in industrial processes to purify materials, test trace amounts of contaminants, isolate chiral compounds and quality control test products. Chromatography is the physical process of separating or analyzing complex mixtures.

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5. What is an example of chromatography?

Ans: An example of chromatography is when a chemical reaction is used to separate into their own parts on a piece of paper each of the different size molecules in a liquid compound.

Ion-Exchange Chromatography

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Ion exchange chromatography: Ion-exchange chromatography is a versatile, high resolution chromatography techniques to purify the protein from a complex mixture. In addition, this chromatography has a high loading capacity to handle large sample volume and the chromatography operation is very simple.

Principle: This chromatography distributes the analyte molecule as per charge and their affinity towards the oppositively charged matrix. The analytes bound to the matrix are exchanged with a competitive counter ion to elute. The interaction between matrix and analyte is determined by net charge, ionic strength and pH of the buffer. For example, when a mixture of positively charged analyte (M, M^{+}, M^{-1}, M^{-2}) loaded onto a positively charged matrix, the neutral or positively charged analyte will not bind to the matrix where as negatively charged analyte will bind as per their relative charge and needed higher concentration of counter ion to elute from matrix (Figure 30.1).

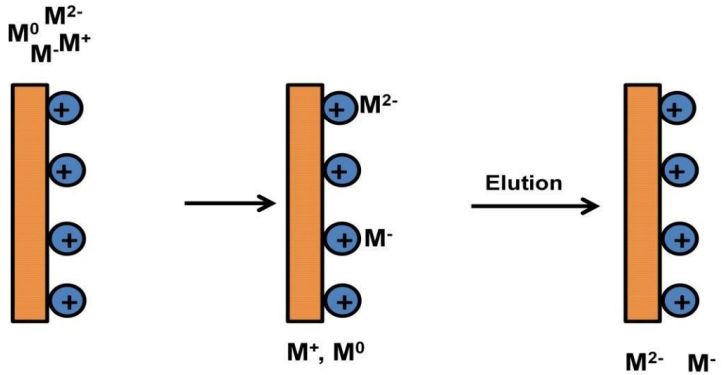

Figure 30.1: Affinity of analytes (M, M^{+}, M^{-1}, M^{-2}) towards positively charged matrix.

The matrix used in ion-exchange chromatography is present in the ionized form with reversibly bound ion to the matrix. The ion present on matrix participitate in the reversible exchange process with analyte. Hence, there are two types of ion-exchange chromatography:

1. **Cation exchange chromatography-** In cation exchange chromatography, matrix has a negatively charged functional group with a affinity towards positively charged molecules. The positively charged analyte replaces the reversible bound cation and binds to the

matrix (Figure 30.2). In the presence of a strong cation (such as Na^+) in the mobile phase, the matrix bound positively charged analyte is replaced with the elution of analyte. The popular cation exchangers used are given in Table 30.1.

2. **Anion Exchange chromatography-** In anion exchange chromatography, matrix has a positively charged functional group with a affinity towards negatively charged molecules. The negatively charged analyte replaces the reversible bound anion and binds to the

matrix (Figure 30.2, B). In the presence of a strong anion (such as Cl^-) in the mobile phase, the matrix bound negatively charged analyte is replaced with the elution of analyte. The popular anion exchangers used are given in Table.

Isoelectric point and charge on a protein: Protein is a polymer made up of amino acids with ionizable side chain. At a particular pH, these amino acid side chain ionizes differentially to give a net charge (positive/negative) to the protein. The pH at which the net charge on a protein is zero is called as Isoelectric point (pI). The protein will have a net positive charge below the pI where as it has net negative charge above the pI value .

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Choice of a Ion-exchange column matrix-Before starting the isolation and purification of a substance, a choice for a suitable ion-exchange chromatography is important. There are multiple parameter which can be consider for choosing the right column matrix.

1. **pI value and Net charge-** The information of a pI will be allow you to calculate the net charge at a particular pH on a protein. As discussed above, a cation exchange chromatography can be use below the pI where as an anion exchange chromatography can be use above the pI value.

2. **Structural stability-**3-D structure of a protein is maintained by electrostatic and

vander waal interaction between charged amino acid, Π-Π interaction between hydrophobic side chain of amino acids. As a result, protein structure is stable in a narrow range around its pI and a large deviation from it may affect its 3-D structure.

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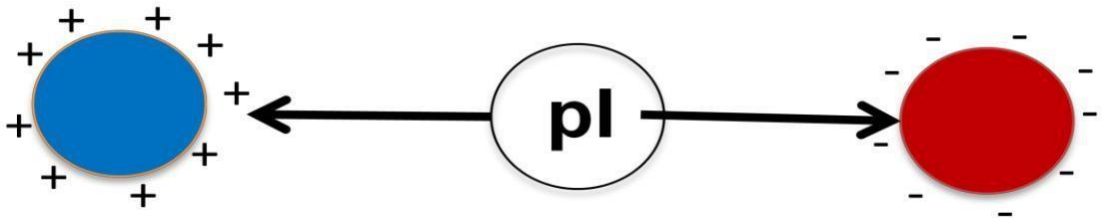
3. **Enzymatic activity**-Similar to structural stability, enzymes are active in a narrow range of pH and this range should be consider for choosing an ion-exchange chromatography.



Operation of the technique-Several parameters needs to be consider to perform ion-exchange chromatography

1. **Column material and stationary phase**-Column material should be chemically inert to avoid destruction of biological sample. It should allow free low of liquid with minimum clogging. It should be capable to withstand the back pressure and it should not compress or expand during the operation.

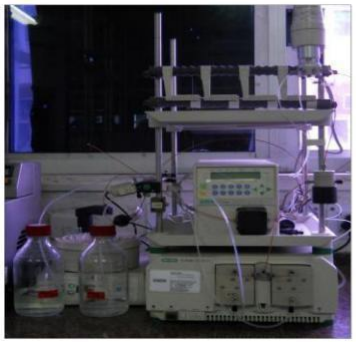
2. **Mobile Phase**-The ionic strength and pH are the crucial parameters to influence the property of the mobile phase.

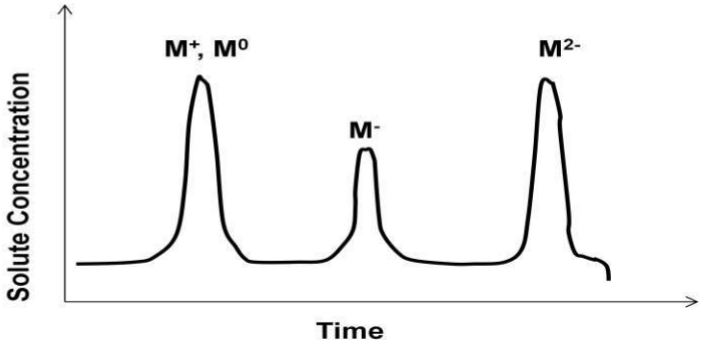
3. Sample Preparation- The sample is prepared in the mobile phase and it should be free of suspended particle to avoid clogging of the column. The most recommended method to apply the sample is to inject the sample with a syringe.

4. Elution- There are many ways to elute a analyte from the ion-exchange column. (1) Isocratic elution (2) Step-wise gradient (3) Continuous gradient either by salt or pH (4) affinity elution (5) displacement chromatography

5. Column Regeneration- After the elution of analyte, ion-exchange chromatography column require a regeneration step to use next time. column is washed with a salt solution with a ionic strength of 2M to remove all non-specifically bound analytes and also to make all functional group in ionononized form to bind fresh analyte.

A **B**





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Operation of the Ion-exchange Chromatography. (A) Chromatography system to perform gradient elution of analytes to give an (B) elution profile.

Capillary Electrophoresis

Capillary Electrophoresis (CE) is one of the possible methods to analyse complex samples. In High Performance Liquid Chromatography (HPLC) and Gas Chromatography (GC) the separating force is the difference in affinity of the sample components to a stationary phase, and or difference in boiling point. With both techniques the most important factor is the polarity of a sample component. In CE the separating force is the difference in charge to size ratio. Not a flow through the column, but the electric field will do the separation.

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In Capillary Electrophoresis a capillary is filled with a conductive fluid at a certain pH value. This is the buffer solution in which the sample will be separated. A sample is introduced in the capillary, either by pressure injection or by electrokinetic injection. A high voltage is generated over the capillary and due to this electric field (up to more then 300 V/cm) the sample components move (migrate) through the capillary at different speeds. Positive components migrate to the negative electrode, negative components migrate to the positive electrode. When you look at the capillary at a certain

place with a detector you will first see the fast components pass, and later on the slower components.

Mobility

As mentioned before the speed of a component (the mobility) is dependable on size and charge. The size is a combination of the sample component and the shield of water that is bound to the component. Even a small ion (as Fluoride, F-) can be big due to a large water shield. In general, the bigger the component, the slower it will migrate through the buffer.

The charge of ions can be strongly dependent on pH value. That is the reason why a buffer at certain pH is used for separations. For example Acetic Acid (pK value 4.756) will be almost completely negative charged at pH 7. The mobility (speed) of the acetic ions will be big. At pH 3, where about 80 % of the acetic acid is neutral, the mobility will be much lower. By changing the pH of a buffer system, the mobilities of the different components can be altered to achieve the best separation. In general the best pH for a separation is between the pK values of the sample components.

Endo Osmotic Flow (EOF)

In most applications the capillary that is being used is made of bare fused silica. This material has at its surface silanol groups (Si - O - H). These groups are slightly acidic. In buffers at higher pH value there are a lot of negative charges at the capillary wall (Si – O-). In the buffer fluid positive charges will be present because of the law of electrical neutrality. When a high voltage is generated over the capillary, these positive charges will start to migrate through the capillary towards the negative electrode. They will drag along the buffer fluid with them. This flow is called the (Electro) Endo

Osmotic Flow (EOF). It is well possible to calculate a mobility of this EOF. The higher the pH, the more negative charges on the capillary wall and the more positive charges in the fluid. This will generate a stronger EOF.

Because the positive charges are all located close to the capillary wall, and there is no pressure force in the middle of the capillary, the flow profile of the EOF is completely flat. This will cause no peak broadening like the parabolic flow profile in HPLC and GC, and that is one of the reasons why such a high resolution can be achieved in CE. As mentioned before the EOF is towards the negative electrode. This flow drags along neutral components (which would not migrate without any fluid flow), and even positive components, whose mobility is lower than the mobility of the EOF, will migrate towards the negative electrode. In this way in one run negative, neutral and (slow) positive components can be separated and detected.

Different modes of Capillary Electrophoresis

In the paragraphs below some general used modes of capillary electrophoresis are explained.

Capillary Zone Electrophoresis (CZE or FSCE)

Capillary Zone Electrophoresis (CZE), also known as free-solution CE, is the most standard form of CE. Buffer is flushed through the capillary by pressure, sample is injected and high voltage is applied. Dependable on the polarity the EOF is towards the inlet or the outlet. Each sample component will migrate through the capillary at its own speed. Only the difference in mobility will cause the separation.

Capillary Gel Electrophoresis (CGE)

With this technique there is a gel matrix inside the capillary. Components with different size but the same mobility are separated with this technique. Components of bigger size will be slowed down more by the gel, and will migrate later through the capillary. Especially with protein and DNA separations this technique is frequently used.

Micellar ElectoKinetic Chromatography (MECC or MEKC)

In this way of electrophoresis micelles are generated in the buffer. These micelles have a non polar inside, and a polar (or charged) surface. Sample components in the buffer solution will be divided over the micelles and the buffer solution, dependable on the affinity to the micelles. Just like in HPLC and GC, there will be a certain stable diversion between buffer and micelles. When the migration speed of the buffer differs from the speed of the micelles, it is possible to separate different components on the fact that there is a different affinity for the micelles. In this way, there is a lot of synergy with HPLC and GC.

Non-Aqueous Capillary Electrophoresis (NACE)

With this technique components that are insoluble in water are separated, mainly depending on the use of organic solvents. The viscosity and dielectric constants of organic solvents affect both sample ion mobility and the level of electroosmotic flow.

Iso Electric Focussing (IEF)

When a pH gradient is applied across the capillary, and a voltage is applied from positive voltage at low pH to negative voltage at high pH, components will migrate to the pH value that equals their pI value. At lower pH value, the components are positively charged, at higher pH values the components are negatively charged. In this way, each component migrates to a different position in the capillary. When pressure is applied, the complete pH gradient moves through the capillary, and subsequently the components will pass the detection window.

Capillary Electro Chromatography (CEC)

With this technique a capillary is partly packed with silica based particles with a stationary phase. When high voltage is applied over the capillary, the buffer fluid will start to migrate due to the EOF that is present because of the silica. The sample will have, just as in HPLC, more or less affinity for the stationary phase. This is the separating force in this technique. The only difference between HPLC and CEC is that not a pressure pump is being used to force the mobile phase through the packed bed (HPLC), but a high voltage is used for this purpose.

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Electro Chromatography (EKC)

With this technique there is a differential interaction of enantiomers with the cyclodextrins, which allows the separation of chiral compounds. This enantiomer analysis is used for the analyses of natural products, such as pharmaceutical/herbal products, toxicology compounds, food and food contaminants, forensic, fingerprinting and many more.

Capillary IsoTechoPhoresis (ITP)

With this technique two kinds of buffers are used. One buffer with high mobility as a leading buffer and one buffer with very low mobility as a terminating buffer. The mobility of the sample components must be between the leading and terminating. In the stable situation, all components migrate through the capillary at the same velocity (hence the name itsotacho=same speed). In the figure, two components A and B are positioned between L(eading) and T(erminating). For the mobilities: m(L)> m(A)> m(B)> m(T).

Because all components have the same speed, there will be different electric fields in each zone, as the law v=m*E is everywhere valid. The electric field in the Terminating zone will be highest. These differences in electric field result in the self-correcting behaviour. If a component (say B) is for some reason (diffusion) in zone A, it will be under a higher electric field giving it a higher speed. The result is that the ion migrates back into it's own zone. The same if it would be in the Leading zone, where it is in a lower electric field. The velocity is lower, and it will be caught by the quicker moving zone of B.

In addition, because of electrical laws, the concentrations are related to the concentration of the Leading electrolyte. For this reason, small concentration samples are strongly concentrated into very narrow zones. Especially this effect is used quite often to concentrate large volume injections.

Principle and Protocol of Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

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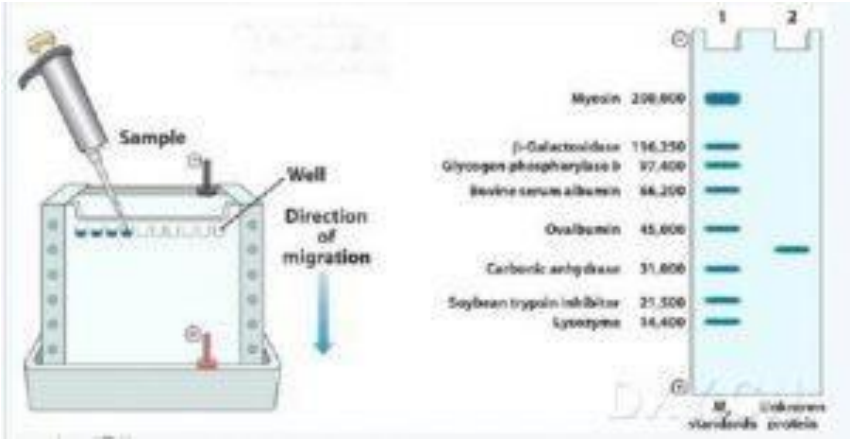
Principle

The concentration of polyacrylamide gels can be prepared as required in two electrophoresis systems —called –continuous system‖ and –discontinuous system‖. The biggest feature of –discontinuous system‖ lies in its greatly improved sample separation resolution. Main features of this electrophoresis are: **(1)** Use of two gel systems with different concentrations; **(2)** Solution composition and pH are different for the preparation of the two gel and are also different from electrophoresis buffer composition and pH in electrophoresis tank. In the experiment, electrophoresis gel is divided into two layers: the upper one is a macroporous gel with low concentration, called stacking gel, buffer for the formulation of this layer is Tris-HCl, pH6.7; the lower one is hole glue with high concentrations, called separating gel or electrophoresis gel , and the buffer for this is Tris-HCl, pH8.9. Electrode buffer in the electrophoresis tank is Tris- glycine, pH8.3. Obviously, the gel

concentrations, compositions, pH and the electrophoresis buffer systems are different from each other, thus forming a discontinuous system.



During the experiment, the protein sample was loaded in the stacking gel. To prevent protein sample diffusing in the electrode buffer, adding an equal volume of 40% sucrose or 50% glycerol to increase the density would be a good choice. To observe the mobility of protein samples, it's better to add bromophenol blue dye or some other tracer dyes into the sample. These colored substances can migrate faster than any macromolecules. As long as the dye dose not move out of the gel, there would be no danger for the sample.

In the discontinuous system, as soon as the power is turned on, Glycine, proteins, chloride ions and bromophenol in HCI would be dissociated into anion, forming an ion flow and moving to the anode. Its mobility depends on the number of electric charges of the ion, molecular size and shape. However, when the glycine ions of electrophoresis buffer (pH8.3) entered into the stacking gel and encountered lower pH (6.7), which lowered down by nearly two units, almost close to the isoelectric point (5.97) of glycine, the dissociation degree of glycine suddenly drop, the amount of charge reduced significantly and then the mobility became slower.Protein sample entered into the stacking gel, pH changes has impact on its dissociation degree either, but the impact is much smaller than on glycine. Thus it has larger mobility than glycine. What's more, the pore size of stacking gel is

too large to cause obstruction to protein molecular. So, in the stacking gel, the mobility of various ions is in the order of: glycine <protein <BPB <Cl.

The decline of dissociation degree after the glycine entering into the stacking gel makes the sudden absence of mobile ions flowing, resulting in reduced conductivity and electric current decline. However, the entire electric current of the other part of the electrophoresis system remain unchanged. On the basis that conductivity is inversely proportional to potential gradient (E=I/n, E stands for potential gradient, I stands for current intensity and N stands for conductivity), there suddenly formed a high local potential gradient between Leading Ion-CI and slow ion-glycine. Protein components in this local potential gradient region quickly migrate to the CI-ions region at different speed under the function of the high electric field. Through this process, the protein sample has been concentrated for several hundred fold and the protein components are arranged in a certain order to form layer.

When the ion flow continued to move forward and entered into the resolving gel prepared by pH8.9 buffer, the protein molecule encountered resistance. Then the mobility became slow. At the same time, under the conditions of pH8.9, glycine would fully

dissociate. Its electricity would increase, eliminating the phenomenon of ion missing. Each section of the gel recovered with a constant electric strength. Proteins begin to migrate at different rates, because of the sieving properties of the gel. Smaller protein-SDS complexes migrate more quickly than larger protein SDS complexes. Within a certain range determined by the porosity of the gel, the migration rate of a protein in the running gel is inversely proportional to the logarithm of its MW.

Seen from the principle above, main advantages of discontinuous polyacrylamide gel electrophoresis is that when the protein samples go through the stacking gel, they can form a tightly compressed layer and flow into the separating gel. With the protein components separated previously and compressed into layer, it can reduce the interference caused by the zone overlapping, thus improving the distinguish ability of electrophoresis. Just because of this advantage, a small amount of protein samples can be well separated too.

Materials and Reagents

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1. **30% acrylamide**: weigh 29g acrylamide, 1g N, N – methylene bis-acrylamide. Add 60 ml warmed deionized water and heat to 37 ℃. Add deionized water to make a final volume of 100ml; filter; Then we have 30% (w / v) acrylamide stock solution; Acrylamide and bis-acrylamide were transformed slowly into acrylic acid and double acrylic acid during storage, so the pH of the solution should be no more than 7.0 and it should be placed in a brow bottle at 4 ℃.

2. **10% sodium dodecyl sulfate (SDS)**: weigh 10g SDS and 90ml deionized water; heat to 68 ℃ and add a few drops of concentrated hydrochloric acid until the pH becomes 7.2; then water to 100ml; after the whole processes, we have 10% (w/v) SDS.

3. **Stacking gel buffer (1mol / L Tris-HCl pH 6.8)**: dissolve 12.12g Tris in 80ml deionized water. Adjust the pH to 6.8 with concentrated hydrochloric acid; add deionized water to 100ml and store at 4℃.

4. **Resolving gel buffer** (1.5mol / L Tris-HCl pH 8.8): dissolve 18.16g Tris in 80ml deionized water; adjust the pH to 8.8 with concentrated hydrochloric acid; add deionized water to 100ml; store at 4 ℃.

5. **10% ammonium persulfate (AP)**: ammonium persulfate provides the free radical necessary for the catalysis of the Polymerization of Acrylamide and Bis-acrylamide; Use deionized water to prepare a small amount of 10% (w/v) solution and store at 4 ℃. Since ammonium persulfate will decompose slowly, it should be freshly prepared every other week.

6. **TEMED (N, N, N, N – tetramethylethylenediamine)**: by catalyzing ammounium persulfate to form free radicals, TEMED accelerated the polymerization of acrylamide and bis-acrylamide. Since TEMED only functions in a free base form, the polymerization reaction would be inhibited when the pH is low.

7. **Tris- glycine electrophoresis buffer**: weigh 15.1g Tris and 94g glycine; Dissolve in 900ml deionized water; then add 50ml 10% (w/v) SDS and deionized water to 1000ml. Dilute 5-fold when using. The final concentration would be: Tris, 25mmol/L; glycine, 250mmol/L; SDS, 0.1% and the pH of the buffer is 8.3.

8. Polyacrylamide gel electrophoresis tank and electrophoresis power supply.

9. Transfer pipette and tip, etc.

Operating Method

1. Assemble glass plate according to the vertical electrophoresis tank instructions; determine the concentration and volume of the separating gel; prepare the desired separating gel according to the ingredients listed for the preparation of Tris- glycine SDS-polyacrylamide gel electrophoresis.

2. Inject the separating gel into the gap of the two glass sheets quickly, leaving space for the infusion of stacking gel (comb teeth length plus 1cm); cover the separating gel with 0.1% SDS carefully(when the concentration of acrylamide ≤ 8%) or

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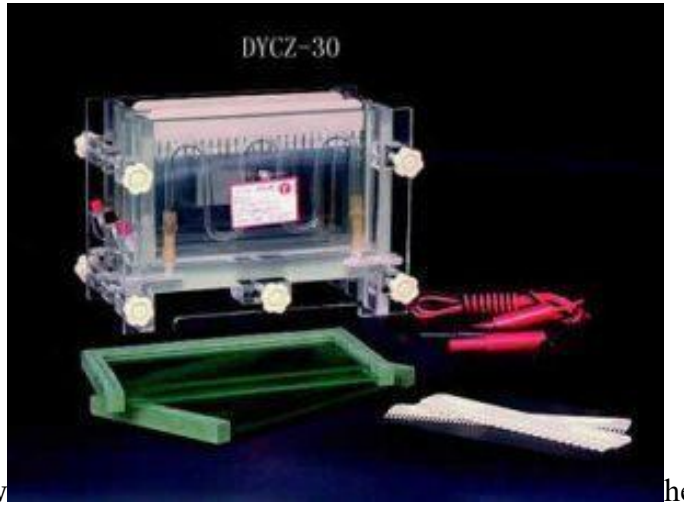

isobutanol or water (w hen the acrylamide concentration ≥10%); the cover layer can prevent the diffusion of oxygen into the gel and inhibit the polymerization of the gel; place the gel vertically at room temperature.

3. When the polymerization of the separating gel completed, pour the cover liquid; wash the top of the gel for several times to remove the acrylamide that were unpolymerized; exclude the liquid on the gels as far as possible.

4. Determine the volume of the stacking gel in need; prepare the desired stacking gel according to the ingredients listed for the preparation of Tris- glycine SDS-polyacrylamide gel electrophoresis of stacking gel; Pipette the stacking gel directly on the separating gel, and insert clean supporting comb immediately, to avoid air bubbles; then add stacking gel solution to fill the gap between comb. Remove the comb after stacking gel polymerization, then the sample hole is formed.

5. Dilute 5-fold of the Tris- glycine electrophoresis buffer stock solution with deionized water; pour the solution into electrophoresis tank; fill the sample hole so that the bubbles in the sample holes can be ruled out through the electrophoresis buffer.

An Introduction to Gel Electrophoresis – DNA, RNA & Proteins

The term gel electrophoresis refers to a technique used for separation and analysis of DNA, RNA , and proteins based on their size and charge. The suffix *phoresis* means –migration‖ or –movement‖, while the prefix *electro* indicates the use of electricity as a mean to separate molecules.

In a typical gel electrophoresis system, an electric field is generated by connecting the two opposite ends of a gel tank to a power supply. One end will become positively charged, while the opposite end negatively. This electric current will cause DNA, RNA and protein fragments to migrate along the gel.

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DNA Gel Electrophoresis

DNA gel electrophoresis is incorporated into various techniques as a preparative technique in molecular biology such as PCR, DNA sequencing, genome mapping and Southern blotting. A gel electrophoresis can run both horizontally and vertically, however, standard DNA and RNA gels run horizontally.

DNA and RNA are negatively charged molecules, once they are loaded into the gel from the negative end of the gel and exposed to an electric field, they migrate through the gel pores towards the positively charged end of the gel.

Gels for DNA or RNA separation are often made out of a polysaccharide called agarose that makes up the gel matrix. When the agarose is heated in a buffer and cooled down, it will form a solid gel. The gel matrix will act as a sieve; the greater the agarose concentration, the smaller the pores created in the gel matrix, and the more difficult it is for large linear DNA molecules to move through the matrix.

Different-size fragments will migrate at different rates, therefore allowing the researcher to identify the fragment of interest among a mixed sample. After electrophoresis, the gel can be visualized under UV lights thanks to the addition of DNA intercaletor, such as Ethidium Bromide to the running gel.

Preparing the Gel

Agarose powder is mixed with an electrophoresis buffer and heated to a high temperature until all of the agarose powder has melted (a microwave oven is generally used at this step).

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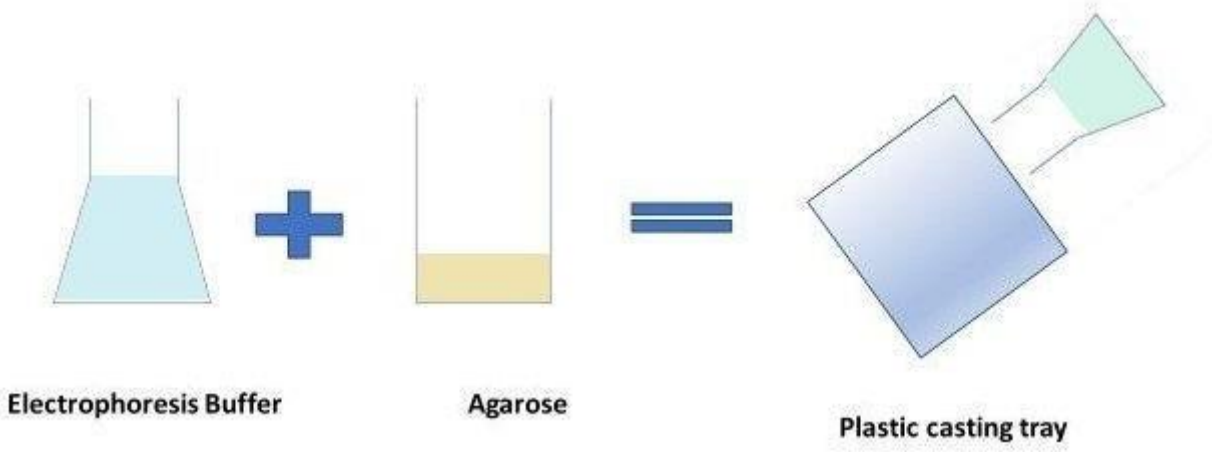

Figure 1: Schematic representation of DNA gel electrophoresis making.

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Electrophoresis buffers are used to provide ions that carry a current and to maintain the pH at a relatively constant value. The most common electrophoresis buffers for nucleic acids are Tris/Acetate/EDTA (TAE), Tris/Borate/EDTA (TBE). In addition, 0.2-0.5 μg/mL of Ethidium bromide will be added to the buffer.

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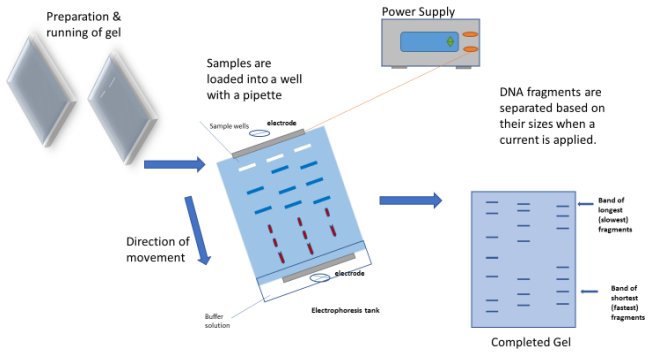

Figure 2: DNA gel electrophoresis

Visualizing the DNA Fragments

When the run has ended, the gel is placed under under a UV light source. Thanks to the addition of Ethidium bromide, intercaeletor-bound DNA fragments will glow and become visible as distinct bands along the gel's length. The exact size of each DNA band is detected by adding a molecular marker made up of several bands of known size.

The molecular weight of linear double stranded DNA run in agarose gel is estimated by the addition of DNA molecular markers often referred to as **DNA Ladders**. These ladders contain precisely quantified and measured linear double stranded fragments of DNA pre-mixed with loading dye making them ready to use.

three DNA ladders:

1. The 50bp PLUS ladders (from 50bp up to 1000bp)
2. The 100bp ladders (from 0.1kb to 3kb bp)
3. The 1kb PLUS (from 0.1kb to 10kb)

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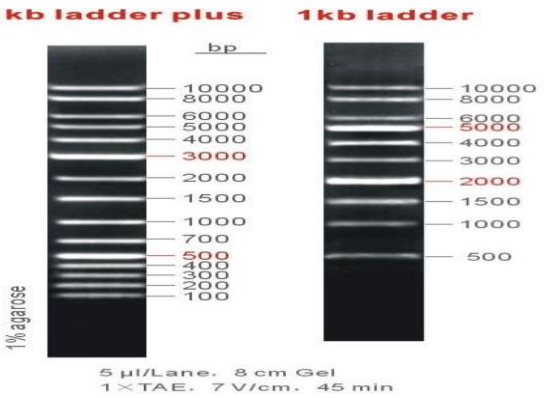

Figure 3: DNA Ladders contain precisely quantified and measured linear double stranded fragments of DNA pre-mixed with loading dye

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Protein Electrophoresis

Proteins are not negatively charged and as such cannot be separated by the application of an electric field. To overcome this issue, detergent sodium dodecyl sulfate is added to the protein solution in order to separate proteins using gel electrophoresis. This treatment causes the proteins to unfold into a linear shape while covering them with a negatively charged coat. This negative coat allows proteins to migrate towards the positively charged end of a gel and therefore be separated by molecular weight.

The purpose of using stain is to have a clear background with blue-stained protein bands. Some premade and traditional home-made protein Coomassie R-250 stains can take 3 hours or more to fully stain gels and it is necessary to fix and wash the gel before adding the stain.

Figure 2: Protein sample preparation workflow

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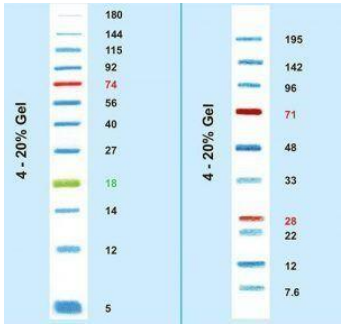

To overcome this issue, we offer easy to use Expedeon's InstantBlue stain which is a ready to use Coomassie protein stain for polyacrylamide gels. Its unique mechanism of action stains proteins in 15 minutes while leaving a clear background, eliminating the need to fix, wash or destain.

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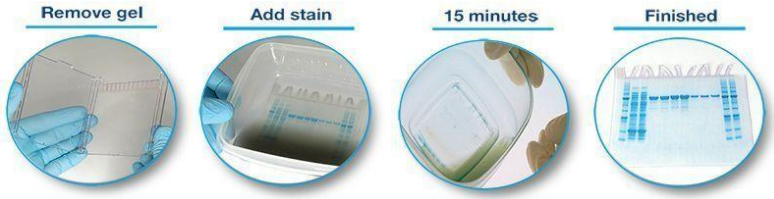

While DNA fragments can be immediately visualized under UV lights, protein fragments need to be labeled with target-specific antibodies, a process known as immunostaining. This reaction cannot happen on a gel substrate; proteins must be transferred onto a membrane (mostly PVDF or Nitrocellulose) then blotted with a solution containing an antibody that will specifically recognize and bind the protein of interest.

The target-antibody conjugation can be visualized by the addition of a label to the chosen antibody..

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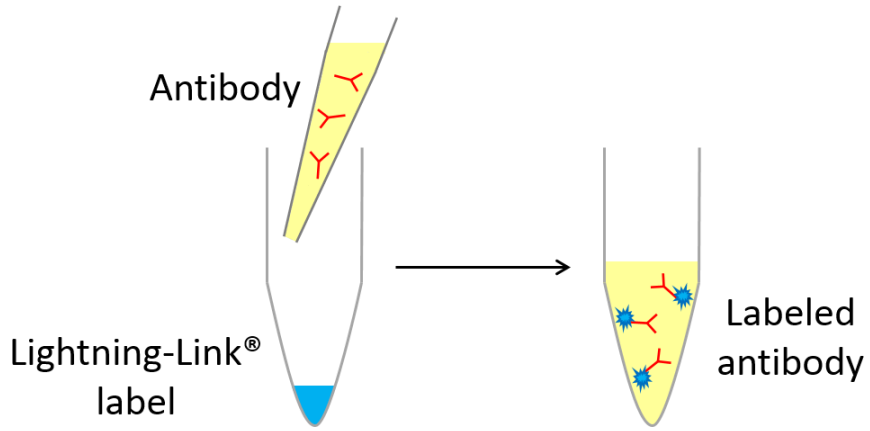

Figure 2: Process diagram for Lightning-Link®

2D GEL ELECTROPHRESIS

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Two-dimensional electrophoresis (**2-D electrophoresis**) is a powerful and widely used method for the analysis of complex protein mixtures extracted from cells, tissues, or other biological samples.

Based on two independent properties :

the first-dimension step**, isoelectric focusing (IEF),** separates proteins according to their isoelectric points (pI);

The second-dimension step, SDS- polyacrylamide gel electrophoresis (SDS-PAGE), separates proteins according to their **molecular weights** (Mr, relative molecular weight).

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EXPERIMENTAL PROCESS :

SAMPLE PREPARATION IPG STRIP

REHYDRATION IEF

IPG STRIP EQUILIBRATIION SDS-PAGE

VISUALIZATION

ANALYSIS

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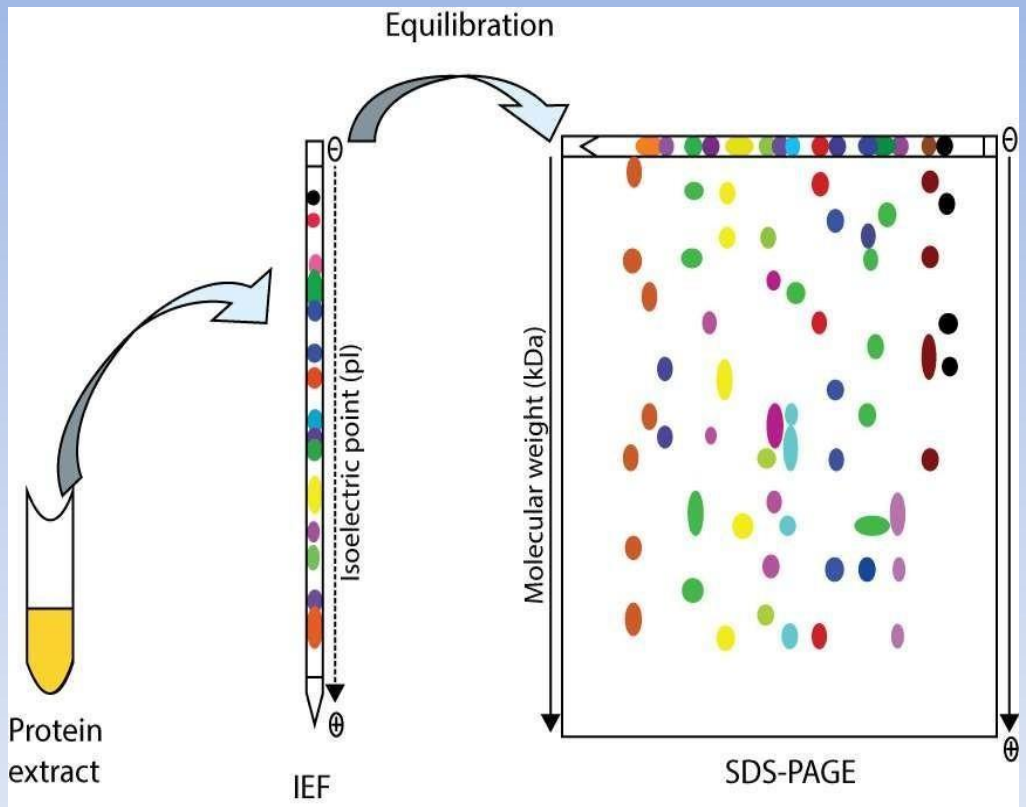
SUB NAME: BIOPROCES



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DEPARTMENT OF BIOTECHNOLOGY

SUB NAME: BIOPROCESS ENGINEERING –II UNIT -III SUBJECT CODE: SBT1306

1. SAMPLE PREPARATION

- **Must break all non-covalent protein-protein, protein- DNA, proteinlipid interactions, disrupt S-S bonds**
- Must prevent proteolysis, accidental phosphorylation, oxidation, cleavage, deamidation
- Must remove substances that might interfere with separation process such as salts, polar detergents (SDS), lipids, polysaccharides, nucleic acids
- Must try to keep proteins soluble during both phases of electrophoresis process

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CELL DISRUPTION

Gentle Lysis Method – Osmotic lysis, Freeze thaw lysis, Detergent lysis,

Enzymatic lysis.

Vigorous Lysis Method – Sonication, Grinding, Mechanical homogenization,

PROTEASE INHIBITORS

-PMSF(phenyl methylsulfonyl fluride), AEBSF(aminoethylbenzylsuonyl fluride),1mMEDTAetc..

PRECIPITATION

- Ammonium sulfate, TCA, acetone, TCAin acetone etc..

CONTAMINANT REMOVAL

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— Filtration, Centrifugation, Chromatography, Solvent Extraction

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PROTEIN SOLUBILIZATION

– 8 M Urea (neutral chaotrope)

– 4% CHAPS (zwitterionic detergent)

– 2-20 mM Tris base (for buffering)

– 5-20 mM DTT (to reduce disulfides)

– Carrier ampholytes or IPG buffer (up to 2% v/v) to enhance protein solubility and reduce charge-charge interactions

- 0.002% bromophenol blue

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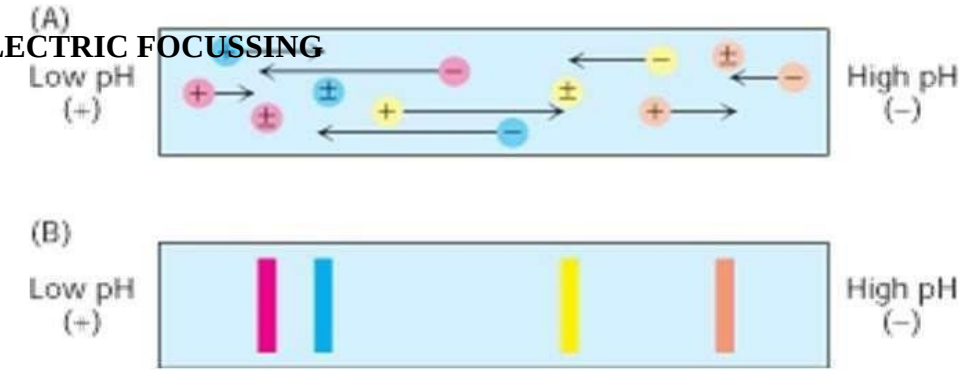
1ST DIMENSION : ISOELECTRIC FOCUSSING



The Principle of Isoelectric Focusing. A pH gradient is established in a gel before loading the sample. (A) The sample is loaded and voltage is applied. The proteins will migrate to their isoelectric pH, the location at which they have no net charge. (B) The proteins form bands that can be excised and used for further experimentation.

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IEF & IPG (IMMOBILIZED pH GRADIENT)

- Separation on the basis of pI, not MW
- Available in different pH ranges

  3-10

  4-8

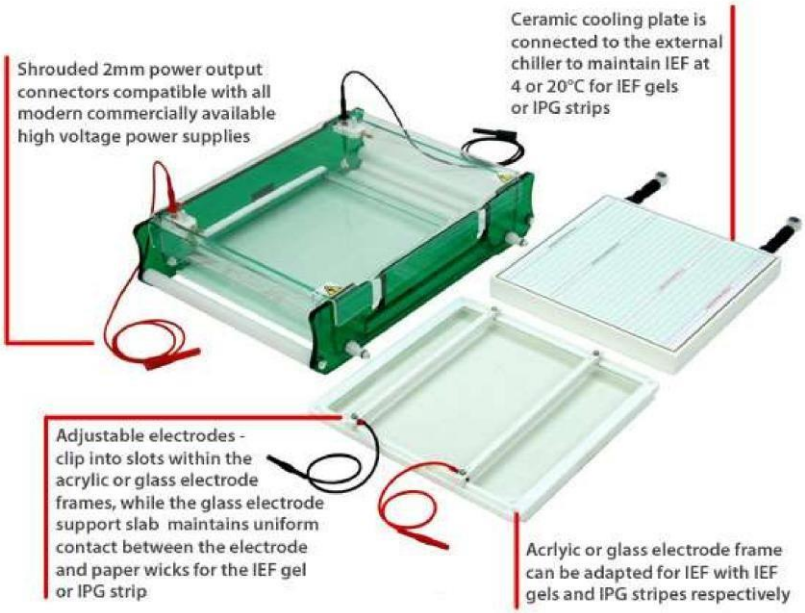
  5-7

- Requires very high voltage

Acrylamide monomer

R- weakly acidic or basic buffering group

SUB NAME: BIOPROCESS



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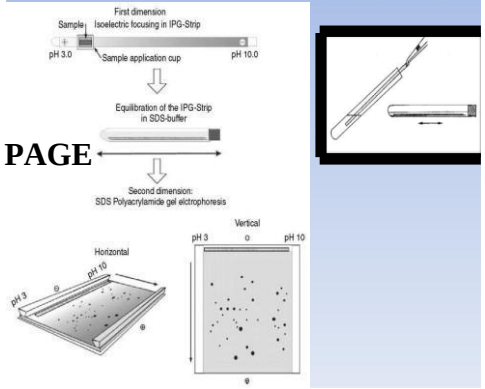
SUB NAME: BIOPROCESS ENGINEERING –II UNIT -III SUBJECT CODE: SBT1306

2nd Dimension – SDS PAGE



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Casting IPG on 2nd Dimension

Visualization……

Desired features……..

- High sensitivity
- Wide linear range for quantification

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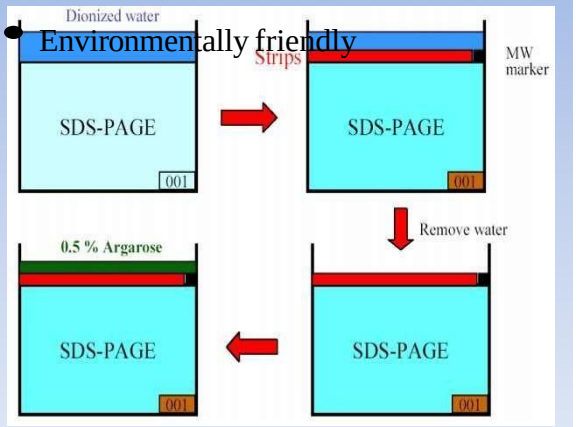
- Compatibility with mass spectrometry
- Low toxicity and environmentally safe
- Environmentally friendly



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Autoradiography and fluorography are the most sensitive detection methods. To employ these techniques, the sample must consist of protein radiolabelled in vivo using either 35S, 14C, 3H or, in the case of phosphoproteins, 32P.

For autoradiographic detection, the gel is simply driedand exposed to X-ray film or—for quicker results and superior dynamic range of quantification—to a storage phosphor screen.

Fluorography is a technique that provides extra sensitivity by impregnating the gel in a scintillant such as PPO (2,4-diphenyloxazole) prior to drying.

Stain	Sensitivity (ng/spot)	Advantages
Coomassie R-250	50-100	Simple, fast, consistent
Colloidal Coomassie	5-10	Simple, fast
Silver stain	1-4	Very sensitive, awkward
Copper stain	5-15	Reversible, 1 reagent negative stain
Zinc stain	5-15	Reversible, simple, fast high contrast neg. stain
SYPRO ruby	1-10	Very sensitive, fluorescent

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SUB NAME: BIOPROCESS ENGINEERING –II UNIT -III SUBJECT CODE: SBT1306

- Picking the spots

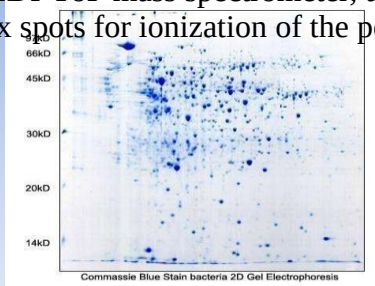
Ettan Spot picker is a robotic system that automatically picks selected protein spots from stained or destained gels using a pick list from the image analysis, and transfers them into microplates.

- Digestion of the proteins

supernatant peptides are mixed with MALDI matrix material and spotted onto MALDI slides using Ettan Spotter.

- MALDI-ToF mass spectrometry

In the Ettan MALDI-ToF mass spectrometer, a laser beam is fired into the dried peptide-matrix spots for ionization of the peptides. De novo sequencing of proteins.



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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

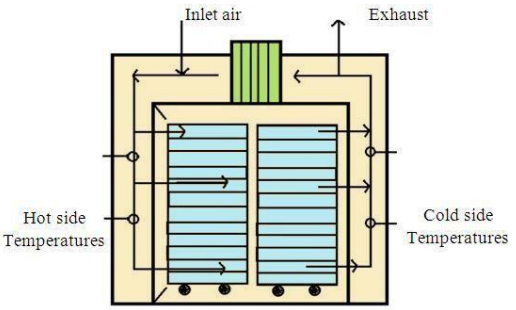
B.TECH – BIOTECHNOLOGY

UNIT –IV EVAPORATION - SBT1306

DRYING

Schematic of a typical batch dryer is shown in figure. Tray dryers usually operate in batch mode, use racks to hold product and circulate air over the material. It consists of a rectangular chamber of sheet metal containing trucks that support racks. Each rack carries a number of trays that are loaded with the material to be dried. Hot air flows through the tunnel over the racks. Sometimes fans are used to on the tunnel wall to blow hot air across the trays. *Even b*affles are used to distribute the air uniformly over the stack of trays. Some moist air is continuously vented through exhaust duct; makeup fresh air enters through the inlet. The racks with the dried product are takento a tray-dumping station.

Inlet air Exhaust



These types of dryers are useful when the production rate is small. They are used to dry wide range of materials, but have high labor requirement for loading and unloading the materials, and are expensive to operate. They find most frequent application for drying valuable products. Drying operation in case of such dryers is slow and requires several hours to complete drying of one batch. With indirect heating often the dryers may be operated under vaccum. The trays may rest on hollow plates supplied with steam or hot water or may themselves contain spaces for a heating fluid. Vapour from the solid may be removed by an ejector or vacuum pump. *Freeze-drying* involves the sublimation of water from ice under high vacuum at temperatures well below 0^{o}C. This is done in special vacuum dryers for drying heat-sensitive products.

Pan Dryer

The atmospheric pan drier has a jacketed round pan in which a stirrer or mill revolves slowly, driven from below. The slow moving stirrer exposes fresh surfaces and thereby raises the rate of evaporation and, hence, of drying. The pan drier is a batch machine and is limited to small batches. Pan driers may be used first to evaporate a solution to its crystallizing concentration and then can function as a crystallizer by sending cold water instead of steam into the jacket. The effect of the stirrer during crystallization prevents the growth of large crystals and promotes formation of small, uniform crystals. The mother liquor is then drained off and the crystals dried in the same apparatus.

Agitated Vacuum Dryer

The agitated vacuum dryer is one of the most versatile in the range and is similar in principle to a pan dryer. The dryer essentially consists of a jacketed cylindrical vessel arranged for hot water, steam or a suitable thermal fluid flow through the jacket for heating. Doors are provided on the shell, at the top for loading the feed material and at the bottom for discharging. The dryers are available in variety of sizes. The entire drying chamber is well machined to insure small clearance with the agitator blade. Thus ensures proper shuffling of the material and avoids localized over heating. Due to the agitation of the product in the agitated vacuum dryer the drying time is substantially reduced. A choice of the agitator design which can be arranged with or without heating depends on the material characteristics and process requirements. While designing the shell one has to consider the external pressure and the shaft designing includes fatigue consideration. Designing the impeller needs consideration of characteristics of the material before and after drying.

Continuous Dryer

Rotary Dryer

The rotary drier is basically a cylinder, inclined slightly to the horizontal, which may be rotated, or the shell may be stationary, and an agitator inside may revolve slowly. In either case, the wet material is fed in at the upper end, and the rotation, or agitation,

advances the material progressively to the lower end, where it is discharged. Figure shows a direct heat rotary drier. Typical dimensions for a unit like this are 9 ft diameter and 45 ft length. In direct-heat revolving rotary driers, hot air or a mixture of flue gases and air travels through the cylinder. The feed rate, the speed of rotation or agitation, the volume of heated air or gases, and their temperature are so regulated that the solid is dried just before discharge.

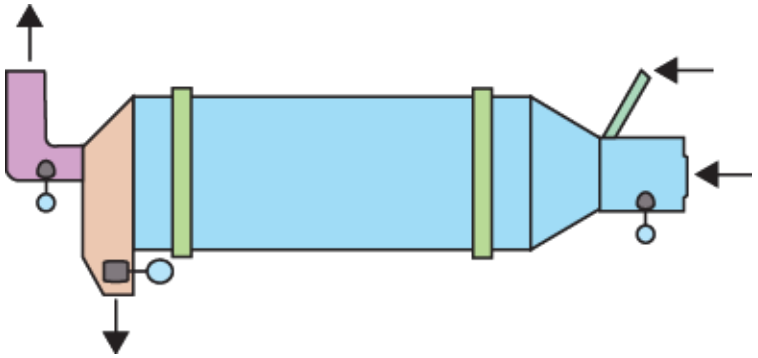


Figure Counter current direct heat rotary dryer

The shell fits loosely into a stationary housing at each end. The material is brought to a chute that runs through the housing; the latter also carries the exhaust pipe. The revolving shell runs on two circular tracks and is turned by a girth gear that meshes with a driven pinion. The inclination is one in sixteen for high capacities and one in thirty for low ones. As the shell revolves, the solid is carried upward one-fourth of the circumference; it then rolls back to a lower level, exposing fresh surfaces to the action of the heat as it does so. Simple rotary driers serve well enough when fuel is cheap. The efficiency is greatly improved by placing longitudinal plates 3 or 4 in. wide on the inside of the cylinder. These are called lifting flights. These carry part of the solid half-way around the circumference and drop it through the whole of a diameter in the central part of the cylinder where the air is hottest and least laden with moisture. By bending the edge of the lifter slightly inward, some of the material is delivered only in the third quarter of the circle, producing a nearly uniform fall of the material throughout the cross section of the cylinder. The heated air streams through a rain of particles. This is the most common form of revolving rotary cylinder. It has high capacity, is simple in operation, and is continuous.

INTRODUCTION AND TYPES OF DRIERS (CONT.)

Drum Dryer **SATHYABAMA**

In drum dryers (Fig 2.3a, b) a liquid containing dissolved solids or slurry carrying suspended solids forms a thin layer on the outside surface of a large rotating drum. For a single drum unit thickness of the film can be controlled by an adjustable scraping blade. In case of a double drum unit thickness can be controlled by the gap between the drums (figure 2.3a). A gas, normally air may be blown over the surface for rapid removal of moisture. The rotation of the drum adjusted so that all of the liquid is fully vaporized and a dried deposit can be scrapped off with the help of flexible or adjustable knife. This type of dryer mainly handles the materials that are too thick for a spray dryer and too thin for a rotary dryer. The solid collects on an apron in front of the knife and rolls to a container or to a screw conveyor. The operation of the drum drier is continuous. The drum is rotated continuously by a gear driven by a pinion that receives its motion through a belt, a chain, or a reduction gear from. The speed of the drum may be regulated by a variable-speed drive to adopt the speed to any slight variation in the feed quality. The speed of the drum regulated depending upon the nature of materials (i.e wet or dry), if the product material is wet/dry quite a distance before the knife is reached, the speed should be decreased/increased. The design of the components is similar to that of drum filter. The knife may be held just against the surface. It may be brought closer by turning the adjusting wheels. The knife supports may be turned through part of a circle so that the angle of the blade of the knife relative to the drum surface may be selected for the greatest shearing effect. In recent years, double drum dryers have replaced single drum dryer in several applications (figure 2.3b), due to their more efficient operation, wide range of products and high production rates.

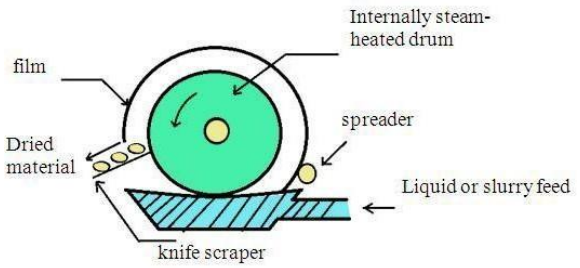


Figure : Single drum dryer

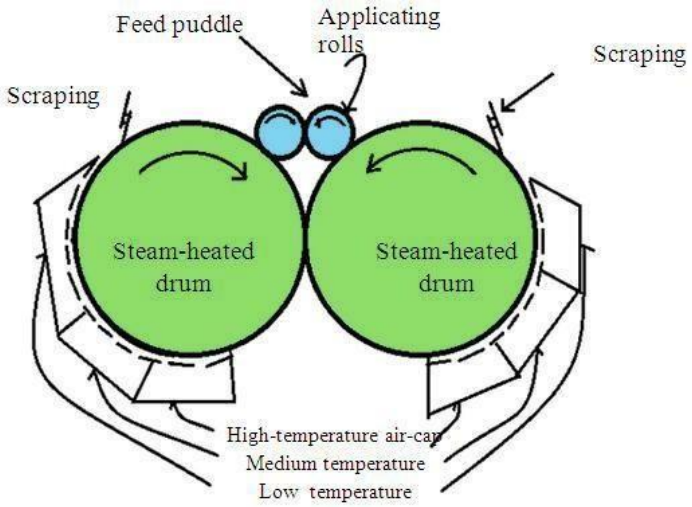


Figure : Double drum dryer

Flash Dryer

The flash driers (), also called pneumatic dryers, are similar in their operating principle to spray dryer. The materials that are to be dried (i.e. solid or semisolid) are dispersed in finely divided form in an upward flowing stream of heated air. These types of dryer are mainly used for drying of heat sensitive or easily oxidizable materials. The wet materials that are to dried can be passed into a high- temperature air stream that carries it to a hammer mill or high-speed agitator where the exposed surface is increased. The drying rate is very high for these dryers (hence the term *flash dryers*), but the solid temperature does not rise much because of the short residence time. A flash dryer is not suitable for particles which are large in size or heavy particles. The special advantage of this type of dryer is that no separate arrangement is required for transporting the dried product. The fine particles leave the mill through a small duct to maintain the carrying velocities (drying gas) and reach a cyclone separator. A solid particle takes few seconds to pass from the point of entry into the air stream to the collector. The inlet gas temperature is high and varies from 650^{O}C to 315^{O}C, for example, in 2 seconds, or from 650^{O}C to 175^{O}C in 4 seconds. The

thermal efficiency this type of dryer is generally low. A material having an initial moisture content of 80 % may be reduced to 5 or 6 % in the dried product.

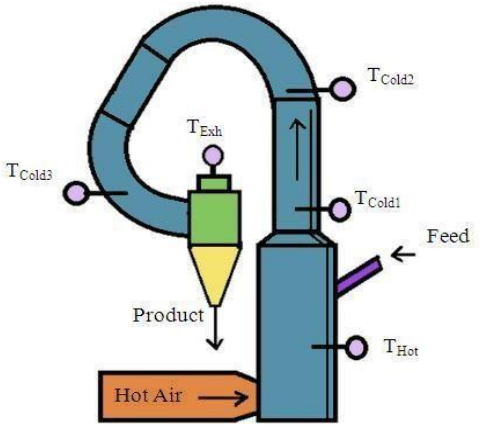


Figure : Flash dryer Fluidised Bed Dryer

Fluidized bed dryer consist of a steel shell of cylindrical or rectangular cross section. A grid is provided in the column over which the wet material is rests. In this type of dryer, the drying gas is passed through the bed of solids at a velocity sufficient to keep the bed in a fluidized state. Mixing and heat transfer are very rapid in this type of dryers. The dryer can be operated in batch or continuous mode (figure 2.5). Fluidized bed dryer are suitable for granular and crystalline materials. If fine particles are present, either from the feed or from particle breakage in the fluidized bed, there may be considerable solid carryover with the exit gas and bag filters are needed for fines recovery. The main advantage of this type of dryer are: rapid and uniform heat transfer, short drying time, good control of the drying conditions.

In case of rectangular fluid-bed dryers separate fluidized compartments are provided through which the solids move in sequence from inlet to outlet. These are known as *plug flow dryers;* residence time is almost the same for all particles in the compartments. But the drying conditions can be changed from one compartment to another, and often the last compartment is fluidized with cold gas to cool the solid before discharge.

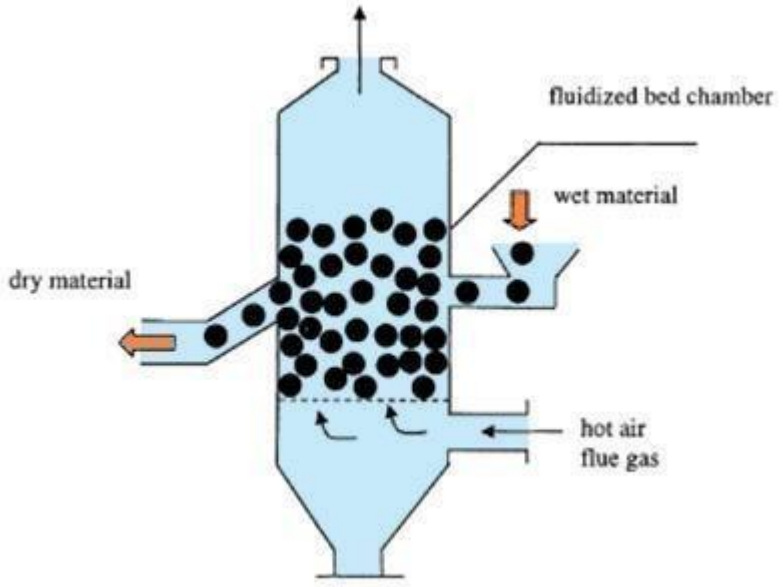


Figure 2.5: Continuous fluidized bed dryer

Screen Conveyor Dryers

Screen conveyor dryer is also called a direct heat continuous type dryer. The solid to be dried are fed on to endless, perforated, conveyor belt through which hot air is forced. The belt is housed in a long rectangular drying chamber or tunnel (figure 2.6). The chamber is divided into series of separate sections, each with its own fan and air heater. Air may be recirculated through, and vented from each section separately or passed from one section to another counter current to the solid movement. The solid is carried through the tunnel and discharged at the opposite end. In order to prevent the higher flow rate of hot air through thinner regions of the bed a uniform feeding rate and distribution of the material over the conveyor is necessary. Coarse granular, flakey, or fibers materials can be dried by through circulation without any pretreatment and without loss of material through the screen. High drying rate can be achieved with good product quality control. Thermal efficiency of this type of dryer is high and with steam heating, the steam consumption for heating the drying gas can be as low as 1.5 kg per kg of water evaporated. Only disadvantage of this type of dryer are high initial cost and high maintenance cost due to the mechanical belt.

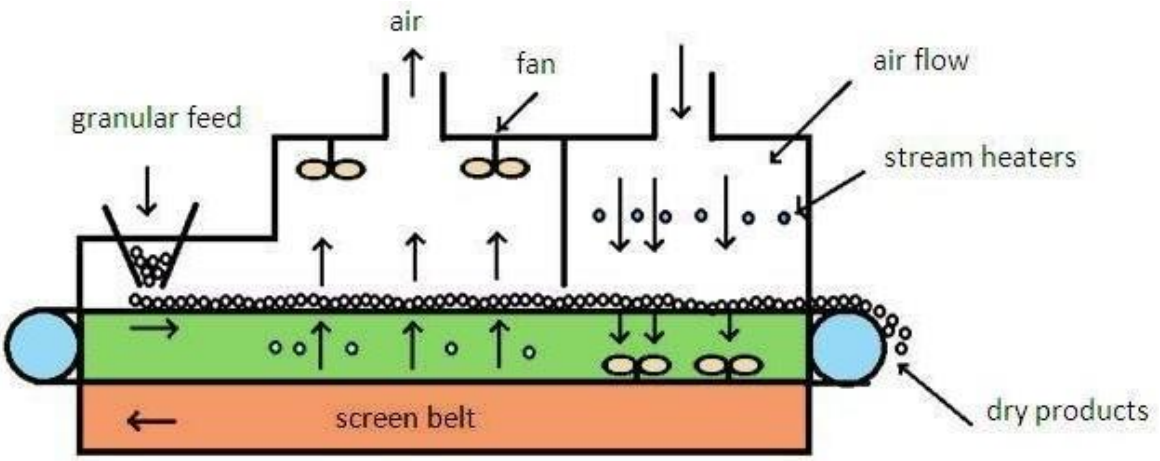


Figure Screen conveyor dryer

NOVEL DRYING TECHNOLOGIES

Newer technologies focus on saving in energy consumption that result in considerable overall improvement in energy efficiency. In addition, the final quality of the product is greatly influenced by the drying technique and strategy. A brief overview of some novel drying techniques is given below:

Microwave Drying

Microwave heating is a direct drying method. High-frequency radio waves are utilized in microwave drying. A high-frequency generates the waves and wave channel guides them in to an oven that is designed to prevent the waves from leaving the chamber. In microwave drying, heat is generated by directly transforming the electromagnetic energy in to kinetic molecular energy, thus the heat is generated deep within the material to be dried. Selection of proper wavelength is necessary to ensure thorough penetration into the material. Apart from these, other parameters such as material type and depth of material being exposed also affect the penetration. Therefore, selection of proper wavelengths and dehydration condition for each product is selected individually.

This type of heating is instantaneous, uniform and penetrating throughout the material, which is a great advantage for the processing of pharmaceutical compounds. In case of microwave drying the waves bounce from wall to wall, until the product absorbs eventually all of the energy, generating heat within the material, resulting in dehydration. Vapour from the liquid evaporating inside the product is emitted through the pore structure of the solid material‟s macro-capillary system, resulting in a high drying rate. This type of dryer is highly efficient and power utilization efficiencies are generally greater than 70 %. Important commercial aspects of this dryer includes the ability to maintain colour, moisture and quality of the natural food.

Supercritical Fluid Extraction and its application to Drying

The supercritical fluid (SCF) is a substance at a temperature and pressure above its critical point. It can effuse through solids like a gas, and dissolve materials like a liquid. Supercritical fluids possess unique properties that enable them to extract

components selectively from a mixture. This ability has been investigated as an alternative to currently used separation processes such as distillation or liquid extractions. In addition, close to the critical point, small changes in pressure or temperature result in large changes in density, allowing many properties of a supercritical fluid to be "fine-tuned". Above the critical point, this increased density produces enhanced solvency, approaching that of a liquid. It is this solvency that makes SCF extraction a feasible alternative. Mass transfer properties resembling that of gases are also a significant factor in SCF extraction. An application of SCF extraction that has seemingly gone unexplored is to the drying of food products. Since moisture content influences texture, chemical reactions, and susceptibility to microbial spoilage, drying is a way to retain quality and prolong shelf life. A complication associated with drying of food products is that they may undergo changes that alter the physical or chemical structure, thus changing the integrity of the product. SCF extraction avoids this problem because it allows the food product to be dehydrated without undergoing a phase change from liquid water to water vapour. Also, if a solvent such as supercritical carbon dioxide is used, it will not be necessary to heat the product above ambient temperatures.

SELECTION OF DRYING EQUIPMENT

In view of the enormous choice of dryer types one could possibly deploy for most products, selection of the best type is a challenging task that should not be taken lightly. The first consideration in selecting a dryer is its operability. Above all else, the equipment must produce the desired product in the desired form at the desired rate. The quality required in a finished product, and its necessary physical characteristics, are determined by its end use. A wrong dryer for a given application is still a poor dryer, regardless of how well it is designed. Although variety of commercial dryers are available in the market, the different types mare largely complementary, not competitive, and the nature of the drying problem dictates the type of dryer that must be used, or at least limits the choice to perhaps two or three possibilities. The final choice is then made on the basis of capital and operating costs. Attention must be paid, however, to the costs of the entire drying system, not just the drying unit alone.There are some general guidelines which need to be followed to select a dryer, but it should be recognized that the rules are far from rigid and

exceptions not uncommon. Often batch dryers are used when the production rate of dried product is less than 150 to 200 kg/h, while continuous dryers are suitable for production rates greater than 1 or 2 tons/h. To handle intermediate production rates other factors must be considered.

The dryer must also operate reliably, safely, and economically. Operation and maintenance costs must not be excessive; pollution must be controlled; energy consumption must be minimized. As with other equipment these requirements may be conflict with one another and a compromise needs to be reached in finding the optimum dryer for a given service. As far as the drying operation itself is concerned, adiabatic dryers are generally less expensive than non-adiabatic dryers, in spite of the lower thermal efficiency of adiabatic units. Unfortunately there is usually a lot of dust carry over from adiabatic dryers, and these entrained particles must be removed from the drying gas. Elaborate particle-removal equipment may be needed, equipment that may cost as much as the dryer itself. This often makes adiabatic dryers less commercially attractive than a –buttoned-up‖ non-adiabatic system in which little or no gas is used.

DESIGN CONSIDERATION OF DRIERS

DESIGN OF DRYER

Design of a rotary dryer only on the basis of fundamental principle is very difficult. Few of correlations that are available for design may not prove to be satisfactory for many systems. The design of a rotary dryer is better done by using pilot plant test data and the full scale operating data of dryer of similar type if available, together with the available design equations. A fairly large number of variables are involved such as solid to be dried per hour, the inlet and exit moisture contents of the solid, the critical and equilibrium moisture contents, temperature and humidity of the drying gas. The design procedure based on the basic principles and available correlations is discussed below. In this case we assume that the solid has only unbound moisture and as shown in fig 2.7 in stage II the solid is at the wet bulb temperature of the gas.

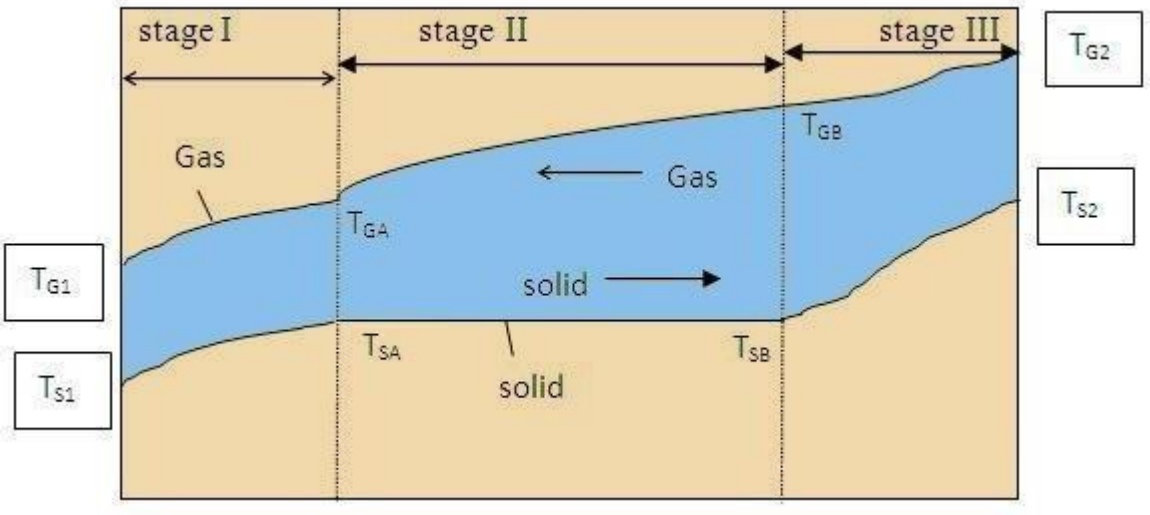


Figure Temperature profile for solid and gas in a counter current rotary dryer

1. Heat losses from dryer surfaces are neglected.

2. Once the capacity of the dryer is known, the drying gas flow rate, its temperature and humidity are decided considering a number of factors. And the following moisture & enthalpy balances need to be satisfied.

$$G_s (Y_1 - Y_2) = M_s$$
$$(X_1 – X_2)$$

$$G_s (Hg_2 – Hg_1) = M_s$$
$$(H_{S2} – H_{s1})$$

Here, G_S = flow rate of air (dry basis, kg/h), Ms = flow rate of solid (kg/h, dry basis), H_S = humidity of air (kg/H_2O/kg dry air)

3. The gas and solid temperatures at the stage boundaries are obtained by moisture and energy (enthalpy) balances. The number of heat transfer unit for each zone is calculated. for the stage II. The number of heat transfer units is given by

$$(NtG)h,II \times \Delta Tm = (TGB – TGA)$$

4. The total length of dryer is given by

$$L = (LT)1\ (NtG)1 + (LT)II\ (NtG)II + (LT)III\ (NtG)III$$

5. The shell diameter is calculated from the dry gas flow rate (from step I) and suitable gas flow velocity or gas mass flow rate
   Some useful correlations for the design of a rotary dryer are given below.
   Volumetric gas-solid heat transfer coefficient.
   $\bar{U}_a = (W/m^3.K) = 237\ (G‟)^{0.67}/d$

Here, G‟ = gas mass flow rate (kg/m^2.h) and d, dryer diameter

Length of transfer unit $L_T = G'C_H / \bar{U}_a$

$$L_T = \frac{0.0063\ C_H \cdot d_s}{G^{0.84}}$$

Here, c_H = average humid heat, and d = dryer diameter

Solid retention time:

$$\theta = \frac{0.23\ L}{S\ N^{0.9}\ d} \pm 1.97 \frac{B\ L\ G'}{F}$$

(+ve sign is for counter flow; –ve sign is for parallel flow of the gas and solid)

Where,

θ = retention time (min); L = dryer length (m)

S = slope of the dryer (m/m); N = speed (rpm)

G‟ = gas mass flow rate (Kg/m^2.h)

F = feed rate (Kg/m^2. h) dry basis

$B = 5\ (d_p)^{-0.5}$

d_p = weight average particle diameter (micron)

d = dryer diameter (m)

= 45.08 BHP

= 33.62 KW

INTRODUCTION AND TYPES OF DRIERS

The term drying refers generally to the removal of moisture from a substance. It is one of the oldest, most commonly used and most energy consuming unit operation in the process industries. Drying is often necessary in various industrial operations particularly in chemical process industries to remove moisture from a wet solid, a solution or a gas to make it dry and choice of drying medium is depends on the chemical nature of the materials. Three basic methods of drying are used today 1) sun drying, a traditional method in which materials dry naturally in the sun, 2) hot air drying in which matrials are exposed to a blast of hot air and 3) freeze drying, in which frozen materials are placed in a vacuum chamber to draw out the water. The fundamental nautre of all drying porcess is the removal of volatile substances (mainly moisture) from mixture to yield a solid product. In general drying is accomplished by thermal techniques and thus involves the application of heat, most commonly by convection from current of air. Throughout the convective drying of solid materials, two processes occur simultaneously namely, transfer of energy from the local environemnt in the dryer and transfer of moisture from within the solid. Therefore this unit operation may be considered as simultaneous heat and mass transfer operation. Drying processes and equipment may be categorised according to several criteria, incuding the nature of material and the method of heat supply and the method of operation. For example In the sugar industry washed and centrifuged sugar crystals are dried to get finisehd product for packing. Drying is an important operation in food processing. Milk is dried in a spray chamber to produce milk powder. All the above examples indicates that wet material loses moisture in direct contact with hot air/gas. The hot air/gas supplies the energy required for drying and also carries away the moisture released by the solid. For heat sensitive materials much of the resistance to drying resides within the material. Unduly high heat and mass transfer rates applied at the surface only result in overheating or over drying of the surface layer resulting in quality problems without major increase in the drying kinetics. The rate of migration

of the moisture from within the solid to the evaporation front often controls the overall drying rate. Therefore, drying may be defined as an operation in which the liquid, generally water, present in a wet solid is removed by vaporization to get a relatively liquid free solid product. Drying of a solid does not demand or ensure complete removal of the moisture. Sometimes it is desirable to retain a little mositure in the solid after drying. Dryer and drying process selection for a specific operation is a complex problem, and many factors have to be taken into account. Though, the overall selection and design of a drying system for a perticular material is dictated by the desire to achieve a favourable combination of a product quality and process

economics. In general, with respect to the rate and total drying time, dryer performance is dependent on the factors such as air characteristics, product cheracteristics, eqipment characteristics. But despite the many commercially available drying techniques at present most dehydrated products (i.e. fruits and vegetables) are still produced by the method of hot air drying. Because this is regarded as the simplest and most economical . There are other water/liquid removal processes such as filtration, settling, centrifugation, supercritical extraction of water from gels etc. In all these operations liquid is removed by mechanical means but a considerable amount of liquid is still retained in the solid. This residual liquid can be removed by drying. One such example is the production of condensed milk involves evaporation, but the production of milk powder involves drying. The phase change and production of a soild phase as end product are essential features of the drying process. Drying is an essential operation in chemical, agricultural, biotechnology, food, polymer, pharmaceutical, pulp and paper, mineral processing, and wood processing industries.

2. PHYSICAL MECHANISM OF DRYING

Drying does not mean only removal of the moisture but during the process, physical structure as well as the apperance has to be preserved. Drying is basically governed by the principles of transport of heat and mass. When a moist solid is heated to an appropriate temperature, moisture vaporizes at or near the solid surface and the heat required for evaporating moisture from the drying product is supplied by the external drying medium, usually air or a hot gas. Drying is a diffusional process in which the transfer of moisture to the surrounding medium takes place by the evaporation of surface moisture, as soon as some of the surface moisture vaporizes, more moisture is transported from interior of the solid to its surface. This transport of moisture within a solid takes place by a variety of mechanisms depending upon the nature and type of the solid and its state of aggregation. Different types of solids may have to be handled

for drying crystalline, granular, beads, powders, sheets, slabs, filter-cakes etc. The mechanism of moisture transport in different solids may be broadly classified into (i) transport by liquid or vapour diffusion (ii) capillary section, and (iii) pressure induced transport. The mechanism that dominates depends on the nature of the solid, its pore structure and the rate of drying. Different mechanisms may come into play and dominate at different stages of drying of the same material.

The following term are commonly used in designing of drying systems.

Moisture content of a substance which exerts as equilibrium vapour pressure less than of the pure liquid at the same temperature is refered to as *bound moisture.*.

Moisture content of the solid which exters an equillibrium vapour pressure equal to that of pure liquid at the given temperature is the *unbound moisture.*

The moisture content of solid in excess of the equilibrium moisture content is refered as *free moisture.* During drying, only free moisture can be evporated. The free moisture content of a solid depends upon the vapour concentration in the gas.

The moisture contents of solid when it is in equilibrium with given partial pressure of vapour in gas phase is called as *equilibrium moisture content*. Similalry, the moisture content at which the constant rate drying peroid ends and the falling rate drying period starts is called *critical moisture content*. During the *constant rate drying period*, the moisture evporated per unit time per unit area of drying surface remains constant and in *falling rate drying period* the amount of moisture evporated per unit time per unit area of drying surface continuously decreases.

3. CLASSIFICATION OF DRYERS

Drying equipment is classified in different ways, according to following design and operating features.

It can be classified based on mode of operation such as batch or continuous, In case of batch dryer the material is loaded in the drying equipment and drying proceeds for a given period of time, whereas, in case of continuous mode the material is continuously added to the dryer and dried material continuously removed. In some cases vacuum may be used to reduce the drying temperature. Some dryers can handle almost any kind of material, whereas others are severely limited in the style of feed they can accept. Drying processes can also be categorized according to the physical state of the feed such as wet solid, liquid, and slurry. Type of heating system i.e. conduction, convection, radiation is another way of categorizing the drying process. Heat may be supplied by direct contact with hot air at atmospheric pressure, and the water vaporized is removed by the air flowing. Heat may also be supplied indirectly through the wall of the dryer from a hot gas flowing outside the wall or by radiation.

Dryers exposing the solids to a hot surface with which the solid is in contact are called adiabatic or direct dryers, while when heat is transferred from an external medium it is known as non-adiabatic or indirect dryers. Dryers heated by dielectric, radiant or microwave energy are also non adiabatic. Some units combine adiabatic and non adiabatic drying; they are known as direct-indirect dryers.

To reduce heat losses most of the commercial dryers are insulated and hot air is recirculated to save energy. Now many designs have energy-saving devices, which recover heat from the exhaust air or automatically control the air humidity. Computer control of dryers in sophisticated driers also results in important savings in energy.

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Crystallization:

A **crystal** is a solid bounded by plane surfaces. Crystallization is important as an industrial process because a large number of commodity chemicals, pharmaceuticals, and specialty chemicals are marketed in the form of crystals. The wide use of crystallization is due to the highly purified and attractive form in which the compounds can be obtained from relatively impure solutions by means of a single processing step. Crystallization can be performed at high or low temperatures, and it generally requires much less energy for separation of pure materials than other commonly used methods of purification do. While crystal-lization may be carried on from vapor or a melt, the most common industrial method is from a solution. A solution is made up of a liquid (solvent) — most commonly water — and one or more dissolved species that are solid in their pure form (solute). The amount of solute present in solution may be expressed in several different units of concentration. For engineering calculations, expressing the solu- bility in mass units is the most useful. The solubility of a material is the maximum amount of solute that can be dissolved in a solvent at a particular temperature. Solubility varies with temperature and,
with most substances, the amount of solute dissolved increases with increasing temperature.

For crystallization to occur, a solution must be supersaturated. **Supersaturation** means that, at a given temperature, the actual solute concentration exceeds the concentration under equilibrium or saturated conditions. A supersaturated solution is metastable, and all crystallization occurs in the metastable region. A crystal suspended in saturated solution will not grow. Supersaturation may be

expressed as the ratio between the actual concentration and the concentration at saturation [Equation (62.1)] or as the difference in concentration between the solution and the saturated solution at the same temperature [Equation (62.2)].

$$S = C / C_S \quad (62.1)$$

$$\Delta C = C - C_S \quad (62.$$

where C is the concentration (g/100 g of solution), and C_S is the concentration (g/100 g of solution) at saturation. This difference in concentration may also be referenced to the solubility diagram and expressed as degrees (∞C) of supersaturation.

Nucleation is the birth of a new crystal within a supersaturated solution. Crystal growth is the layer- by-layer addition of solute to an existing crystal. Both of these phenomena are caused by supersaturation. Nucleation is a relatively rapid phenomenon that can occur in a matter of seconds. Growth is a layer- by-layer process on the surface of an existing crystal and takes considerably more time. The ratio of nucleation to growth controls the size distribution of the crystal product obtained. Generating a high level of supersaturation spontaneously leads to both nucleation and growth. The competition between these two processes determines the character of the product produced.

Methods of Creating Supersaturation

Supersaturation may be created by cooling a solution of normal solubility into the metastable zone. Typically, the amount of supersaturation that can be created in this way without causing spontaneous nucleation is in the range of 1 to 2∞C.

Evaporation of solvent at a constant temperature also produces supersaturation by reducing the amount of solvent available to hold the solute. The reaction of two or more chemical species, which causes the formation of a less soluble species in the solvent, can also produce supersaturation. Finally, the addition of a miscible nonsolvent in which the solute is not soluble to a solvent will cause a decrease in the solubility of the solute in the solution. This technique is most often used in pharmaceutical operations involving the addition of alcohol or similar solvents to the primary solvent (water).

Reasons for the Use of Crystallization

Crystallization is important as an industrial process because a large number of materials can be marketed in the form of crystals that have good handling properties. Typically, crystalline materials can be separated from relatively impure solutions in a single processing step. In terms of energy requirements, the energy required for crystallization is typically much less than for separation by distillation or other means. In addition, crystallization can often be performed at relatively low temperatures on a scale that involves quantities from a few pounds up to thousands of tons per day.

Solubility Relations

Equilibrium relations for crystallization systems are expressed in the form of solubility data, which are plotted as phase diagrams or solubility curves. The starting point in designing any crystallization process is knowledge of the solubility curve, which is ordinarily plotted in terms of mass units as a function

of temperature. An example is given in Figure 62.1 for the solubility of magnesium sulfate in water as a function of temperature. At any concentration and temperature, the information on the diagram allows one to predict the mixture of solids and solution that exists. Note that, in the case of magnesium sulfate, a number of different hydrates can exist in addition to the solution itself, or ice plus the solution. The line that forms a boundary between the solution area and the various crystal hydrate areas is a solubility curve. Starting from point (1) at 50∞C and cooling to 30∞C at point (2) is a path that crosses the solubility line. During the cooling process, crossing the line in this manner indicates that the solution has become supersaturated for the concentration in question. If the supersaturation is within the metastable range — which is approximately 1∞C — then growth can occur on existing crystals, but no substantial amount of nucleation will occur. If the cooling proceeds further, the system can become unstably supersaturated, and spontaneous nucleation takes place. If spontaneous nucleation takes place, very small crystals or nuclei form, and they will grow as long as the solution remains supersaturated.As growth takes place, the concentration drops in the direction of point (3), and, as it approaches the solubility line, growth ceases because the driving force approaches zero. Organic and inorganic materials

have similar solubility curves and they vary in concentration and temperature for each compound. Some materials have no hydrates and others exhibit a wide range of hydrates similar to those shown in Figure
62.1. Solubility information on most compounds is available from the literature in publications, such as the *International Critical Tables* [Campbell and Smith, 1951] and *Lang's Solubility of Inorganic and Organic Compounds* [Linke, 1958], and in various software packages that are becoming available.

Product Characteristics

The shape and size of a crystal are determined by its internal structure as well as external conditions that occur during its growth cycle. These external conditions include growth rate, the solvent system, the level of agitation, and the effect of impurities that may be present. Crystalline material is almost always

separated from its mother liquor before the crystal can be dried or used. The filterability of the crystals, whether separation is done on a centrifuge or filter, is an important characteristic of the product. Generally, larger particles filter more readily, but the average particle size by itself is not an unfailing indication of filterability. The particle size distribution is important because, if it is very broad, small particles may be trapped between the larger particles, making the drainage rates much lower. This could lead to retention of mother liquor, which will degrade the purity of the final product. Broad distributions that increase the amount of mother liquor retained also make the cake less pervious to wash liquids. Products crystallized from continuous crystallizers typically have a coefficient of variation of 45 to 50%. Products made from batch crystallizers, which are fully seeded, often show narrower size distributions with a coefficient of variation of approximately 25 to 30%.

The bulk density of the dried material is affected not only by the crystal density itself, but also by the size distribution. A broader distribution leads to tighter packing and, therefore, a higher bulk density. The flow properties of a crystal product are affected by the crystal shape. Rounded crystals that are formed under conditions of relatively high attrition flow very well, particularly if the particles are in the size range of -8 to +30 U.S. Mesh.

Impurities Influencing the Product

Since crystallization is generally performed to produce high-purity products, it is important that the crystal be grown in such a way that the impurities that are part of the mother liquor are not carried out with the crystalline particles. Impurities can affect the growth rate and nucleation rate during crystalli- zation

and, as a consequence, affect both the mean particle diameter and the habit of the particles being crystallized. Most habit modifiers cause a change in the crystal shape because they are absorbed on one or more of the crystal faces, thereby altering the growth rate of that face and causing that face to either become predominant or largely disappear. Impurities that have this influence can be either ionic, surface- active compounds or polymers.

Under some conditions, the impurities in a product can be increased by lattice incorporation, which occurs when an impurity in the mother liquor substitutes for molecules in the product crystal lattice. Mixed crystals — which are really two separate species crystallizing at the same time — can also be produced. Surface absorption of species that are in the mother liquor not only can add to the impurity level of the product, but can also alter the growth rate and, therefore, the habit of the crystals. Solvent inclusion can occur when rapidly growing crystals form around small volumes of mother liquor that become trapped inside the crystal lattice. The liquor in these inclusions may or may not find its way to the surface during the subsequent drying operations.

Solvent inclusion probably accounts for the largest increase in impurity levels in a crystal, with lattice incorporation generally less, and surface absorption accounting for only very minute amounts of con- tamination. Normally, recrystallization from a relatively pure solution will eliminate virtually all the impurities, except for a material whose presence is due to lattice incorporation.

Kinds of Crystallization Processes

Crystallization can be carried on in either a batch or continuous manner, irrespective of whether evap- oration, cooling, or solvent change is the method of

creating supersaturation. Batch processes are almost always used for small capacities and have useful application for large capacities when a very narrow particle size distribution is required, such as with sugar, or when materials (e.g., pharmaceuticals) that require very accurate inventory control are being handled.

A continuous crystallization process normally must operate around the clock because the retention times typically used in crystallizers range from about 1 to 6 h. As such, it takes at least four to six retention times for the crystallizer to come to equilibrium, which means there may be off-spec product when the system is started up. To minimize this, the unit should be kept running steadily as long as

possible. The cost of at least three operators per day and the instrumentation required to continuously control the process represent a substantially greater investment than what is required for batch processing. This disadvantage can only be overcome by utilizing that labor and investment at relatively high production rates.

62.7 Calculation of Yield in a Crystallization Process

In order to calculate the yield in a crystallization process, it is necessary that the concentration of feed, mother liquor, and any change in solvent inventory (evaporation) be known. In most crystallization processes, the supersaturation in the residual mother liquor is relatively small and can be ignored when calculating the yield. With some materials, such as sugar, a substantial amount of supersaturation can exist, and under such circumstances the exact concentration of the solute in the final mother liquor must be known in order to make a yield calculation. The product crystal may be hydrated, depending on the compound and temperature at which the final crystal is separated from the mother liquor.

Shown below is a formula method for calculating the yield of a hydrated crystal from a feed solution

[Myerson,
1993].

$$P = R\frac{100W - \quad - E)}{S(H}\frac{o}{o}\frac{}{100 - S(R - 1)}$$

(62.3)

wher

e P = weight of product

R = mole weight of hydrate crystal / mole weight of anhydrous crystal

S = solubility at the mother liquor (final) temperature in units/100 units of solvent

W_O = weight of anhydrous solute in feed

H_O = weight of solvent in feed

E = evaporation

62.8 Mathematical Models of Continuous Crystallization

Randolph and Larsen [1988] developed a method of modeling continuous crystallizers in which the growth rate is independent of size and the slurry is uniformly mixed. Such crystallizers are often referred to as the mixed-suspension mixed-product removal (MSMPR) type. For operation under steady condi- tions, the population density of an MSMPR crystallizer (FC and DTB types shown in Figure 62.2 and Figure 62.3. respectively) is

$$n = n^o e^{-L/GT} \quad (62.4)$$

wher

e n = population density, number/mm

G = mm/h

growth rate, T = retention time, h

L = characteristic length, mm

n^o = nuclei population density (i.e., intercept at size $L = O$)

A plot of the ln n versus L will be a straight line if the system is operating under the conditions assumed above. The nucleation rate and the mean particle size (by weight) are

$$B^o = Gn^o \quad (62.5)$$

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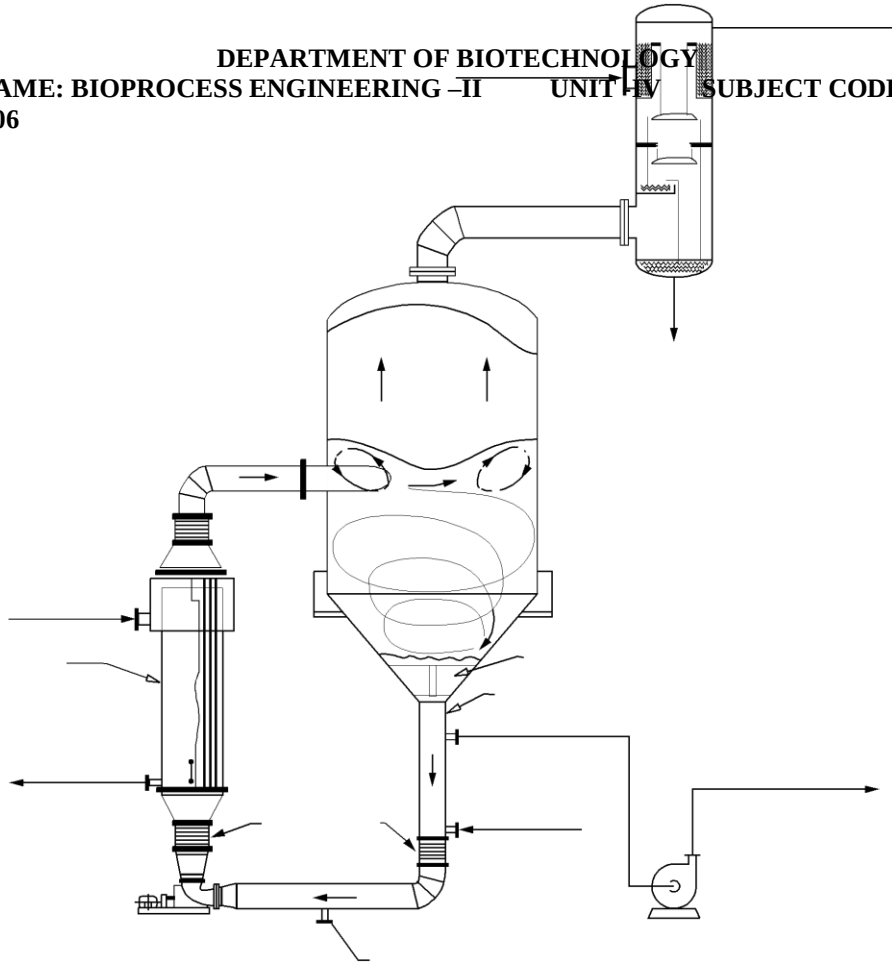

FIGURE 62.3 Swenson draft-tube baffle crystallizer. (*Source:* Courtesy of Swenson Process Equipment Inc.)

In solving Equation (62.4) and Equation (62.5), it must be remembered that the growth rate and the nucleation rate must be measured under the same conditions. In evaluating performance of crystallization equipment, it is necessary to know the heat balance, material balance, and population balance of the particles being used as seed (when used), as well as the product population balance.

Equipment Designs

While many solvent systems are possible, most large-scale industrial crystallizers crystallize solutes from water. Organic solvents are sometimes encountered in the petroleum industry, and alcohol solutions or mixtures of alcohol and water are found in pharmaceutical applications. Typically, water solutions haveviscosities in the range of 1 to 25 cp and boiling point elevations from 1 up to 12∞C. The viscosity of a solution is very important because it determines the settling rates of particles within the solution and heat transfer rates in heat exchange equipment required for heating or cooling the solution. The boiling point elevation represents a temperature loss in an evaporative system where condensation of the vapor in a multiple-stage evaporative crystallizer or condenser is required.

The evaporation rate is determined from the basic process requirements and the heat balance around the system. The evaporation rate and the temperature at which evaporation occurs determine the min- imum body diameter. The specific volume of water vapor is strongly influenced by pressure and tem- perature. Low

temperatures, which represent relatively high vacuum for water at its boiling point, require larger bodies than do systems operating at atmospheric pressure. The other consideration in sizing the body is the minimum volume required to provide the retention time required for crystal growth.

Shown in Figure 62.2 is a forced-circulation evaporator-crystallizer, which is often used for the pro- duction of sodium chloride, citric acid, sodium sulfate, sodium carbonate, and many other inorganic compounds produced by evaporative crystallization. The body diameter and straight side are determined by the vapor release rate and retention time required to grow crystals of the desired size. The sizes of the circulating pipe, pump, heat exchanger, and recirculation pipe are based on the heat input required to cause the evaporation to take place. Crystals in the solution circulated throughout the body are kept in suspension by the action of the recirculating liquor. Tube velocities, heat transfer rates, and circulation rates are determined by the particular application and the physical properties of the solution. Slurry leaving the crystallizer is pumped by the product discharge pump into a centrifuge, filter, or other separation equipment. This type of crystallizer is often referred to as an MSMPR type, and the crystal size distribution can be described by the mathematical model described in Equation (62.4) through Equation (62.7). The crystal size typically produced in equipment of this type is in the range of 30 to
100 Mesh, and slurry discharge densities typically handled in such equipment range from about 20 to
40% by weight
solids.

Shown in Figure 62.3 is a draft-tube baffle (DTB) crystallizer of the evaporative type, including an elutriation leg. Slurry within the crystallizer body is

pumped to the surface by means of a slow propeller and recirculates to the suction of the propeller where it is mixed with heated solution exiting the heating element. Surrounding the body of slurry in the crystallizer is an annular space between the skirt baffle and the settler. Liquid is pumped from this annular space at a controlled rate so that small crystal particles from the body can be removed, but the bulk of the circulated liquor and crystals enters the propeller suction. The flow from the annular area is pumped through a circulating pipe by a circulating pump through the heat exchanger, where the temperature rise destroys small particles that are present. This continuous removal and dissolution of small particles by temperature increase serves two purposes:

1. The heat required for the evaporation is transferred into the liquid so that a constant vaporization rate can be maintained.
2. Small particles are continuously removed so as to limit the seed crystals in the body to values low enough so that the production can be obtained in a coarse crystal size.

When the crystals become too large to be circulated by the propeller, they settle into the elutriation leg, where they are washed by a countercurrent stream of mother liquor pumped from behind the baffle. Crystals leaving the leg are therefore classified generally at a heavier slurry density than would be true if they were pumped from the body itself. This combination of removal of unwanted fines for destruction and classification of the particle size being discharged from the crystallizer encourages the growth of larger particles than would be obtained in a crystallizer such as the forced circulation type in Figure 62.2. Typically, the

DTB crystallizer is used for products in the range of 8 to 20 Mesh with materials such as ammonium sulfate and potassium chloride.

Shown in Figure 62.4 is a surface-cooled crystallizer, which is frequently used at temperatures close to ambient or below. Slurry leaving the body is pumped through a heat exchanger and returns to the body through a vertical inlet. Surrounding the circulating slurry is a baffle that permits removal of unwanted fine crystals or provides for the removal of clarified mother liquor to increase the slurry density

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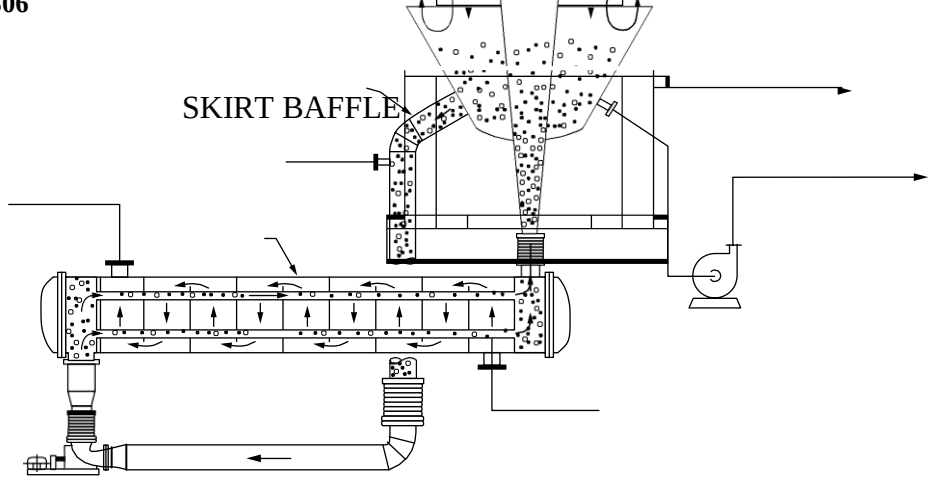

within the crystallizer body. Slurry pumped through the tubes of the cooler is chilled by a coolant that is circulated outside the tubes. The temperature difference between the coolant and the slurry flowing through the tubes must be limited to approximately 3 to 8∞C. The temperature drop of the slurry passing through the tubes is normally about 0.5∞C. These very low values are required in order to minimize the growth of solids on the tubes. Crystallizers of this type produce a product that ranges between 20 and
150 Mesh in size. Common applications are for the production of copper sulfate pentahydrate, sodium chlorate, sodium carbonate decahydrate, and sodium sulfate decahydrate.

Shown in Figure 62.5 is a reaction-type DTB crystallizer. This unit, while in many respects similar to the DTB crystallizer shown in Figure 62.3, has the important difference that no heat exchanger is required to supply the heat required for evaporation. The heat of reaction of the reactants injected into the crystallizer body supplies this heat. Typically, this type of equipment is used for the production of ammonium sulfate, where sulfuric acid and gaseous ammonia are mixed in the draft tube of the crys- tallizer so as to produce supersaturation with respect to ammonium sulfate. The heat of reaction is removed by vaporizing water, which can be recirculated to the crystallizer and used for the destruction of fines. Whenever a chemical reaction causes a precipitation of crystalline product, this type of equipment is worth considering because the conditions used in crystallization are compatible with low temperature rises and good heat removal required in reactors. By combining the reactor and crystallizer, there is better control of the particle size with an obvious decrease in equipment costs.

Evaporation

When a solution is boiled (evaporated) at constant pressure, the total pressure above the solution represents the sum of the partial pressures of the liquids that are boiling. If only water is present, then the pressure above the solution at any temperature corresponds to water at its boiling point at that pressure. If there is more than one component present and that component has a vapor pressure at the temperature of the liquid, then the total pressure represents the vapor pressure of water plus the vapor

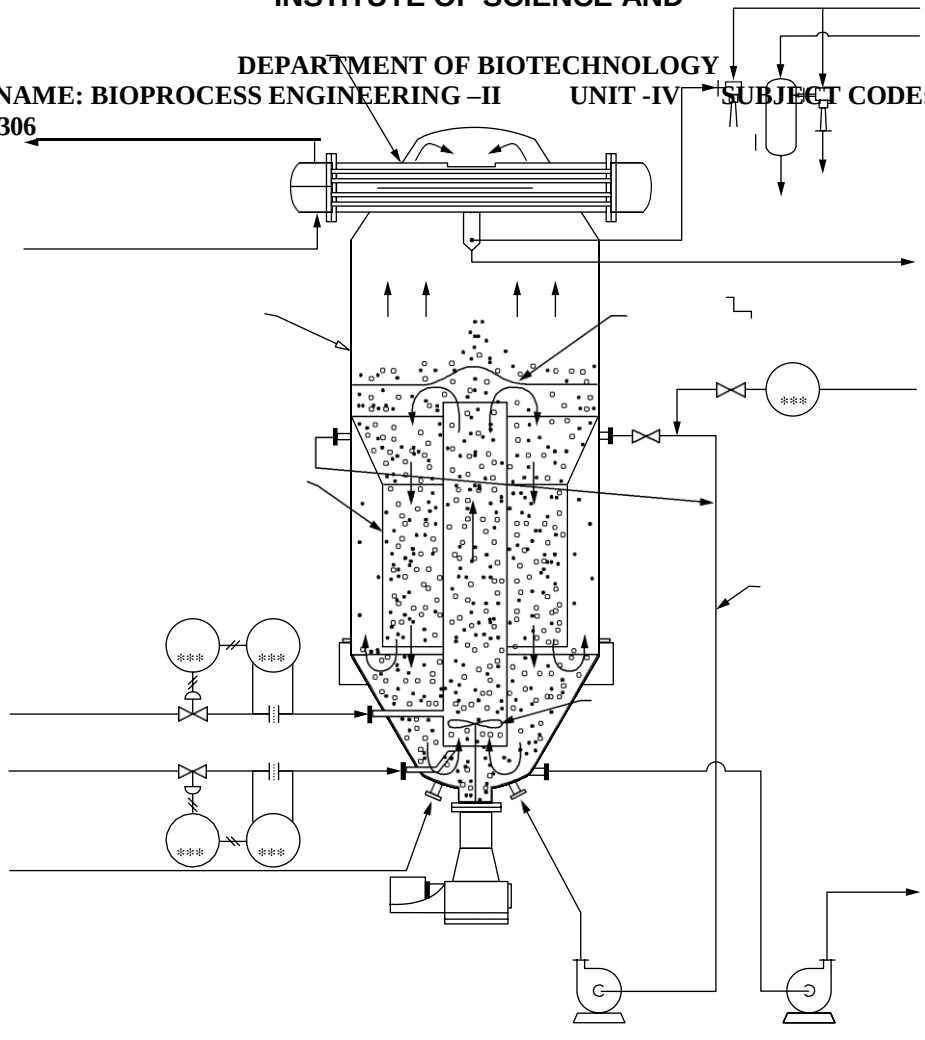

FIGURE Swenson reaction-type DTB crystallizer. (*Source:* Courtesy of Swenson Process Equipment Inc.)

pressure of the other component. Vapor leaving such a system, therefore, represents a mixture of solvents in the ratio of their partial pressures. In a sense, an evaporator is a single plate distillation column. In most applications, the vapor

pressure of the solute is negligible and only water is removed during boiling, which can be condensed in the form of a pure solution. However, when volatile compounds are present (e.g., H_3BO_3, HNO_3), some of the volatile material will appear in the overhead vapor.

Since the heat required to vaporize water is approximately 556 cal/kg (1000 Btu/lb), it is important

to reduce the amount of energy required as much as possible so as to improve the economics of the process. For this reason, multiple-effect evaporators were developed in the middle of the 19th century and continue today as an important means for achieving good economy during evaporation or crystal- lization. A multiple-effect falling-film evaporator consisting of three vessels and a condenser is shown in Figure 62.6. In this type of equipment, the vapor boiled from the first effect (the vessel where the steam enters) is conducted to the heat exchanger of the second effect, where it acts as the heating medium. Vapor boiled in the second effect is conducted to the third effect, where it again acts as the heating medium. Vapor leaving the third effect, in this case, is condensed in a condenser utilizing ambient-

temperature water. The flow of feed solution to the evaporator can be either forward, backward, or parallel. In a forward feed evaporator, the feed enters the first effect, then passes to the second effect, and is ultimately removed from the third effect as concentrated liquor. With this type of flowsheet, heat exchange means must be employed to minimize the sensible heat required for the liquid fed to the first effect. In a backward feed evaporator, this is not normally done.

An alternative means for reducing energy consumption during evaporation is shown in the recom- pression evaporative crystallizer in Figure 62.7. The technique can be employed on both evaporation and crystallization equipment. In

this case, a single vessel is employed, and the vapor boiled out of the solvent is compressed by a centrifugal compressor and used as the heating medium in the heat exchanger. The compressed vapor has a higher pressure and a higher condensing temperature so that there is a change in temperature between the vapor being condensed in the heater and the liquid being heated in the heat exchanger. In utilizing this technique, it must be remembered that the boiling point elevation decreases the pressure of the vapor above the liquid at any given temperature and, thereby, represents a pressure barrier that must be overcome by the compressor. The efficiency of this process varies greatly with the boiling point elevation. As a practical matter, such techniques are limited to those liquids which have boiling point elevations of less than about 13∞C. Typically, such compressors are driven at constant speed by an electric motor. The turndown ratio on a constant speed compressor is about 40%. A variable-speed drive would give a greater range of evaporative capacity.

During the last 100 years, a wide variety of evaporator types has evolved, each offering advantages for certain specific applications. The forced-circulation crystallizer shown in Figure 62.2 is utilized for many applications where no crystallization occurs, but the liquids being handled are viscous, and use of the circulation system is needed to promote heat transfer. A number of evaporator types have been developed that require no external circulating system. For the most part, these rely upon thermo-syphon effects to promote movement of liquid through the tubes as an aid to heat transfer. The calandria evaporator (or Roberts type) shown in Figure 62.8 is a design that has been widely used since the 19th century for both crystallization and evaporation applications. It relies on natural circulation in relatively short tubes (1 to 2 m) to maintain heat transfer rates; a relatively large amount of recirculation occurs through the tubes. Since there is

no recirculation pump or piping, this type of equipment is relatively simple to operate and requires a minimum of instrumentation. The volume of liquid retained in this vessel is much larger than in some of the rising or falling film designs and, therefore, in dealing with heat-sensitive materials where concentration must proceed at relatively short retention times, the calandria would be

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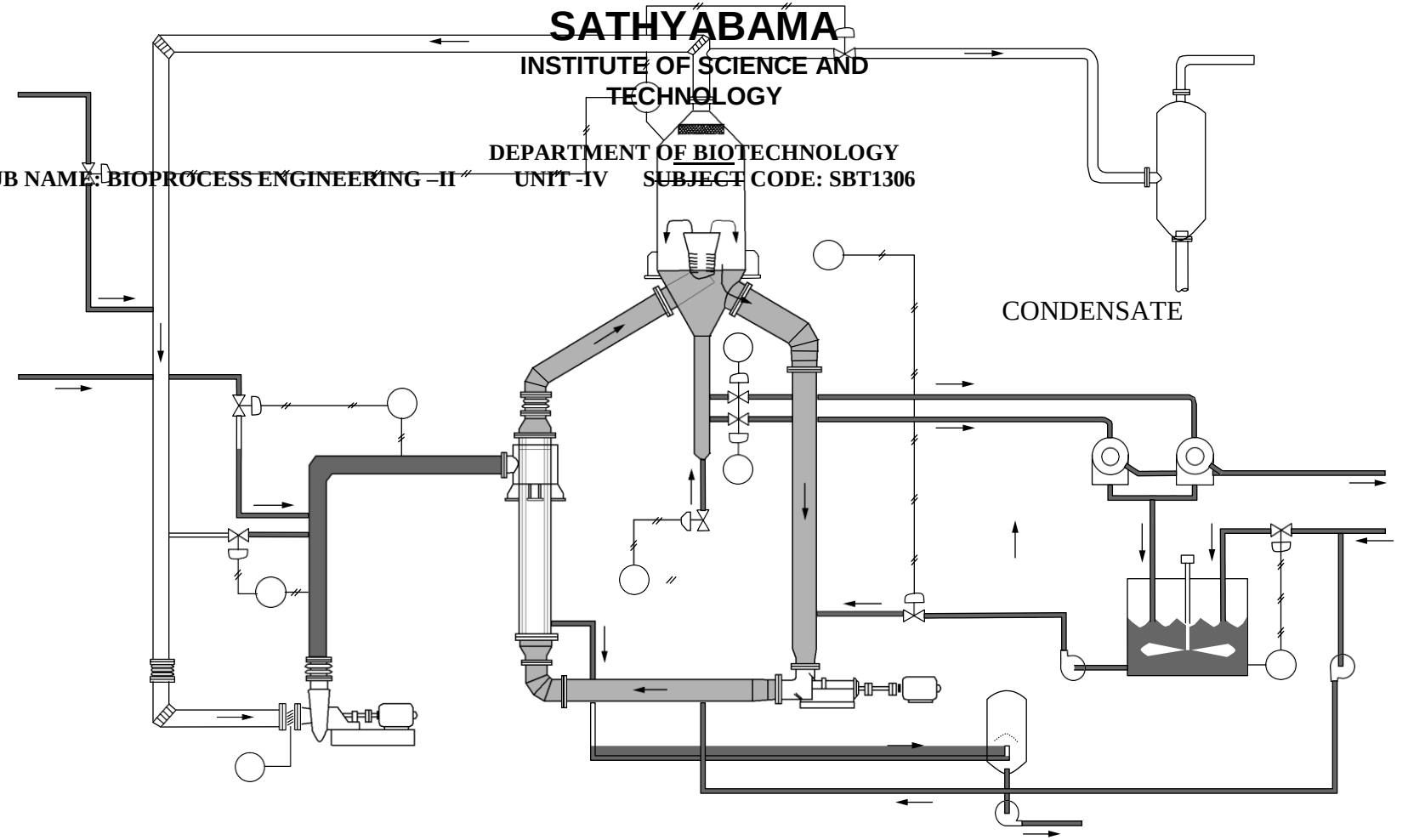

FIGURE Swenson recompression evaporator-crystallizer. (*Source:* Courtesy of Swenson Process Equipment Inc.)

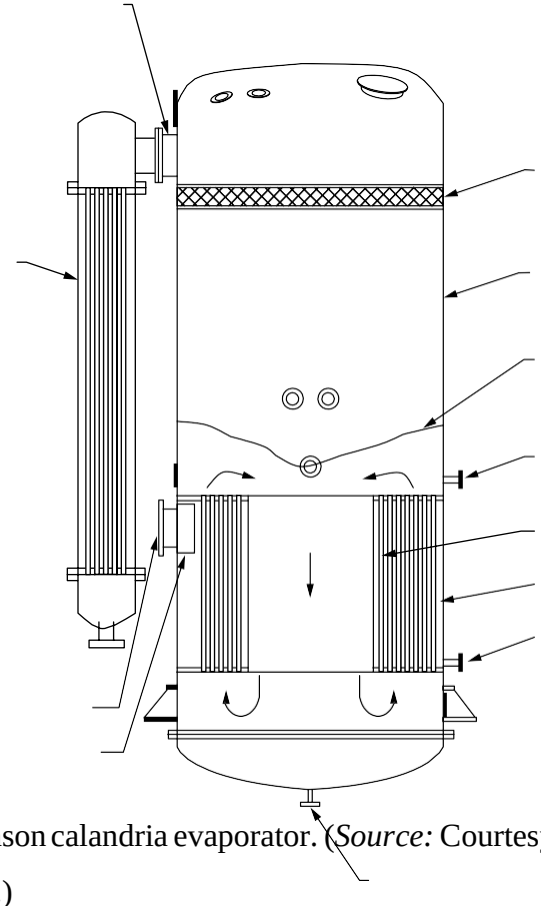

FIGURE Swenson calandria evaporator. (*Source:* Courtesy of Swenson Process Equipment Inc.)

a poor choice. In many situations, however, especially where some crystallization may occur, this evap- orator may be operated successfully in a semi-batch or continuous manner.

The falling-film evaporator shown in Figure 62.9 is similar to the rising-film evaporator, except that there must be sufficient liquid at all times entering the heater at the feed inlet to wet the inside surface of the tubes in the heat

exchanger bundle. With insufficient circulation, solute material can dry on the tubes and cause a serious reduction in heat transfer. Many falling-film evaporators operate with a recir- culating pump between the concentrated liquor outlet and the feed inlet to be certain that the recirculation rate is adequate to maintain a film on the tubes at all times. If this is done, the system can operate stably through a wide range of capacities and achieve very high rates of heat transfer, often 50 to 100% more than are obtained in a rising-film evaporator. The other advantage of the falling film evaporator is that it can operate with very low temperature differences between the steam and the liquid since there is no hydrostatic pressure drop of consequence within the tubes to prevent boiling at the inlet end of the heat exchanger. As a result, this type of design has found wide application as a recompression evaporator.

Even though evaporators are typically used where no precipitation of solids occurs, there is often a trace of precipitation in the form of scaling components that coat the inside of the tubes over a relatively long period of time. This scaling is analogous to that which occurs in boilers and many other types of heat transfer equipment. Typically it is due to either a small amount of precipitation or, because of the composition of the materials being concentrated, some inverted solubility components. Such scaling may often be reduced by a technique known as ‒sludge recirculation.‖ This is commonly done in cooling tower blowdown evaporation and in the evaporation of salt brines where scaling components are present.

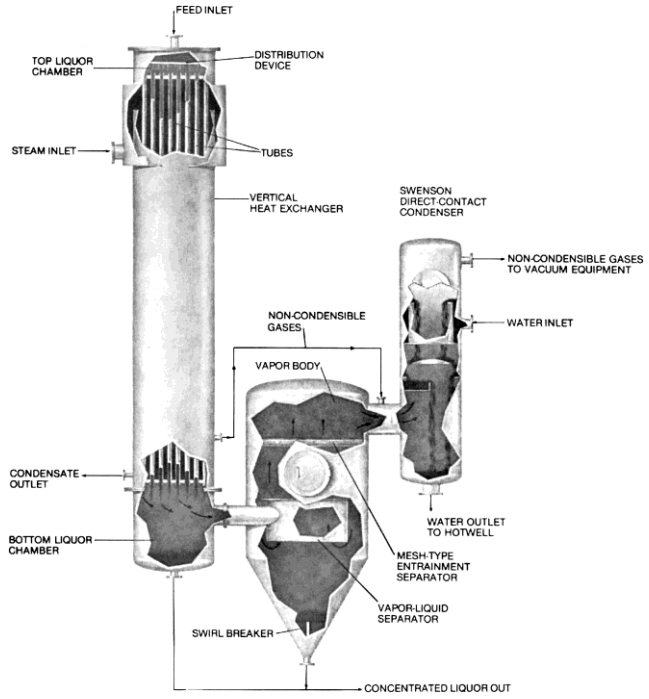

FIGURE Swenson falling-film evaporator. (*Source:* Courtesy of Swenson Process Equipment Inc.)

In these cases, the evaporator flowsheet is designed in such a way that a thickened slurry of the scaling component can be recirculated from the discharge of the evaporator back to the feed side. By maintaining an artificial slurry density of the scaling component, which is higher than the natural slurry density, it is often possible to reduce the growth of scale which occurs on heat transfer surfaces.

Defining Terms

Crystal — A solid bounded by plane surfaces that has an internal order with atoms or molecules in a fixed lattice arrangement.

Crystallizer — An apparatus for causing the crystallization of solutes from solvents by means of changes in heat or solvent inventory.

Evaporator — An apparatus for causing water or other solvents to be removed from a solution in order to increase the concentration of the solution.

Nucleation — The birth of a new crystal within a supersaturated solution.

Recompression — A process for collecting the vapor boiled from the solution in an evaporator or crystallizer and compressing to a higher pressure, where it can be used as the heating medium for said evaporator or crystallizer.

Supersaturation — A metastable condition in a solution that permits nucleation and growth of crystals to occur.

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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

B.TECH – BIOTECHNOLOGY

UNIT –V COMMERCIAL PRODUCTS - SBT1306

Blood products

- A blood product is any therapeutic substance prepared from human blood. This includes: whole blood; blood components; and plasma derivatives. Whole blood is not commonly used in transfusion medicine. Blood components include: red blood cell concentrates or suspensions; platelets produced from whole blood or via apheresis; plasma; and cryoprecipitate. Plasma derivatives are plasma proteins prepared

under pharmaceutical manufacturing conditions, these include: albumin; coagulation factor concentrates; and immunoglobulins.

- lood products may also be called blood-based products to differ from blood substitutes, which generally refer to artificially produced products. Also, although many blood products have the effect of volume expansion, the group is usually distinguished from volume expanders, which generally refer to artificially produced substances and are thereby within the scope of *blood substitutes*
- The term cryosupernatant (also called cryo-poor plasma, cryoprecipitate depleted, cryoprecipitate reduced plasma) refers to plasma from which the cryoprecipitate has been removed. It is used to treat thrombocytopenic purpura.
- Fresh frozen plasma (FFP) is a blood product made from the liquid portion of whole blood. It is used to treat conditions in which there are low blood clotting factors (INR>1.5) or low levels of other blood proteins.[3][2] It may also be used as the replacement fluid in plasma exchange. Using ABO compatible plasma, while not required, may be recommended. Use as a volume expander is not recommended.[3] It is given by slow injection into a vein.
- Side effects include nausea and itchiness. Rarely there may be allergic reactions, blood clots, or infections. It is unclear if use during pregnancy or breastfeeding is safe for the baby. Greater care should be taken in people with protein S deficiency, IgA deficiency, or heart failure.[1] Fresh frozen plasma is made up of a complex mixture of water, proteins, carbohydrates, fats, and vitamins.When frozen it lasts about a year.

- Plasma first came into medical use during the Second World War. It is on the World Health Organization's List of Essential Medicines, the safest and most effective medicines needed in a health system. In the United Kingdom it costs about £30 per unit. A number of other versions also exist including plasma frozen within 24 hours after phlebotomy, cryoprecipitate reduced plasma, thawed plasma, and solvent detergent plasma
- Plasma frozen within 24 hours after phlebotomy, commonly called FP24,PF-24, or similar names, is a frozen human blood plasma product used in transfusion medicine. It differs from fresh-frozen plasma (FFP) in that it is frozen within 24 hours of blood collection, whereas FFP is frozen within 8 hours. The phrase "FFP" is sometimes used to refer to any frozen blood plasma product intended for transfusion.
- PF24 is stored, thawed, and infused with the same procedures used for FFP. Although it is technically a different product, most healthcare providers continue to refer to FFP when the actual component is PF24.
- Platelet transfusion, also known as platelet concentrate, is used to prevent or treat bleeding in people with either a low platelet count or poor platelet function. Often this occurs in people receiving cancer chemotherapy. Preventive transfusion is often done in those with platelet levels of less than 10×10^9/L. In those who are bleeding transfusion is usually carried out at less than 50×10^9/L. Blood group matching (ABO, RhD) is typically recommended before platelets are given. Unmatched platelets, however, are often used due to the unavailability of matched platelets. They are given by injection into a vein.
- Side effects can include allergic reactions such as anaphylaxis, infection, and lung injury. Bacterial infections are relatively more common with platelets as they are stored at warmer temperatures.[2] Platelets can be produced either from whole blood or by apheresis.[1] They keep for up to five to seven days.

- Platelet transfusions came into medical use in the 1950s and 1960s. It is on the World Health Organization's List of Essential Medicines, the safest and most effective medicines needed in a health system. In the United Kingdom it costs the NHS about £200 per unit. Some versions of platelets have had the white blood cells partially removed or been gamma irradiated which have specific benefits for certain populations.
- **Packed red blood cells**, also known as **packed cells**, are red blood cells that have been separated for blood transfusion. The packed cells are typically used in anemia that is either causing symptoms or when the hemoglobin is less than usually 70–80 g/L (7–8 g/dL).In adults, one unit brings up hemoglobin levels by about 10 g/L (1 g/dL). Repeated transfusions may be required in people receiving cancer chemotherapy or who have hemoglobin disorders.Cross matching is typically required before the blood is given.[1] It is given by injection into a vein.
- Side effects include allergic reactions such as anaphylaxis, red blood cell breakdown, infection, volume overload, and lung injury.With current preparation methods in the developed world the risk of viral infections such as hepatitis C and HIV/AIDS are less than one in a million. However, the risks of infection are higher in low income countries. Packed red blood cells are produced from whole blood or by apheresis. They typically last for three to six weeks.
- The widespread use of packed red blood cells began in the 1960s.It is on the World Health Organization's List of Essential Medicines, the safest and most effective medicines needed in a health system. In the United Kingdom they cost about £120 per unit.[11] A number of other versions also exist including whole blood, leukocyte reduced red blood cells, and washed red blood cells.
- **Cryoprecipitate**, also called **cryo** for short, is a frozen blood product prepared from blood plasma.[1] To create cryoprecipitate, fresh frozen plasma thawed at 1–6 °C, is then centrifuged and the precipitate is collected. The precipitate is resuspended in a small amount of residual plasma (generally 10–15 mL) and is then re-frozen for storage. It is

often transfused to adults as two 5-unit pools instead of as a single product. One of the most important constituents is factor VIII (also called antihaemophilic factor or AHF), which is why cryoprecipitate is sometimes called **cryoprecipitated antihaemophilic factor** or **cryoprecipitated AHF**. In many clinical contexts, use of whole cryoprecipitate has been replaced with use of clotting factor concentrates made therefrom (where available), but the whole form is still routinely stocked by many, if not most, hospital blood banks. Cryo can be stored at −18 °C or colder for 12 months from the original collection date.[2] After thawing, single units of cryo (or units pooled using a sterile method) can be stored at 20–24 °C for up to 6 hours. If units of cryo are pooled in an open system, they can only be held at 20–24 °C for up to 4 hours.[2] Presently cryo cannot be re-frozen for storage after it is thawed for use if it is not transfused.

- **Interleukins**

  Interleukins (ILs) are a group of cytokines (secreted proteins and signal molecules) that were first seen to be expressed by white blood cells (leukocytes). ILs can be divided into four major groups based on distinguishing structural features. However, their amino acid sequence similarity is rather weak (typically 15–25% identity). The human genome encodes more than 50 interleukins and related proteins.

  The function of the immune system depends in a large part on interleukins, and rare deficiencies of a number of them have been described, all featuring autoimmune diseases or immune deficiency. The majority of interleukins are synthesized by helper CD4 T lymphocytes, as well as through monocytes, macrophages, and endothelial cells. They promote the development and differentiation of T and B lymphocytes, and hematopoietic cells.

- Interleukin receptors on astrocytes in the hippocampus are also known to be involved in the development of spatial memories in mice

- **Interferons**

Interferons are a group of signaling proteins[2] made and released by host cells in response to the presence of several viruses. In a typical scenario, a virus-infected cell will release interferons causing nearby cells to heighten their anti-viral defenses.

IFNs belong to the large class of proteins known as cytokines, molecules used for communication between cells to trigger the protective defenses of the immune system that help eradicate pathogens. Interferons are named for their ability to "interfere" with viral replication by protecting cells from virus infections. IFNs also have various other functions: they activate immune cells, such as natural killer cells and macrophages; they increase host defenses by up-regulating antigen presentation by virtue of increasing the expression of major histocompatibility complex (MHC) antigens. Certain symptoms of infections, such as fever, muscle pain and "flu-like symptoms", are also caused by the production of IFNs and other cytokines.

More than twenty distinct IFN genes and proteins have been identified in animals, including humans. They are typically divided among three classes: Type I IFN, Type II IFN, and Type III IFN. IFNs belonging to all three classes are important for fighting viral infections and for the regulation of the immune system.

Industrial enzymes

Industrial enzymes are enzymes that are commercially used in a variety of industries such as pharmaceuticals, chemical production, biofuels, food & beverage, and consumer products. Due to advancements in recent years, biocatalysis through isolated enzymes is considered more economical than use of whole cells. Enzymes may be used as a unit operation within a process to generate a desired product, or may be the product of interest. Industrial biological catalysis through enzymes has experienced rapid growth in recent years due to their ability to operate at mild conditions, and exceptional chiral and positional specificity, things that traditional chemical processes lack. Isolated enzymes are typically used in hydrolytic and isomerization reactions. Whole cells are typically

used when a reaction requires a co-factor. Although co-factors may be generated in vitro, it is typically more cost-effective to use metabolically active cells.

Enzymes as a Unit Operation		
Enzyme	**Industry**	**Application**
Palatase	Food	Enhance cheese flavor
Lipozyme	Food	Interesterification of vegetable oil
Lipase	Pharmaceutical	Synthesis of chiral compounds
Lipopan	Food	Emulsifier
Cellulase	Biofuel	Class of enzymes that degrade cellulose to glucose monomers
Amylase	Food/biofuel	Class of enzymes that degrade starch to glucose monomers
Xylose isomerase	Food	High fructose corn syrup production
Resinase	Paper	Pitch control in paper processing
Penicillin amidase	Pharmaceutical	Synthetic antibiotic production
Amidase	Chemical	Class of enzymes used for non-proteinogenic enantiomerically pure amino acid production

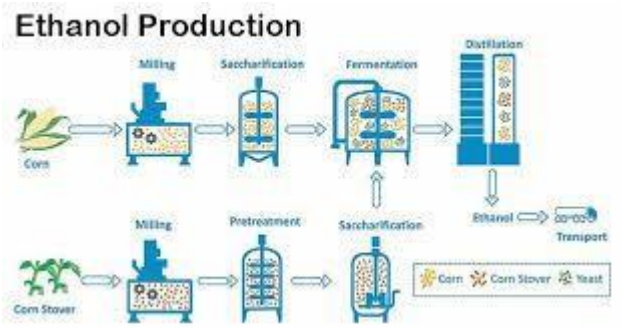
NON CATALYTIC INDUSTRIAL PROTEINS:

A variety of non-catalytic bulk proteins are produced in very significant quantities by the biotech sector. These proteins, which are largely derived from food sources and/or find application in the food sector, are the focus of this chapter. Such proteins are added to food either for obvious nutritional reasons or more commonly because they exhibit specific desirable functional properties. The chapter then focuses specifically on the biochemistry, industrial production and applications of the major milk proteins, namely: caseins, α-lactalbumin, β-lactoglobulin, serum albumin, immunoglobulin, lactophorin,

lactoferrin, and various enzymes (e.g. peroxidase, lysozyme). Animal-derived proteins are considered next in this chapter. A number of plant-derived proteins find actual or potential use in the food industry for functional purposes. Prominent among these are soy proteins, as well as rapeseed/canola proteins. Several proteins (and peptides) have been identified which, if tasted, are perceived as being intensely sweet.

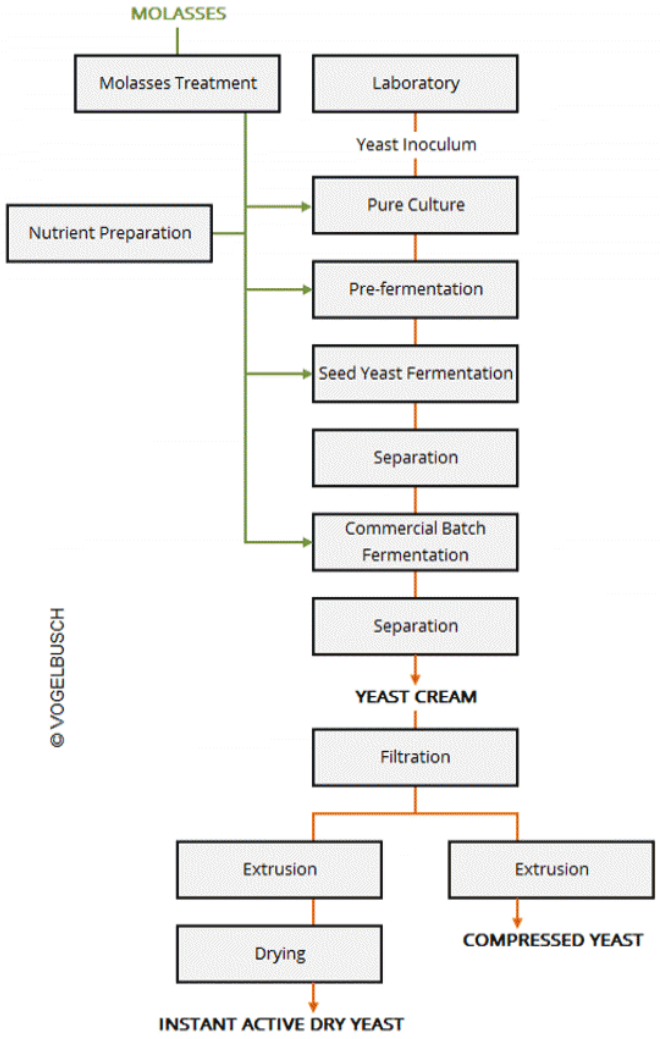
Bioethanol

Bioethanol is **produced** using familiar methods, such as fermentation, and it can be distributed using the same petrol forecourts and transportation systems as before. **Bioethanol Production**. **Ethanol** can be **produced** from biomass by the hydrolysis and sugar fermentation processes.



BAKERS YEAST

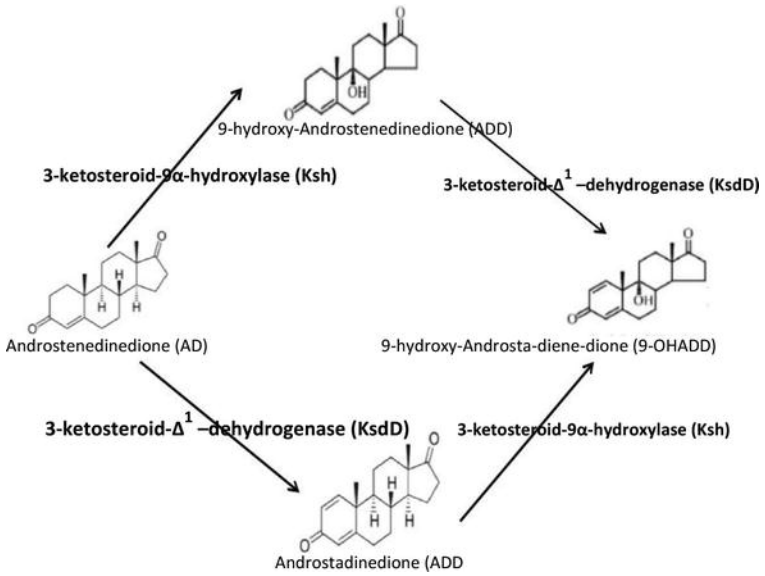
The first stage of yeast production consists of growing the yeast from the pure yeast culture in a series of fermentation vessels. The principal raw materials used in producing baker's yeast are the pure yeast culture and molasses. The yeast strain used in producing compressed yeast is Saccharomyces cerevisiae.



MICROBIAL STEROIDS

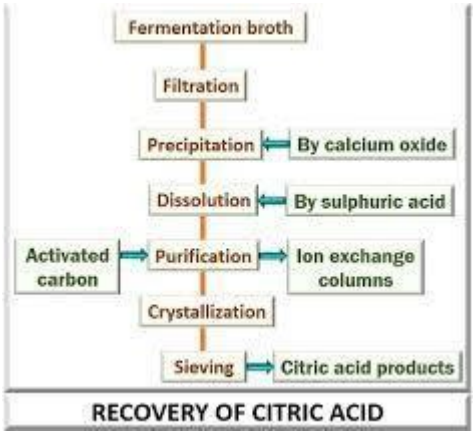
Steroids are terpenoid lipids of specific structure that contain the nucleus of four cycloalkane rings. Androstenedione (AD) is a natural steroid which belongs to the 17-ketosteroid family. It is produced by the adrenal cortex and gonads. In the body, cholesterol leads to the formation of steroidal hormones. AD is a compound specifically

used as a precursor for the majority of pharmaceutically active steroids such as testosterone, estradiol, ethinylestradiol, testolactone, progesterone, cortisone, cortisol, prednisone and prednisolone . The steroid pharmaceuticals are of great importance for their role in the management of human fertility, osteoporosis, menopause and blood pressure regulation . Commercially, steroid production represents one of the largest sectors of medical products manufactured by the pharmaceutical industry . Production of AD and androstadienedione (ADD) exceeds 1000 tons per year in the world. Therefore, production of AD on a large scale with cost effective process becomes an area of demanding research in biotechnology. Apart from the broadly used natural compounds, phytosterols gained an increasing importance as raw materials for the synthesis of steroidal drugs such as pregnenolone, boldenone, androstenedione and androstadienedione. The utilization of cholesterol and phytosterol as the sole carbon source by *Mycobacterium* sp. for growth and proliferation led to a serious development in microbial biotransformation processes for production of large number of steroidal compounds .



Citric acid

Citric acid is the most important organic **acid produced** in tonnage and is extensively used in food and pharmaceutical industries. It is **produced** mainly by submerged **fermentation** using Aspergillus niger or Candida sp. from different sources of carbohydrates, such as molasses and starch based media.



PENICILLIN PRODUCTION AND RECOVERY PROCESS

- INTRODUCTION

  ANTIBIOTICS: A substance's ability to kill or inhibit only the growing cells not matured cells.

  Penicillin is Produced by *Penicillium chrysogenum, Penicillium notatum, Some bacteria also can be produced.*

  Streptomyces griseus

  Arthrobacter sp

  Nocardia sp

  This is the first antibiotic, which was discovered by Alexander fleming (1928).

- Types:

  Penicillin V

  Penicillin G

  Ampicillin

  Amoxcillin

- **Antibiotics - Mode of action**

  Affecting cell wall - Penicillin

  Damaging cell membrane -Polymyxin

  Inhibiting Protein synthesis -Tetracycline

  Inhibiting Nucleic acid synthesis -Rifambin

  Blocking cell metaboloism – Sulfanilamide

  Narrow spectrum of antibiotics – kills only specific,Broad spectrum of antibiotics – kills all pathogens

- **MODE OF ACTION - PENICILLIN**

  Penicillin contains Beta – lactam Ring

  It inhibits cell wall synthesis

  Inhibits Transpeptidase enzyme involved in the cross linking of peptidoglycan.

  Production process

  Produced by Penicillum chrysogenum and P.notatum.

- Medium Components:

  Lactose 6%,molasses, soya meal,Nitrogen source(ammonium sulfate, acetate,lactate, ammonia gas,corn steep liquor may be used).minerals(phosphours,sulphur, mg,zn,fe,cu)

  pH – 6.5 ,Temp -26°C to28°CDuration –

  7 days

  An aerobic fermentation.

  2ºmetabolites,produced in the stationary phase.

- **Production process**

  The most important naturally occurring penicillin is Penicillin – G. Based on the precursors type of penicillin can be changed.

  Example: Phenyl acetic acid – Penicillin G,Phenoxyacetic acid – Penicillin V,

  Recovery Process

- USES

  Used in the treatment of throat infections,meningitis and syphilis.

  The side effects of penicillin are hypersensitivity reaction, skin rash,swelling.

XANTHAN GUM PRODUCTION AND RECOVERY PROCESS

- INTRODUCTION

- Xanthan gum is a type of exopolysaccharids. Produced by Xanthomonas campestris ,gram negative, Pseudomonas family.
- It contains 5 sugars. Glucose, mannose, gluconic acid with acetate and Pyruvate (gives viscosity).
- Mol.wt 2 – 15 *10^4 Daltons.
- It was first discovered by Allene rosalind jeanes in 1950 at northern regional centre and became commercial at 1964.
- The gum was approved as one of the common food additives in 1968 by FDA.
- Because of non toxic, highly water insoluble, resist to enzymatic digestion.

- **APPLICATIONS**

APPLICATIONS

- Xanthan gum is a food additives used to thicken, emulsify and stabilize water based foods.
- In foods like pudding, whipped cream and yogurts it works to improve their bonding and thickness.
- It is used in different types of foods including salad dressings, sauces, ice creams and other frozen foods.
- In many food products, xanthan gum prevents the water from segregating and settling at the top that is solid or semi-solid.

IN DRESSINGS

- Xanthan gum stabilizes acids and alkali.
- It works as an excellent stabilizer for oil dressings.
- Xanthan gum clings well to salads. Xanthan gum has been used to improve the flow ability in fungicides, herbicides and insecticides.
- Xanthan gum helps to control spray drift and cling which increase the contact time between pesticide and crop.
- Xanthan gum provides excellent texturing in ceiling tile coatings and paints.

- PRODUCTION OF
  L-LYSINE
- INTRODUCTION
- Amino acids are building block of proteins.
- It contains amino, carboxyl and R group.
- L Lysine is an essential amino acid.
- 146.19g mol wt.
- Water soluble

- It cannot be synthesized by the human body and should be taken from dietary sources such as soybean,fish,lentil,etc
- It is used as a supplement for the nutrients in the form of medicines
- Lysine is present in two forms namely

1.D-form of Lysine

2.L-form of Lysine

- IMPORTANCE OF L-LYSINE
- It is important for the growth of muscle.
- It is used to form carnitine, a substance found in most cells in our body.
- It also helps to transport the fat cells across the body
- It reduces the anxiety by blocking stress response receptors.
- It is responsible for the improvement of calcium absorption and retention.
- L Lysine
- Deficiency of lysine causes – hair loss
- Recommended doses is 3000mg/day for 6 months.
- Side effects:

Cramping , stomach pain,diarrhea, over doses can cause liver damage

- Types of process
- L-lysine can be synthesized by Two ways

- It can be synthesized by naturally or by fermentation.

NATURAL PROCESS:

- ❖ Natural process involves two pathways

1.Diaminopimelic Pathway

2.α-amino-adipate Pathway

FERMENTATION PROCESS:

- Molasses, Ammonia nitrate, other minerals, pH- 7.2, 35 – 37 °C, duration 100 hrs.
- RAW MATERIALS
- Carbon sources : Cane molasses
- Nitrogen sources : Corn steep liquor, Soybean meal
- Mineral and Salts :KH2Po4,K2HPo4,CaCO3
- Trace elements : Corn steep liquor
- Anti foaming agents :PEG-2000,Silicon based oil
- STEPS INVOLVED
- A gram positive bacterium called corynebacterium glutamicum is used for the production of L-lysine
- Media is formulated for the culture growth and inoculum is developed.
- Fermentation process is carried out in two modes namely Fed batch and Batch process.
- Optimum pH is 7.2
- Optimum Temperature is 35-37 °C
- Time Required is around 72 -100 hours in the production cycle
- After fermentation process , L-lysine is separated by ultra filtration
- Further the impurities are extracted from the mixture
- Purification is carried out by chromatographic techniques especially Ion Exchange chromatography.
- Further it is washed with aqueous ammonia solution.
- Purified product is then packed for commercial uses
- INOCULUM DEVELOPMENT

- FERMENTATION

 Culture media prepared is fed into the Fermentor.

 Fermentation is carried out under Aerobic conditions

 When glucose is exhausted, the batch fermentation ends and the fed batch fermentation starts.

 CULTURE + CARBOHYDRATE → AMINO ACID

SOURCE

- USES
- As a Food decorative
- As a Nutritional supplements
- For preventing and curing Cold sores
- For Cosmetics , Baby products , bath products , etc..
- As animal feed and fertilizers
- As intermediate to produce organic chemicals

- **Nucleotides**

Nucleotides are molecules consisting of a nucleoside and a phosphate group. They are the basic building blocks of DNA and RNA.They are organic molecules that serve as the monomer units for forming the nucleic acid polymers deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), both of which are essential biomolecules within all life-forms on Earth. Nucleotides are the building blocks of nucleic acids; they are composed of three sub unit molecules: a nitrogenous base (also known as nucleobase), a five-carbon sugar (ribose or deoxyribose), and at least one phosphate group. The four nitrogenous bases present in DNA are guanine, adenine, cytosine and thymine; in RNA uracil is used in place of thymine.Nucleotides also play a central role in metabolism at a fundamental, cellular level. They carry packets of chemical energy—in the form of the nucleoside triphosphates Adenosine triphosphate (ATP), Guanosine triphosphate (GTP), Cytidine triphosphate (CTP) and Uridine triphosphate (UTP)—throughout the cell to the many cellular functions that demand energy, which include: synthesizing amino

acids, proteins and cell membranes and parts, moving the cell and moving cell parts (both internally and intercellularly), dividing the cell, etc.[1] In addition, nucleotides participate in cell signaling (cyclic guanosine monophosphate or cGMP and cyclic adenosine monophosphate or cAMP), and are incorporated into important cofactors of enzymatic reactions (e.g. coenzyme A, FAD, FMN, NAD, and $NADP^+$).

In experimental biochemistry, nucleotides can be radiolabeled with radionuclides to yield radionucleotides. A nucleotide is composed of three distinctive chemical sub-units: a five-carbon sugar molecule, a nitrogenous base—which two together are called a nucleoside—and one phosphate group. With all three joined, a nucleotide is also termed a "nucleoside *mono*phosphate". The chemistry sources ACS Style Guide[2] and IUPAC Gold Book[3] prescribe that a nucleotide should contain only one phosphate group, but common usage in molecular biology textbooks often extends the definition to include molecules with two, or with three, phosphates.[1][4][5][6] Thus, the terms "nucleoside *di*phosphate" or "nucleoside *tri*phosphate" may also indicate nucleotides.

Nucleotides contain either a purine or a pyrimidine base—i.e., the nitrogenous base molecule, also known as a nucleobase—and are termed *ribo*nucleotides if the sugar is ribose, or *deoxyribo*nucleotides if the sugar is deoxyribose. Individual phosphate molecules repetitively connect the sugar-ring molecules in two adjacent nucleotide monomers, thereby connecting the nucleotide monomers of a nucleic acid end-to-end into a long chain. These chain-joins of sugar and phosphate molecules create a 'backbone' strand for a single- or double helix. In any one strand, the chemical orientation (directionality) of the chain-joins runs from the 5'-end to the 3'-end (*read*: 5 prime-end to 3 prime-end)—referring to the five carbon sites on sugar molecules in adjacent nucleotides. In a double helix, the two strands are oriented in opposite directions, which permits base pairing and complementarity between the base-pairs, all which is essential for replicating or transcribing the encoded information found in DNA.

Unlike in nucleic acid nucleotides, singular cyclic nucleotides are formed when the phosphate group is bound twice to the same sugar molecule, i.e., at the corners of the

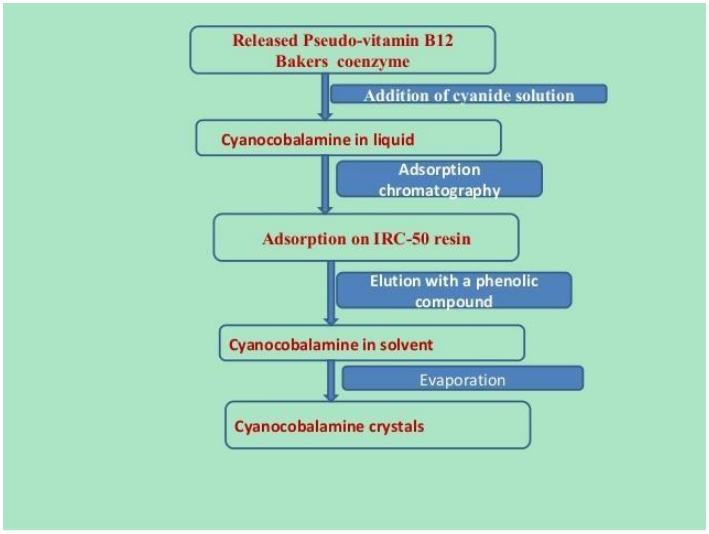
sugar hydroxyl groups.[1] These individual nucleotides function in cell metabolism rather than the nucleic acid structures of long-chain molecules.

Nucleic acids then are polymeric macromolecules assembled from nucleotides, the monomer-units of nucleic acids. The purine bases adenine and guanine and pyrimidine base cytosine occur in both DNA and RNA, while the pyrimidine bases thymine (in DNA) and uracil (in RNA) occur in just one. Adenine forms a base pair with thymine with two hydrogen bonds, while guanine pairs with cytosine with three hydrogen bonds.

- www.healthline.com

- **Vitamin B12**

Vitamin B12 also known as cobalamin, is an important water-soluble **vitamin** . It plays an essential role in the production of your red blood cells and DNA, as well as the proper functioning of your nervous system. **Vitamin B12** is naturally found in animal foods, including meats, fish, poultry, eggs and dairy.



Shikonin

Shikonin is a naturally occurring naphthoquinone found in the dried root of the plant Lithospermum erythrorhizon. **Shikonin** is the first natural colorant that has been commercially **produced** by a plant cell culture method to supplythe cosmetic industry in Japan since 1983