Separation Technologies

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LIST OF ABBREVIATIONS

AGMD	Air Gap Membrane Distillation
BLM	Bulk Liquid Membrane
CD	Catalytic Distillation
CE	Capillary Electrophoresis
CMC	Critical Micellar Concentration
CPE	Cloud Point Extraction
CZE	Capillary Zone Electrophoresis
DCMD	Direct Contact MD
DEHPA	Di(2-Ethylhexyl)Phosphoric Acid
ELM	Emulsion Liquid Membrane
EP	Electrophoresis
GC	Gas Chromatography
GST	Glutathione S-Transferase
HIC	Hydrophobic Interaction Chromatography
HPLC	High-Pressure Liquid Chromatography
IACs	Immunoaffinity Columns
IE	Ion-Exchange
ILs	Ionic Liquids
ITP	Isotachophoresis
L	Ligand
LC	Liquid Chromatography
LPME	Liquid-Phase Microextraction
MD	Membrane Distillation
ME	Micellar Extraction
MLC	Micellar Liquid Chromatography
MS	Mass Spectroscopy
NF	Nanofiltration

PAG	Polyacrylamide Gel
PAGE	Polyacrylamide Gel Electrophoresis
PE	Polyethylene
PTFE	Polytetrafluoroethylene
R	Receptor
RD	Reactive Distillation
RL	Ligand-Receptor
RO	Reverse Osmosis
RPLC	Reversed-Phase Liquid Chromatography
RT	Room-Temperature
SDBSA	Sodium Dodecylbenzene Sulfonic Acid
SDS	Sodium Dodecyl Sulfate
SDSA	Sodium Dodecanesulfonic Acid
SFC	Supercritical Fluid Chromatography
SFE	Supercritical Fluid Extraction
SHWC	Superheated Water Chromatography
SLM	Supported Liquid Membrane
SPME	Solid-Phase Microextraction
SWE	Subcritical Water Extraction
SWGMD	Sweeping Gas MD
TLC	Thin-Layer Chromatography
TOMAC	Tri Octyl Methylammonium Chloride
UF	Ultrafiltration
VMD	Vacuum MD

PREFACE

Separation processes hold a crucial place in most of the contemporary chemical, physicochemical, and pharmaceutical processes. The conventional separation processes include distillation, adsorption, absorption, filtration, chromatography, etc. These separation technologies have been in practice for decades, and their pertinent methodologies are being upgraded regularly. On the contrary, novel separation technologies such as membrane processes, supercritical fluid extraction, and membrane bioreactors processes are getting a reputation in modern plants. This book is predominantly designed to highlight these innovative separation technologies.

The imminent water crisis faced by humans by poor sanitation and water stress signifies one of the utmost challenges for this century. Therefore, it is essential to explore the alternatives for recycling water and preserving water. Various separation technologies have emerged as promising candidates to fight the water crisis. Although there are many books that discuss different separation technologies, but this book contains the latest and comprehensive overview of the contemporary separation techniques. Different chapters of the book provide a unique information that focuses on the rising importance of separation techniques.

The book contains eight chapters which are written in a reader-friendly manner. Each chapter starts with a general introduction of the topic followed by specific details about the topic. Chapter 1 focuses on providing the fundamentals of various separation techniques. Separation configurations and principles are also discussed in the chapter. Distillation is one of the essential separation processes with a wide variety of applications. Chapter 2 offers a detailed discussion about various types of distillation processes.

Chapter 3 discusses the comprehensive overview of chromatographic processes and their principles. A detailed overview of different types of chromatography is presented in the chapter. Solvent extraction processes have gained significant attention in the past few years due to their robust separation methodologies. Chapter 4 discusses the principles and applications of solvent extraction processes for separating different material components.

Most of the separation processes were carried out using mechanical techniques in the past. Presently, those mechanical techniques have been upgraded to meet the current standards of separation technologies. Chapter 5 briefly discusses the mechanical separation processes, which include sedimentation, centrifugation, filtration, and sieving. The separation techniques based on adsorption phenomenon have gained immense attention during the last few decades due to their excellent performance. Chapter 6 focuses on providing a detailed overview of different principles of adsorption separation techniques.

Chapter 7 presents a comprehensive information about electrophoretic separation processes. Different types of electrophoretic processes are discussed in the chapter. Today, the world is shifting towards efficient and green separation technologies for treating wastewater and chemicals. Membrane processes are suitable for providing the required results with reasonable economic costs. Chapter 8 introduces the readers with the fundamentals of membrane processes and their principles.

This book is intended for students (both graduate and undergraduate-level) of chemical engineering, membrane engineering, and environmental engineering. Moreover, the professionals associated with concerned with the pharmaceutical industry, chemical industry, and water industry can also benefit from this book.

—Author

Chapter

1 Introduction to Separation Technology and Processes

CONTENTS

1.1. INTRODUCTION

Biologically active molecules like antibodies, peptides, and proteins are finding more and more therapeutic use; this is increasing the industrial need for cost-efficient and rapid means of purification and separation. Examples of separation methods that are being used in both industrial scale and laboratory scale are crystallization, extraction, aqueous two-phase systems, filtration precipitation, and chromatography. A schematic illustration of separation processes that are typically used for water treatment is shown in Figure 1.1 (Guiochon and Beaver, 2011; Herrera-Herrera et al., 2012).

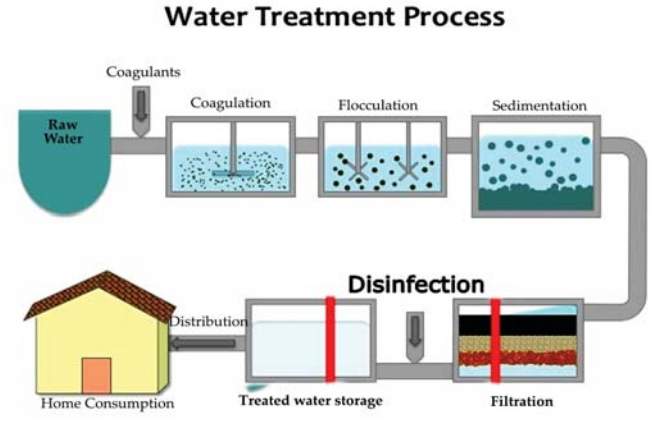

Figure 1.1. Water treatment process.

Source: https://www.onlinebiologynotes.com/steps-of-water-purification-process/.

We use separation processes for converting feed mixtures into products that may differ in composition. Diffusional separation processes are primarily dealing with unit operations that are best for feeds composed of homogeneous mixtures. Following processes are included in diffusional separation processes (Liu et al., 2008; Xiayan and Legido□Quigley, 2008):

- Evaporation;
- Liquid-liquid extraction;
- Sublimation;
- Process-scale chromatography;
- Absorption;
- Adsorption;

- Distillation;
- Biochemical separations;
- Membrane separations.

These are different from heterogeneous mixture's mechanical separation. As in them, the product phases already exist at a microscale. The technique of mechanical separation includes (Nash et al., 2008; Guihen, 2013):

- Filtration;
- Centrifuges, filtering;
- Sedimentation;
- Hydrocyclones;
- Air classifying;
- Dust separation;
- Centrifuges, sedimenting;
- Elutriation;
- Dense-medium separation;
- Electrostatic separation;
- Magnetic separation;
- Screening;
- Gravity concentration;
- Mineral sorting;
- Flotation.

For the fuel and chemical industries, more than 50% of the total equipment investment is constituted by the separation processes. They have applications in food and pharmaceutical industries, recovery of metals and in mineral ore's beneficiation, in processing effluents, and many other industries. There are different purposes for which separation processes are used, and we can classify them as (Eldridge, 1993; Baker, 2002):

1. **Purification:** It is a process in which impurities are removed, thereby obtaining a desirable substance having a higher purity level; e.g., treatment of drinking water and refining of sugar.
2. **Recovery or Concentration:** In this process, the concentration of any required substance is increased in solution. This is done mostly by removing a solvent's substantial fraction; e.g., fruit juice concentrates production and recovering metal values from effluents (Rushton et al., 2008).

3. **Fractionation:** In this process, the desired substance is separated from one another, e.g., chromatographic separations and crude oil's primary distillation.

1.2. DEFINITIONS

The basis of many separation processes depends upon a simple principle stating that at equilibrium, there are different compositions of different phases of matters. The examples include liquid and vapor states in distillation and non-miscible liquid states in liquid-liquid extraction. Separation is achieved when these states are allowed to move on towards equilibrium, therefore, named as equilibration processes (Han and Row, 2010).

Another class, called rate-administered processes, accomplishes separation by methods for contrasts in paces of transport of various species by a medium or hindrance. Therefore, the products are likely completely miscible with feed; the separation annihilates if they are remixed. Examples include ultrafiltration (UF) and vaporous dispersion (Mersmann et al., 2011).

To acquire separation, a separating agent is to be added, which tends to create a second phase in equilibration processes. States of energy or matter are taken by the separating agent. Energy separating agents includes reboiler heat that creates vapor when distilled and refrigeration causes the formation of ice during freeze concentration processes. Mass separating agents examples include ion-exchange (IE) resin that desalts water and absorbent for gas separation. Usually, separating agent is associated with the consumption costs of a separation process, i.e., the steam cost for driving the distillation column's reboiler or maybe, the cost of mass separating agent regeneration required for reuse. According to the restrictions of economics and environmental influences, it is advised to reuse or regenerate a mass separating agent. A second, successive separation process is usually required for a mass separating agent dependent separation process (Higashiyama and Asano, 1998; Wankat, 2006).

The level of separation accomplished between the two substances unconcerned items identifies with the division factor between them. For equilibration measures, the detachment factor a_{ij} is generally characterized as the proportion of separation coefficients (K_i and K_j) of two species between two stages at balance ($a_{ij} = K_i/K_j$). Constants K_i and K_j being the separation coefficients of species i and j, are characterized as the grouping of specific

species categories in stage 2 partitioned by that in stage 1. Any focus units might be utilized for Ki and Kj in one or the other stage, given similar units are utilized for the two species in a given stage. Stages 1 and 2 are typically picked so that aij is more noteworthy than solidarity (Cadotte et al., 1988).

1.3. PROCESS CONFIGURATIONS

Frequently, the species that are separated are separated to an unrequired extent by a basic equilibration or by solitary entry through a boundary. In such cases, the level of partition between species can be maximized by organizing or counter money.

Instances of each have appeared in Figure 1.2. By relocating the item starting with one reaching or stage then onto the next and properly reusing halfway items to past stages (Figure 1.2(A)), the level of detachment between species in definitive items can be improved impressively. For a distillation worked at all out reflux (microscopic feed and item flow), parathion factor for items from a solitary harmony stage is a_{ij}, as effectively noted, while that from N balance stages joined counter as of now is a^{N}_{ij} (the Fenske condition).

Persistent countercurrent contacting can be utilized to achieve a similar impact, as demonstrated in Figure 1.2(B). Two stages stream countercurrent to one another via an open material,

for example, an organized pressing, which makes an interface for the mass exchange between stages. The activity is similar to a countercurrent heat exchanger, according to which a hot stream's exit temperature can be lower than the cool stream's exit temperature. At times, contractors work with components of both discretely organized and ceaseless countercurrent reaching, for instance, turning disk contractors utilized for liquid-liquid extraction (Van de Witte et al., 1996; Geankoplis, 2003).

Another method of upgrading the level of separation feasible in a straightforward contact is the chromatographic mode where, portable stage streams along with a flimsy, fixed phase. The slimness of the fixed stage gives quick paces of mass exchange, giving the impact of numerous stages or "transfer units" per unit length of a fixed stage (Ravanchi et al., 2009).

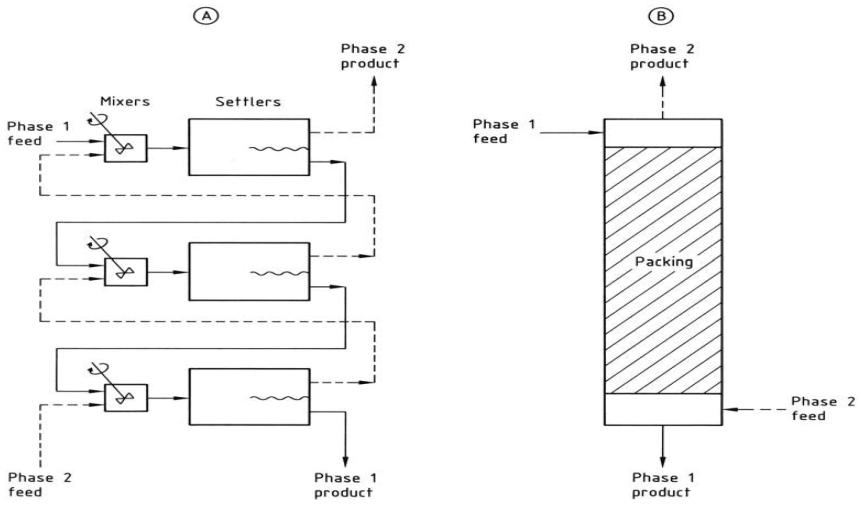

Figure 1.2. Counter currency and staging: (A) staged mixer-solvent extraction settler system; (B) continuous countercurrent extractor.

Source: https://onlinelibrary.wiley.com/doi/abs/10.1002/14356007.b03_01.

In elution chromatography, segments of blend infused as a heartbeat at the gulf end of the contactor continue along the fixed stage at various rates, controlled by diverse balance distributions among stages, and radiate as a progression of secluded pinnacles (Figure 1.2). Field-stream fractionations are rate-administered measures worked in a similar to, elution-chromatographic mode (Ravanchi et al., 2009).

A constant arranged or countercurrent contactor gives a high feed limit yet creates just two results of high virtue. (Moderate items, or side streams, of lower immaculateness, can likewise be available.) Elution-chromatography mode isolates an unpredictable combination into numerous unadulterated pinnacles or products, yet its innate limit is low on the grounds that the feed is infused irregularly as heartbeats (Bruschke, 1995).

At the point when a strong feed is utilized (e.g., draining of meal and ground coffee to shape coffee extricate; Liquid-Solid Extraction) or a solid mass the differentiating specialist is utilized (e.g., an adsorbent; Adsorption), development of the mass ought to for the most part be stayed away from in light of the fact that steady loss and unwanted blending may result. Fixed-bed measures are usually utilized (e.g., the home water conditioner loaded up with particle IE adhesive or bed of enacted alumina for drying of air) when a solid feed or mass differentiating specialist is utilized. A recovered

fixed-bed separation can deal with a significant feed rate however in its least complex structure gives just one profoundly cleaned item, the underlying gushing, before a subsequent segment leaves the bed. Among the prominent developments in the modern division during the previous few decades have been viable methods of reproducing counter money with fixed beds (Figure 1.3) (Humphrey, 1995; Seader et al., 1998).

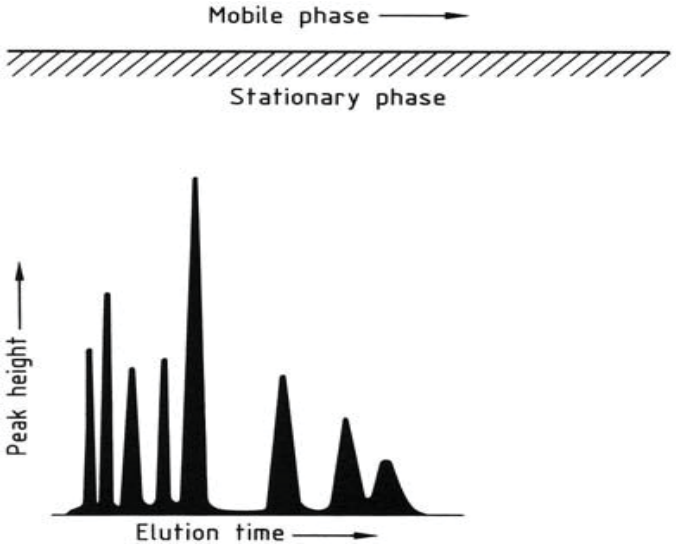

Figure 1.3. Typical output from a liquid or gas chromatography (sensed peak height by any of different methods used for detection, vs. elution time).

Source: https://onlinelibrary.wiley.com/doi/abs/10.1002/14356007.b03_01.

One more generally unique way to deal with partition includes the relocation of various species to harmonious positions inside a slope field. One model is isoelectric centering. These cycles are characteristically sluggish however can give partitions that are unrealistic by different methods (Magnussen et al., 1981).

1.4. SEPARATION PRINCIPLES

For a feed of a specific stage condition, an equilibration separation process is to be founded on the development of, or interaction with, any non-miscible second state of matter. For a fluid feed, the subsequent stage might be gaseous (stripping), a non-miscible fluid (extraction), or any solid (crystallization, adsorption). Equilibration might be with the heft of the subsequent stage or

with a surface. Surface-based separation measures incorporate adsorption, bubbling, foaming, and emulsion fractionation.

For rate-represented processes, contrasts in any type of transport can be abused. These incorporate paces of saturation through a solid membrane, Knudsen dispersion in a permeable medium (as utilized for detachment of uranium isotopes in UF_6), heat diffusion, electrophoresis (EP), or pressing factor dispersion (Sørensen et al., 1979).

Frequently, two separation standards utilized above can work synergistically. Examples include the utilization of cross fields for rate-represented separation and the improvement of relative unpredictability in refining by adding a substance that adjusts the harmony between stages, as done in azeotropic and extractive refining (Skiborowski et al., 2013).

1.5. DISADVANTAGES AND ADVANTAGES OF VARIOUS SEPARATION METHODS

At the point when similar division elements can be accomplished, equilibration measures utilizing energy isolating specialists will, in general, be less expensive than the ones utilizing a mass separating agent, on account of the requirement for coursing and recovering the mass separating agent or, in all likelihood discarding and supplanting it. Additionally, when organizing or counter money is required, equilibration measures will in general be more appealing financially than rate-represented cycles that give an identical partition factor since gear and energy can be used all the more productively in the previous (Hansen et al., 1991; Sirkar, 2014).

Separation measures including a solid stage can endure disadvantages related to low or vanishingly little mobile rates in the solid stage and with the allure of keeping the solid stage fixed. Some strategies for separation are more qualified for specific scopes of feed fixation. Specifically, fixed-bed activities are best for eliminating moderately weaken solutes, as the diluteness requires less bed volume and less regular recovery. Hence, adsorption, and particle trade processes will in general be utilized to recuperate solutes from moderately weaken takes care of or to eliminate debasements. Along with this range of feed focus, extraction (with fluids) and absorption (gasses) are normally considered for a center scope of fixation, on the grounds that the dissolvable stream rate required will, in general, be moderately autonomous of the feed solute focus. Operational maximum cutoff points on feed solute focus can emerge out of the need to keep fluid stages non-miscible in the

extraction or to stay away from too enormous a rate diminishing of the stream pace of the feed stage. Distillation functions admirably over a wide scope of feed fixations yet can encounter low stage efficiencies at extremely low solute focuses (Reid et al., 1987; de Haan et al., 2020).

Membrane separation gives high selectivity to eliminating or thinking (UF) high sub-atomic mass or macromolecular solutes, for eliminating water salts or focusing them (reverse osmosis (RO), electrodialysis), and for dividing solutes of high and low sub-atomic mass (dialysis). Prudent, high-limit films that viably eliminate polar natural solutes of lower sub-atomic mass presently can't seem to be grown, yet are a region of exploration. The solute tested through limits and selectivity given by the membranes are upgraded by impregnation of a membrane with fitting extractant (solid upheld fluid membrane, encouraged mobility). Membrane measures are most appropriate for low molar solute fixations, on the grounds that giving the main impetus to move across the layer is generally troublesome and costly (Kieffer, 1982; Prausnitz et al., 1998).

Price and Scaleup. Various strategies for division have intrinsically various expenses. A backward relationship will in general exist between the estimation of the item and the size of creation. For high-esteem substances created on a limited scale, a lot more extensive scope of separation strategies are to be considered than for substances of lower esteem. Regularly the expense or estimation of a substance is impacted firmly by the trouble of separation; accordingly, for a high-esteem substance, use of more up to date or more uncommon methods for division might be important to play out the ideal separation by any means. Besides, certain detachment measures (e.g., distillation, extraction) can be level up more promptly than others. The techniques that depend on exceptionally slight stages or thin stream channels (chromatography), laminar stream (field-stream cycles), or prepared dissemination of warmth (EP) are especially hard proportional up to enormous limits (Chernik, 1995; Lewis et al., 2013).

Thermosensitive product. Much of the time, the feed or items are delicate to warmth degradation, defilement, or changes because of an adjustment in the substance climate, for example, protein denaturation. These imperatives are especially regular in the food and drug ventures. Strategies for detachment that evade these issues will have a preferred position. To maintain a strategic distance from the heat, low temperatures and short home occasions ought to be utilized. Measures that try not to warm the feed (extraction, sorption, crystallization, and so forth) are profitable; if

vaporization should be utilized, activity under vacuum is useful. Worries about tainting and synthetic climate limit the sorts of mass separating agents that can be utilized. For example, in bioprocessing, numerous solvents have harmful impacts, so precipitation and the utilization of solid mass sorbents are moderately beneficial. Additionally, proteins may denature in the event that they are taken into a non-aqueous stage (Brunner et al., 1987; Chapman et al., 1988).

Reversible Chemical Reactions in Processes. Most modern divisions are completed by measures that don't include chemical reactions, in light of the utilization of reagents needed to achieve the response, the expense of reagents expected to recover the first wanted substance, and the need to discard undesirable response items. Nonetheless, chemical complexation or affiliation responses (benefactor-acceptor, chelation, clathrate, and so on) are substantially more promptly reversible and can also be utilized beneficially in divisions to expand the selectivity among solutes or the limit with regards to an ideal solute (Heric and Brewer, 1967). Reversible chemical communications can be executed in extraction; sorption; particle trade (ion exchange); azeotropic and extractive refining; impregnated membranes; and froth, bubble, and emulsion fractionation. At last, the regular cycles for gathering a solute in an arrangement by evacuation of dissolvable (vanishing, switch assimilation, UF, freeze focus) bring about the cost with respect to the measure of dissolvable that should be taken out. Extraction or sorption of the solute(s) of interest can prompt fixation also, in light of the fact that solitary a restricted measure of the dissolvable will go with the solute into or onto the extractant or sorbent. Reversible substance connections can be utilized viably here also (Bechta et al., 2008).

1.6. SELECTION OF A SEPARATION PROCESS

In looking for at least one proper strategies for separating a specific combination, the principal thought is the size of the separation factor liable to be accomplished by various techniques for separation. Contrasts in instability (distillation, evaporation, stripping), dissolvability (crystallization, extraction, assimilation), charge-to-mass proportion (particle trade, EP), atomic size and shape (adsorption with sub-atomic strainers, crystallization, gel saturation, clathrate, dialysis), and synthetic reactivity would all be able to be utilized for separations (Seidell, 1958; Fourie et al., 2008).

Then, regardless of whether conventional or outrageous conditions (extremely high or low pressing factor, exceptionally high or low

temperature, and so on) are expected to get appealing separation factors should be resolved. Techniques that require inordinate temperature-time mixes or bring about tainting or substance change might be precluded by the idea of the feed or item. Likewise, as effectively noticed, the estimation of the substance and the ideal size of operation can decide the number and kinds of choices to be thought of, as a result of the changing expense of various detachment strategies and their reasonableness for scaleup. At long last, a down to earth thought is the measure of past involvement in a specific interaction (Wisniak and Herskowitz, 1984; Pfohl et al., 1999).

Among close to rises to, distillation has a preferred position since it stays away from solids, it is not difficult to stage and level up, and an immense overabundance of involvement exists. Rate-represented cycles are normally viewed as genuinely just when the ideal separation can be accomplished in a solitary stage. The detachment of uranium isotopes by vaporous dispersion is a remarkable special case for this speculation, in any case. With measures using a mass isolating specialist, simplicity of recovery turns into a predominant factor. At last, as effectively noted, fixed-bed measures acquire a remunerating advantage when substances at generally low focus are to be taken out (Wisniak and Tamir, 1987).

1.7. FACTORS INFLUENCING PHASE EQUILIBRIA

A few general components impact equilibria and can be used in the rationale of choosing measures and picking mass separating agents. A summed-up equilibration separation interaction might be viewed as having a source (feed) stage and a second, getting stage. For the solute(s)of interest to move the ideal way, the substance capability of the solute in the getting stage should be not exactly that in the source stage (Schweitzer, 1988).

The partition coefficient K_i for solute between two mass stages is free of solute fixation when ideal arrangements exist in the two stages or, in many examples, when the solute is exceptionally weakened in the two stages. The partition coefficient is essentially the proportion of the action coefficient in the source stage to that in the acceptance stage, if the movement coefficients and the partition coefficient depend on similar concentration units (moles or mass per volume, weight division, and so forth) On the off chance that special cooperation happen among solute and dissolvable [defined as the major component(s)] in one or the other stage, the circulation of the segment toward that stage will be upgraded. Essentially, if less partiality exists between solute and dissolvable particles than between dissolvable atoms

themselves, the solute will be headed toward the other stage (Taft et al., 1985a, b). The estimations of K_i for some stage circulation measures are overwhelmed by the nonidealities in one of two stages. This is especially valid for measures that eliminate nonpolar or low-extremity solutes from a fluid arrangement in which the watery stage is profoundly nonideal (huge movement coefficients) and the getting stage is substantially more almost ideal. In this way, interactions among numerous natural mixtures depend on a unique set of chemical factors are related to polarity polarizability, and dispersion forces. In this way, interactions among numerous natural mixtures depend on a unique set of chemical factors are related to polarity polarizability, and dispersion forces. These interactions serve to associate different partitioning properties of fluids (Kamlet et al., 1985).

Dimerization or Polymerization of solute, or arrangement of solute particles into anionic micelles in one of the stages, serves to expand the circulation (i.e., increment K_i) of the solute in that stage at higher solute fixations (Dragoescu et al., 2013).

Another regular equilibrium highlight is the immersion of a stage. In adsorption measures, the limited measure of the mass surface region gives a maximum cutoff on the measure of solute that can be taken up inside the limit of the particular layer contiguous to the surface. The equivalent is valid for a gas-fluid surface limit in froth or air pocket fractionation. In these cases, the partition coefficient toward the surface stage diminishes as immersion is drawn nearer. This adds on as another motivation behind why surface-based separations measures are more valuable when the solute to be taken out is available in low fixation (Hirata et al., 1975; Knapp et al., 1982).

A comparable circumstance exists which includes chemical reactions (e.g., particle trade, reversible chemical complexation). The reactant in the accepting stage has a restricted limit, in that the stoichiometry of the hidden reaction(s) can't be surpassed. Accordingly, a dissolvable stage containing a responsive natural extractant of atomic mass 400 at 40 wt.% in a natural diluent can all things considered take up 5 wt.% of a solute of sub-atomic mass 50, with which the extractant structures a 1:1 complex. The complexation of extra solute atoms with a reactant particle induces extra take-up (Gmehling et al., 1977).

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Chapter

2 Fundamentals of Distillation

CONTENTS

2.1. INTRODUCTION

The distillation process is studied in this chapter. The significant substances will be enabled to be understood to the reader together with distillation calculations. The procedure of separation of two or more components within an overhead distillate and bottoms is known as distillation. The distillate may be a vapor or liquid or both, whereas the bottoms product is almost particularly liquid (Gryta, 2005, 2008).

The separation of liquid mixtures into their different parts is one of the primary operations in the process industries. For accomplishing separation, the most extensively used method is the distillation and is the crucial process in any crude oil process refinery. In an attempt to carry out the need for purer products, along with the desire for greater efficiency, continued research has been promoted by the refinery operators into the approach of distillation (Sommer et al., 1985). This chapter presents the fundamental principles, methods of distillation, equations, relationships, and calculation of the number of stages needed for both multi-component and binary systems. Fundamental issues of design are considered related to chemical, physical characteristics, and hydraulic phenomenon within the unit; including the column internal configurations. In equilibrium, the composition of the vapor with a liquid of given composition is determined on trial with the help of an equilibrium still (Figure 2.1) (Gryta and Tomaszewska, 1998).

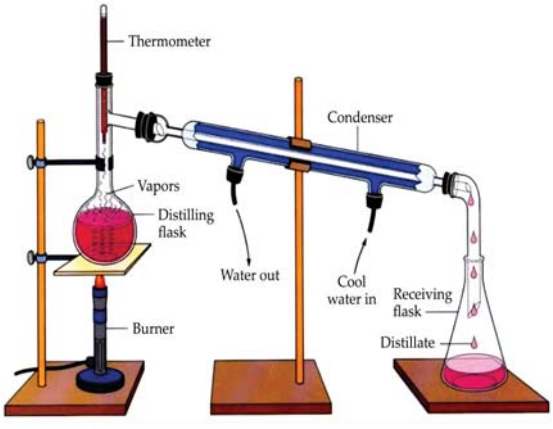

Figure 2.1. Conventional distillation process.

Source: https://www.worldofchemicals.com/605/chemistry-articles/distillation-process-of-chemicals.html.

The distillation, distillation types, applications, and process are studied in this chapter. The significant points about the distillation will be enabled to understand by the reader. Distillation is an extensively used procedure for separating mixtures depending on divergence in the conditions needed to change the state of elements of the mixture. There are various kinds of distillation, e.g., simple, steam, fractional, zone, and vacuum. In addition, the distillation method, which has a broad range of uses plays a significant part in science, industrial, and technology studies (Grethlein and Lynd, 1992; Gerbaud et al., 2019).

Distillation being a chemical method for separating the elements of a liquid mixture by accumulating and boiling condensed vapors. For the separation of mixtures of solutes or for the purification of compounds in solution, distillation is used by the chemists. For instance, there are different boiling points for different compounds. This characteristic implies that a more volatile compound will vaporize at a lower temperature as compared to a less volatile compound. In the chemical industry, for the separation of liquid mixtures, distillation must be well recognized; it gives the reason for approximate 95% of all liquid separations (El-Bourawi et al., 2006). Hither, there is a heat transfer within the bottom and top of the column and the large driving force for mass participating to an enormous energy loss. Under this plot, the economics and also socio-environmental welfare will be improved by any participation to minimize energy consumption using energy-efficient technologies (Lawson and Lloyd, 1997). Note that distillation is not a chemical reaction, however, it can be assumed as a physical separation process. In the typical distillation process, much enough energy is given to the reboiler, and much of the energy is rejected in the condenser. VRHP can maximize the energy level to satisfy the concern of high-grade energy. Due to the less input of external heat and higher thermodynamic efficiency, the energy expenditure of the VRHP process can be minimized significantly (Agreda and Partin, 1984). In large-scale chemicals manufacturing, separation operations elucidate about 50–70% of the energy used (Srisurichan et al., 2006).

2.2. DISTILLATION COLUMN

Three things are required by the separation process. (i) A second phase should be created so that both vapor and liquid phases exist and can approach each other on every phase within a separation column. (ii) The components have various volatilities so that they will subdivide within two phases to

a different range. (iii) Gravity or other mechanical means can separate two phases. As the second phase is formed using thermal means, therefore distillation differs from stripping and absorption (Figure 2.2) (Wood and Berry, 1973).

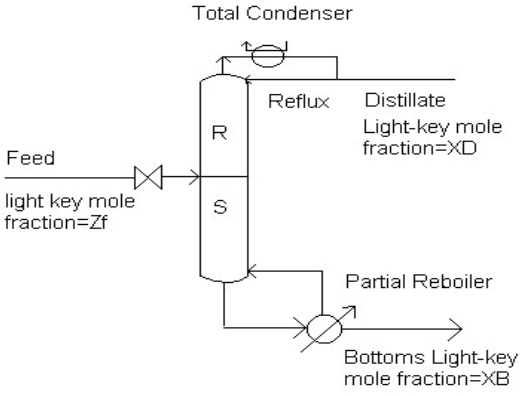

Figure 2.2. Flow diagram of a distillation column.

Source: https://www.pipingengineer.org/arrangement-of-distillation-tower-and-related-equipments/distillation-tower-pfd/.

The distillation column consists of two product streams and one feed stream. The feed comprises a mole percent of the light element, ZF. A composition of XD of the light element present in the product stream leaving the top. The product stream exiting the bottom comprises a composition of Xb of the light element. The column is divided into two sections. The top section is known to be the rectifying section. And the bottom section is referred to as the stripping section (Dhole and Linnhoff, 1993).

The top product stream penetrates a total condenser. This productively condenses all of the vapor distillate into liquid. A partial reboiler is used by the bottom product stream. This permits us for giving energy to our column (Agrawal, 2003).

2.2.1. Limitations

1. **Azeotropes:** A liquid mixture which when evaporates, forms a similar composition as the liquid is known as azeotropes. When the mixture is azeotropic, then highly advanced kinds of separation should be considered (Stephan et al., 1995; Al-Arfaj and Luyben, 2000).
2. **Solids:** If the material to be separated contains resins or tars that could foul or plug a continuous unit or is high in solids, then a batch separation must be considered.
3. **Optimum Pressure:** (Fitzmorris and Mah, 1980; Ogunnaike et al., 1983):

i. **Vacuum Operation:** Use of a vacuum must be advised for polymerizable materials or heat-sensitive compounds. The vacuum is normally not used except for requirements, e.g., a low bottoms temperature is required to bypass thermal decomposition.

ii. **The Distillation Column is Raised Atmospheric Pressure:** The column shell must be thicker to hold the pressure of the column.

iii. If the column pressure needed to achieve aloft condensation along with cooling water is below 250 lb/in^2, then in the overhead condenser, the column pressure must turn over an average temperature driving force of 5–15°C.

iv. If the column pressure needed to accomplish aloft condensation along with cooling water is above 250 lb/in^2, then assume an alternative of utilizing a refrigerant on the overhead and operating the column at minimum pressure.

4. Optimum temperature differences in condensers and reboilers (King, 1980):

i. Reboiler temperatures must be kept short enough to prevent fouling or/and bottoms degradation;

ii. Table 2.1 shows the prevalent temperature differences used for heat exchange across condensers and reboilers.

Table 2.1. Prevalent Temperature Differences Used for Heat Exchange Across Condensers and Reboilers

	Temperature (K)
Reboiler:	
Steam	10–60
Process fluid	10–20
Hot oil	20–60
Condenser:	
Refrigeration	3–10
Pressurized fluid	10–20
Cooling water	6–20
Air	20–50
Boiling water	20–40

2.2.2. Applicability

1. Distillation is the least costly method of separating mixtures of liquids;
2. If relative volatilities of components are below 1.1, then distillation becomes very costly (Douglas, 1988) and reactive or extraction distillation must be considered.

2.3. ROLE OF RAOULT'S LAW AND DALTON'S LAW

The expressions that correlate the vapor fugacity of a constituent in a solution to composition are called Raoult's law and Henry's law. Raoult's Law is not applicable to a non-ideal solution. Henry's law must be used only for the non-ideal solution (Cheng et al., 1992). Raoult's law considers that a component participates to the total vapor pressure of mixture in ratio to its vapor pressure if succinctly, or pure: partial pressure = mole fraction * vapor pressure, if pure; and its percentage of the mixture. The law will fail if the volatility of a component has relied on its percentage in the mixture, or if one constituent changes another constituent's vapor pressure (Figure 2.3) (Willis et al., 2020).

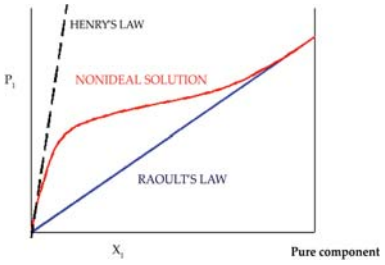

Figure 2.3. Demonstration of Henry's law and Rault's law.

Source: https://chem.libretexts.org/Bookshelves/Physical_and_Theoretical_Chemistry_Textbook_Maps/Supplemental_Modules_(Physical_and_Theoretical_Chemistry)/Physical_Properties_of_Matter/Solutions_and_Mixtures/Ideal_Solutions/Changes_In_Vapor_Pressure%2C_Raoult%27s_Law.

A linear equilibrium relationship is predicted by Henry's law. Yet, a lot of equilibrium relationships are literally non-linear. Henry's law can only be applied on a modest liquid concentration range, particularly on a dilute solution. For solvent purification, the simplest and most aged method is distillation and it is cheap too. It is formed on Raoult's law, which articulates that the partial vapor pressure of a solvent $\propto$ its mole fraction (Hu et al., 2003). There are complex principles engaged in the fractional distillation of liquid mixtures but can be visualized by assuming a system that roughly complies with Raoult's law. In many cases, systems diverge to a lesser or greater extent from Raoult's law, and vapor pressures may be less or greater as compared to the values calculated (Figure 2.4) (Parveen et al., 2009).

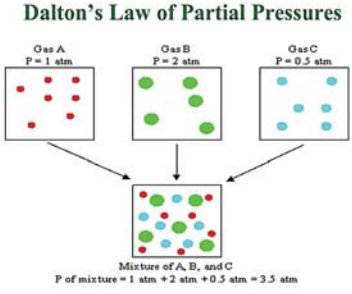

Figure 2.4. Demonstration of Dalton's law of mixtures.

Source: https://slideplayer.com/slide/1710580/.

2.4. WHY IS IT NOT POSSIBLE TO COMPLETELY PURIFY A MIXTURE BY DISTILLATION?

All the volatile constituents simmer at the boiling point of the mixture. The extent of a component in the resultant vapor has relied on its contribution to the total vapor pressure of the mixture. Therefore, the compounds with low partial pressures may be concentrated in the liquid, on the other side, the compounds having higher partial pressures may be concentrated in the vapors (Xu et al., 2014). As the component in the mixture cannot possess zero partial pressure, hence it is not possible to obtain a totally pure component from the mixture through distillation. In this case, when the goal is ultra-pure products, chemical separation should be practiced (Gruber et al., 1994). It is impossible to purify a mixture of compounds using distillation entirely; however, the samples of high purity can be acquired if one of the ingredients in the mixture has partial pressure close to zero. The following sections illustrate various kinds of distillation (Hernandez, 2008; Battisti et al., 2020).

2.5. IDEALIZED DISTILLATION MODEL

It is a general misinterpretation that at a given pressure in a liquid mixture, each element boils at the boiling point matching to the given pressure, and the vapors of each constituent will assemble purely and separately (Zhu et al., 2001; Linek et al., 2004). Withal, this does not happen, not even in an idealized system. Dalton's law and Raoult's law basically govern the idealized models of distillation considering that vapor-liquid equilibria are achieved. When the total vapor pressure is reached to the pressure around the liquid, boiling initiates, and liquid changes to gas during the whole of the bulk of the liquid. In the case of chemically identical liquids, e.g., toluene, and benzene, the idealized model is definite. In other situations, severe changes are noticed from Dalton's law and Raoult's law, most popularly in the mixture of water and ethanol (Burger et al., 2003; Huber et al., 2008).

2.6. SIMPLE DISTILLATION

One of the simplest distillation processes is simple distillation, in which the leading component to be purified is vaporized along with impurities at a high temperature, and the resultant vapor is moved to a container, where it condenses at a lower temperature (Doherty and Perkins, 1978, 1979). The purification effect is because of the difference in composition within

the vapor in equilibrium with it and the liquid mixture to be separated. Usually, the separation effect is evaluated theoretically through the Rayleigh equation. The appropriateness of this equation is restricted by the condition of the entire mixing of the liquid. If there is no mixing or the mixing is not complete, the component purification effectiveness is significantly affected by impurity diffusion in the liquid. Comparable to the Rayleigh equation, the presented diffusion model in a prior study favors one to evaluate the degree of purification of components (Van Dongen, 1983).

Following steps are involved in the distillation process: (i) vaporization, (ii) condensation, and (iii) movement of the vaporized component from the evaporation zone to the condensation zone. All three steps take place one after the other and are based on each other in a restricted vaporization-condensation system. The condensation and vaporization temperatures, condensation compartments, connecting tube, and the hydraulic resistances of the vaporization determine the evaporation rate in such a system.

2.7. FRACTIONAL DISTILLATION

A mixture of liquids is boiled in fractional distillation, and the rising vapors move up to a glass tube known as the "fractionating column" and separate. The fractionating column is situated in between the "Y" adaptor and the flask containing the mixture and is normally filled with plastic beads or glass, which makes better the separation within the liquids being distilled. Fractional distillation turns to an improved separation as compared to simple distillation because in the fractionating column, glass beads give 'theoretical plates' where the vapors can condense, re-evaporate, and again re-condense, actually distilling the mixture repeatedly (Pedersen et al., 2017). One vaporization-condensation cycle and one theoretical plate are equivalent, which is equal to one simple distillation. The highly volatile liquids will slowly transfer towards the peak of the fractionating column, whereas lower boiling liquids will remain at the bottom, providing an improved separation within the liquids. The vapor ultimately approaches the condenser, where it is cooled and then drops into the storage vessel. There are different methods of categorizing the valuable fractions that are distilled from crude oil. One common method is by distributing into three classes: heavy, middle, and light fractions. Heavier substances condense at greater temperatures and are taken out from the bottom of the column. Before the lighter fractions are cooled to their condensing temperature, they are capable to lift higher in the column, permitting them to be taken out at a little higher levels (Figure 2.5) (Kansha et al., 2010; Capunitan and Capareda, 2013).

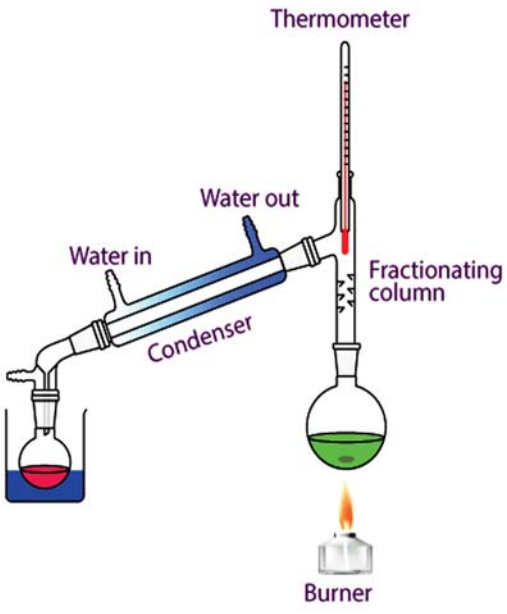

Figure 2.5. Demonstration of the fractional distillation process.

Source: https://byjus.com/chemistry/fractional-distillation/.

2.8. STEAM DISTILLATION

A process whose objective is to separate, purify, or isolate temperature-sensitive substances, i.e., natural aromatic compounds is known as steam distillation. Water or steam must be an add-on to the distillation apparatus, and the process might be operated at low temperatures using the characteristics of unmixable, immiscible liquids (down the components decomposition point). This method is operated through bubbling hot steam with a mixture that is seldomly heated to evaporate some of it. When the organic compounds are distilled, the vapor is condensed as they must be immiscible with each other. After simmering the immiscible liquid through steam to heat, the steam guarantees that the temperature of compounds does not surpass their boiling point, consequently the vapor that is condensed can be gathered (Markham, 1942; Cassel et al., 2009). There are two phases of the resulting fluid, i.e., water that is distilled and an organic compound. These compounds are separate because of the characteristics of non-mixing liquids and can be substantially separated using a separation funnel or by

draining. Generally, the method is used for steam stripping in petroleum refineries to detach organic compounds and to set apart essential oils from natural products (citrus oil from orange peel or lemon, eucalyptus oil from eucalyptus, etc.). The products that are manufactured through steam distillation are broadly used in cologne and perfume production besides some cooking materials (Figure 2.6) (Bremner and Keeney, 1965; Masango, 2005).

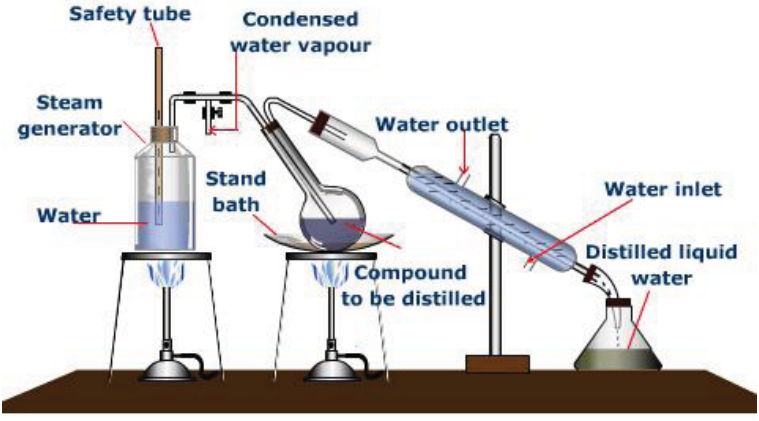

Figure 2.6. A typical system of a steam distillation procedure.

Source: http://ochemlabtechniques.blogspot.com/p/how-steam-distillation-works.html.

2.9. VACUUM DISTILLATION

The method used to isolate higher boiling fractions of crude oil is known as vacuum distillation. The fundamental theory and the process are corresponding to those used to isolate the lighter fractions from the atmospheric distillation process. The distinctness within the two physical separation techniques is that vacuum distillation happens at a quietly reduced pressure; on the other hand, atmospheric distillation happens under atmospheric pressure, hence minimizing the boiling point of a substance (Dankert et al., 1981; Taylor et al., 2010). Therefore, high boiling substances can be boiled at lower temperatures, without the cracking hazard. Vacuum towers are less in height as compared to atmospheric towers in order to reduce the pressure differential from top to bottom. Normally, the range of 50 to 100 mmHg pressure is used in vacuum distillation, despite that some lubricating oil

stocks might need even less pressure operating conditions (Luque et al., 2009). Usually, fractions revived from the vacuum distillation process are divided into the lubricating oil, asphalt, and gas oil. The consolidation of the vacuum and atmospheric distillation processes is a significant initial step in transforming crude oil into beneficial and economically useful products (Figure 2.7) (Yong et al., 2001; An et al., 2018).

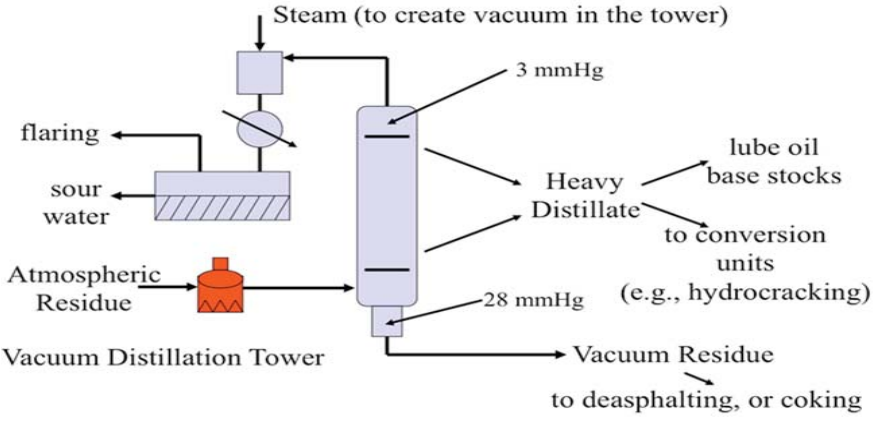

Figure 2.7. *Demonstration of a vacuum distillation model.*

Source: https://www.e-education.psu.edu/fsc432/content/atmospheric-and-vacuum-distillation-units.

2.10. AIR-SENSITIVE VACUUM DISTILLATION

This method is also valuable for compounds that boil at atmospheric pressure beyond their decomposition temperature and therefore by any attempt to boil them under atmospheric pressure, that would be broken down. Some compounds possess very high boiling points. To boil these sorts of compounds, it is generally good to lower the pressure at which these compounds are boiled rather than increasing the temperature (Malik et al., 2001; Liu and Girolami, 2020). After the pressure is kept lower from the vapor pressure of the compound (at a given temperature), boiling, and other distillation processes can begin. This approach is known as vacuum distillation and is generally found in the laboratory being the rotary evaporator. One of the most essential membrane distillation (MD) processes is air gap membrane distillation (AGMD) that is used for separating pure water from saline water. In AGMD configuration, a sluggish air gap is

proposed within the condensing surface and the membrane which serves as a thermal insulation layer to reduce the heat deficit through conduction over the membrane (Kremer and Helquist, 1984).

2.11. SHORT PATH DISTILLATION

For the separation of heat-sensitive and low-volatile substances, e.g., pharmaceutical intermediaries, high-vacuum oils, and vitamins, usually, short-path distillation is used as a suitable method (Yang et al., 2004). There are two major styles of distillation: the centrifugal and the falling-film style. For various thermally unstable organic components, special benefits are still shown by the centrifugal style because the residence time is merely on the order of a few tenths of that in a falling film and the liquid film in a spinning cone is too uniform, which efficiently minimizes the component decomposition (Oterhals et al., 2010; Tovar et al., 2010).

2.12. ZONE DISTILLATION

Zone distillation is a distillation method in a deep container with the condensation of vapor in the solid-state at condensate pulling in a cold area and partial melting of refined substance in a mobile liquid zone. This is a theoretically working process. The solid condensate with non-regular impurity distribution is producing when the zone heater is shifting from the top to the bottom of the container. Next, the much pure piece of the condensate may be obtained as the product (Potts and Thomas, 1992; Podrebarac et al., 1998). The process may be repeated again and again by shifting the received condensate to the bottom area of the container on the location of the refined substance (without turnover). The non-regular impurity distribution in the condensate (i.e., the efficiency of purification) grows with the number of iterations of the process. Zone distillation is a distillation related to zone recrystallization. Impurity distribution in the condensate is expressed by popular equations of zone recrystallization with different numbers of repetitions of the process-including replacement distribution efficient k of crystallization upon separation factor α of distillation (Hsu et al., 2019).

2.13. CATALYTIC DISTILLATION (CD)

Catalytic distillation (CD) is a “green” technology whose goal is to catalyze the reactants during being distilled continually. CD is improvising the field that associates catalysis, kinetics, and mass transfer to yield too selective and

energy-efficient process conveniently. This reactor technology permits us to separate products and reactants using a single distillation column or reactor (Gangadwala et al., 2004; Idris and Engell, 2012). The vital function is, increased the production of heterogeneous catalytic reactions (i.e., refining gasoline, etc.). The liquid reactants are catalyzed in the process during being heated within the CD column, and after that, the products straightforwardly initiate to vaporize and are isolated from the initial solution. The recently produced products boiled out of the system promptly by heating and catalyzing the reactants at the same prompt. According to Le Chatelier's principle, new products are produced from the reactants to take over from the removed products continually. In this case, the system cannot approach the equilibrium due to the loss of products by the system continuously (Figure 2.8) (Lange et al., 2007; Zhang et al., 2011).

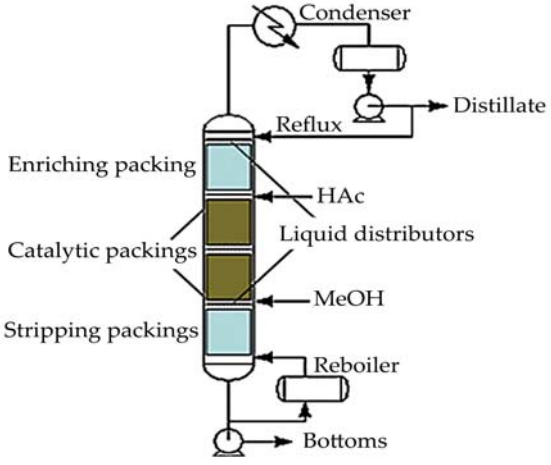

Figure 2.8. Flow-diagram of catalytic distillation.

Source: https://www.researchgate.net/figure/Catalytic-distillation-unit_fig2_292144111.

Due to an internal recycling system (Reflux), the reactants are usually more volatile as compared to the products. The reflux moves the concentrated vapor backward to the catalyst area, and additionally, gives a part of the condensed liquids to the column to ensure that just the products with minimum boiling points are occupied. The usages are hydration of olefins, esterification, alkylations, aldol condensation, hydrolysis, desulfurization, oligomerization of olefins, and hydrogenation. Many CD processes have a

connection with the petrochemical and petroleum industries (Hanika et al., 1999).

2.14. REACTIVE DISTILLATION (RD)

A process intensification method that allows concurrent separation to occur if a chemical reaction happens is known as reactive distillation (RD). RD provides advantages to equilibrium-limited reactions via pulling the chemical equilibrium to the whole transformation and permitting high selectivity (e.g., refraining potential consecutive reactions) because of the constant reduction of products from the RD column (Kiss et al., 2006). Among the rest, RD provides great benefits in cost minimization by simplifying complicated processes and integrating the separation functions and reaction into one unit with minimized needs. The energy requirement is reduced by the usage of the exothermic heat of reaction to stimulate the liquid vaporization. There are also safety, environmental, and health improvements chiefly due to fewer risks of runaway reactions, lower levels of the reactive hold-up, and low emissions from plants (Barbosa and Doherty, 1988).

The implementation of RD is very tempting to other reactions, including ternary systems ($A \rightleftharpoons B + C$ and $A + B \rightleftharpoons C$) and reversible quaternary systems ($A + B \rightleftharpoons C + D$). Some examples are dehydration of glycerol to aceto, diethyl carbonate synthesis using transesterification of ethanol and propylene carbonate, isoamyl acetate production through the esterification of acetic acid and isoamyl alcohol, and the hydration of cyclohexene to obtain cyclohexanol (Figure 2.9) (Noeres et al., 2003).

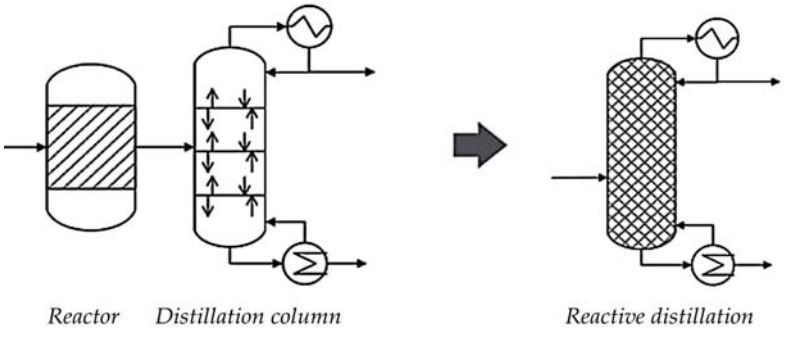

Figure 2.9. Illustration of reactive distillation.

Source: https://www.intechopen.com/books/distillation-modeling-simulation-and-optimization/reactive-distillation-modeling-simulation-and-optimization.

2.15. EXTRACTIVE DISTILLATION

Extractive distillation is one of the prominent distillation processes for the separation of low-relative-volatility mixtures and maximum- or minimum-boiling azeotropes. Different from azeotropic distillation, the entrainer is inputted at a different locale as compared to the primary mixture to be separated, breeding an extractive section inside the column (Luyben, 2008, 2013). For many years, it was supposed that only heavy entrainers were feasible, but light, intermediate, and heterogeneous entrainers are also good enough. Entrainer selection depends on the evaluation of uni-volatility curves and on the information of the residue curve map topology to study the volatility order of the mixture. In many situations, the feasible product is a saddle mark of the residue curve map. A common benchmark indicates that the least or the most volatile component is renewed from an indirect or a direct split-column configuration in the bottom stream or in the distillate, respectively. Continuous or batch operation is regulated by similar entrainer selection rules, however, the operating parameter value range for the reflux ratio and the entrainer-feed flow rate ratio is not the same. In addition, the batch process enables one to stir the motionless path by transforming the reflux policy while operating. Figure 2.10 shows the technique of extractive distillation (Van Dyk and Nieuwoudt, 2000).

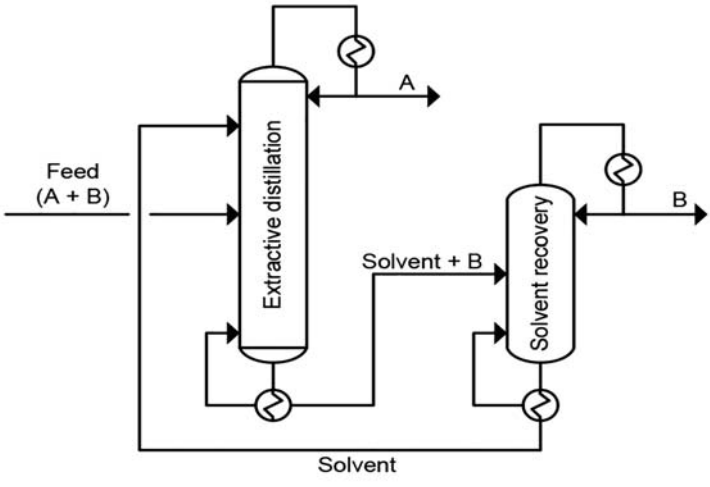

Figure 2.10. *System of the extractive distillation.*

Source: https://link.springer.com/chapter/10.1007/978-3-662-48520-0_2.

2.16. PERVAPORATION

Pervaporation is a membrane separation technology whose selectivity and performance are used for various applications. The applications regarding the end of pipe processes involve solvent recovery (dehydration of ethanol-water solution, etc.). This approach is vital for solvent dehydration due to the high selectivity of water with state of art membranes. Moreover, it is perfect to assess a comparably less amount of concentration range of water that has to flow from the solution. Therefore, it is adopted in the industry (Kober, 1917; Bowen et al., 2004).

2.17. FLASH EVAPORATION

The liquid will be over much heated and by that evaporated when it gets into the system with less saturation pressure comparable to that corresponding to its starting temperature. This event is known as the flash evaporation (Vane, 2005). Considering a strong evaporation manner, flash evaporation is escorted with state changes while absorption of heat. It has been applicable in many fields, including national defense, seawater desalination, aerospace, electronic industry, and health care. High-altitude and vacuum environments are the natural low-pressure environment. Due to this benefit, the aerospace field extensively used flash evaporation. Currently, aerospace technology progresses rapidly. Spacecrafts and aircrafts have vast applications and essential influence in military purposes, scientific exploration, and civil purposes (Junjie et al., 2010). They have turn into the research hotspot globally. The former one covers helicopters, supersonic bombers, and planes. The latter one includes man-made earth satellite, space shuttle, space probe, manned spacecraft, and so on. Usually, air vehicles give services in outer space or high altitude. These spaces are stiff environments with low pressure and low temperature. The high-speed frictions at running over the atmospheric layers and high temperature will be experienced by the air vehicles in these environments. The stable and safe running of air vehicles is influenced by these hostile environments significantly (Figure 2.11) (Saury et al., 2005; Cheng et al., 2016).

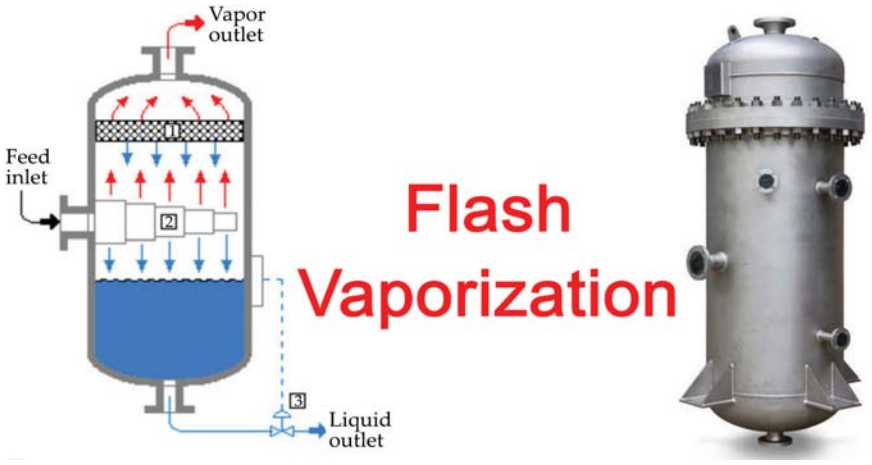

Figure 2.11. Flash evaporation column.

Source: https://chemicalengineeringworld.com/flash-vaporization/.

2.18. CO-DISTILLATION

A distillation that is operated on mixtures in which the two substances are not miscible is known as co-distillation. The Dean-Stark apparatus is utilized for this intention in the laboratory to pull out water from synthesis products. Another example is the Bleidner having two refluxing solvents (Fales et al., 1973; Kim et al., 1982).

2.19. MEMBRANE DISTILLATION (MD)

A separation process in which just vapors are permitted to penetrate a porous hydrophobic membrane is known as membrane distillation (MD). The separation is accomplished due to the vapor pressure differences within the membrane surfaces. There are so many pores of the membrane in which the pores cannot be misted from the feed solution. One side of the membrane contacts directly with hot saline water in the generation to produce the hot side. Another side of the membrane indirectly or directly get in contact with fresh, cold water to produce the cold side. The vapor pervading the membrane compresses on the cold side to form the distillate. Figure 2.12 shows a typical MD unit (Peng et al., 2004).

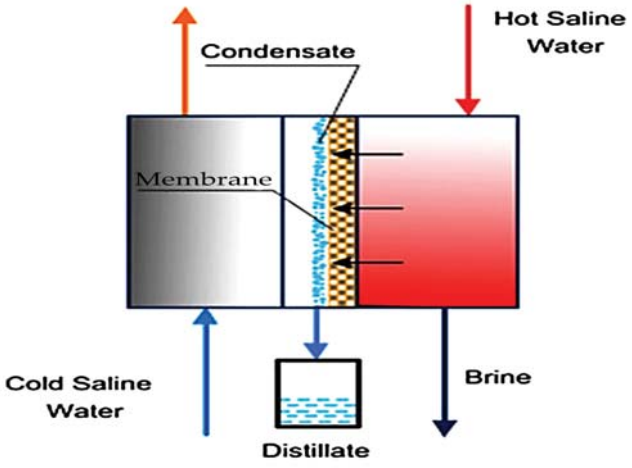

Figure 2.12. Membrane distillation process.

Source: https://wdrc.kaust.edu.sa/Pages/Pub-2016-DME.aspx.

There are various MD techniques. The four key techniques primarily vary by the way in which this channel has functioned or the composition of its distillate channel. These are vacuum MD (VMD), sweeping gas MD (SWGMD), and direct contact MD (DCMD).

2.20. EVAPORATION

2.20.1. Freeze Distillation

A process in which liquor is frozen and then obliged to melt, extracting extra water from it is known as freezing distillation or 'freezing concentration.' 'Distillation' is a little misnomer, as nothing is heated-it can be said rather the opposite. While this process, the concentration of the alcohol content and the liquor both are increased. In contrast with regular distillation, freezing distillation is assumed to be legal globally. The simplest technique is to pour the liquor into a container, e.g., a plastic jug, and let it freeze, either outdoors according to the weather where you live or in the freezer. This may commence up to a few days (but faster than aging), but it can speed up by freeze distilling in some smaller bottles (Lawson and Lloyd, 1997). When it's frozen, unfreeze it slowly and move it into another container. Clear once most of the residue in the jug is ice. Stir carefully and have a savor. The

gravity of the final product is hard to be estimated. It relies on the amount of water that is removed, which consequently relies on the degree of the freezer, etc. Still, if half of the amount of the liquor is lost, enough less than double of the initial alcohol percentage will be kept. Note that there's much enough to do with freeze distillation-the end gravity relies on the number of times freezing distillation is done as well as on the end temperature (El-Bourawi et al., 2006).

2.20.2. Rotary Evaporation

Flow pattern outside and inside a rotating cylinder with a specific known amount of the liquid is a subject that is largely described in the literature (Wang et al., 2012). The method of minimizing the volume of a solvent through distributing it being a thin film over the interior of a container at reduced pressure and increased temperature is known as rotary evaporation. There are mostly four major components of the rotary evaporators: rotor, heat bath, solvent trap, and condenser. In several laboratories, particularly in instances incorporating low boiling point solvents, rotary evaporation is a preferred method for solvent removal because of its speed and competence to manage large volumes of solvent. To cut tools, e.g., drill bits, etc., and to cool electrical motors, rotating cylindrical heat pipes are used. Nonetheless, the heat pipes are normally blocked at their end with a steady volume of liquor within them for operation (Lu et al., 2019).

2.20.3. Dry Distillation or Destructive Distillation

The second-generation biofuels have been constructed by the lignocellulosic resources, e.g., agricultural leftovers, energy crops, and wood that do not contend with the food industry, even though unprocessed feedstocks are grown on arable land. Third-generation biofuels cover those that are derived from algae biomass plus arable land is not necessary for their production (21). Increasing interest is developed for the thermo-chemical methods to change biomass to liquid fuels because of many reasons. These techniques are able of transforming biomass to a vast range of products covering gas, a varied range of liquor biofuels, and even biochar (the solid leftover), which has different applications, i.e., soil productivity improvement and bioremediation (Kumta et al., 1998; Ferhat et al., 2007).

Dry distillation refers to the solid heating technique to the solid material during blocking the air off. By applying this method, organic matter is breaking down to liquid or gas and eventually evaporates and some of such

liquid or gas remains as a solid substance. Additionally, thermo-chemical methods are somehow feedstock-agnostic, simply flexible to operate a range of moisture contents, and easy to conduct.

2.21. AZEOTROPIC DISTILLATION

Azeotropes are described as the mixtures of liquors that boil at a fixed temperature similar to pure liquid and have the alike composition of substances in liquid and also in vapor phase. Azeotropes are known as constant boiling mixtures as well because the whole of the azeotropes transforms into a vapor state at a fixed temperature, and their parts cannot be isolated by fractional distillation. With the decay of the global environment, circumstantial friendly ionic liquids (ILs) have gained much attention like solvents for segregating azeotropes (Laroche et al., 1991). A popular classical example of azeotropic distillation is its utilization in dehydrating water and ethanol mixtures. There are two types of azeotropes: maximum and minimum boiling azeotrope.

Maximum boiling azeotrope: There is a transitional composition for the solutions with negative deviations, for which there is the minimum vapor pressure of the solution, and therefore, the boiling point is increased. The solution distills at a fixed temperature at this composition without any change in composition. This kind of solution is known as a maximum boiling azeotrope.

Minimum boiling azeotrope: There is an in-between composition for the solutions with a positive deviation, for which there is the maximum vapor pressure of the solution, and thus, the boiling point is reduced. The solution distills at a fixed temperature without any variation in composition at this composition. This sort of solution is named minimum boiling azeotrope.

2.22. INDUSTRIAL DISTILLATION

Industrial distillation is usually carried out in huge, vertical cylindrical columns called distillation columns or distillation towers having heights ranging from around 6–90 meters or more and diameters ranging from around 65 cm to 16 m. If the process feed possesses a diverse composition like in distilling crude oil, then the liquor outlets on intervals above the column let the removal of various fractions or products with different boiling ranges or boiling points. The “heaviest” products (those having the highest boiling point) leave from the bottom of the column and are generally known as the

bottoms, and the "lightest" products (those having the lowest boiling point) leave from the lid of the columns (Figure 2.13) (Tyréus, 1979).

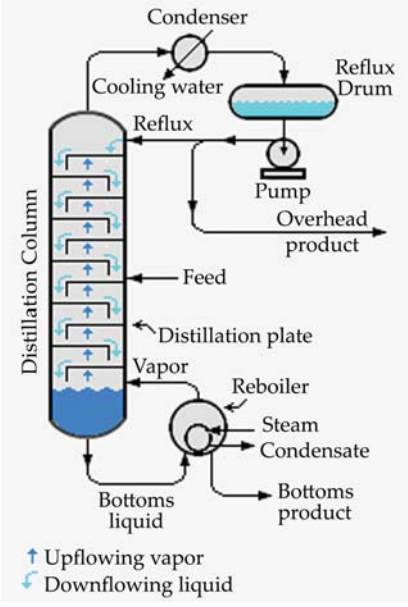

Figure 2.13. Illustration of a typical industrial distillation tower.

Source: https://en.wikipedia.org/wiki/Reflux.

2.23. APPLICATIONS

2.23.1. Scientific Uses

A maximum of around 16% of alcohol content can be produced by the concentration of Alcohol: Fermented grains, which is the upper limit of many wines and beers because the yeast organism utilized in fermentation cannot sustain in more concentrated alcoholic solutions. However, normally around 40% of the alcohol content by volume is used in spirits like gin, rum, and vodka. Distillation is the technique used to make hard liquors and to concentrate fermented solutions. Usually, in academic laboratories, a distillation of fermented grape juice is accomplished (Dhole and Linnhoff, 1993).

A leading problem from which today's world is suffering is freshwater, distilled water: approach to clean. In states adjacent to the ocean, sometimes

seawater desalination is used to serve the state with drinkable water. One of the vital techniques used to clean ocean water is distillation which works well since microorganisms, salt, and other substances of seawater are non-volatile. A lot of energy is required for the process of distilling water which is its major drawback. Economics can be a vital obstacle to using this method unless engineered creatively. For the distillation of seawater, the heat released from a power plant is used often to give the energy. The distilleries and coupled power plants are chiefly used by Israel and Saudi Arabia to get approximately half the freshwater required for their states.

2.23.2. Water Purification

A process whose objective is to purify the dirty water that involves dissolved and solved materials is known as water purification. Distillation aids to do this process. An ideal water purification system was discovered by scientists that take out two kinds of unwanted components from the water. In this method, a membrane is used by using a chemical process, physical barrier, biological process, or optical process. The purified water can be utilized for the applications of food processing, providing drinking water, constructions, swimming pool, industrial processes, and other water-based utilities (Camacho et al., 2013). Kinds of water filters cover screen filters, media filters, granular ambient filters, disk filters, rapid sand filters, slow sand filter beds, biological filters like algae scrubbers, and cloth filters. The granular ambient filters are categorized as pressure and gravity filters based on the driving force for water filtration. The prominent differences between both kinds of filters are the head required to flow water from the filtration rate, the ambient bed, and the type of container used to hold the filter medium.

2.23.3. Alcoholic Beverages

The fermentation by-products like methanol might be involved in diluted spirits or incorrectly distilled, which might be a reason for very severe or even life alarming health problems if taken excessively. While the formation process, the production of dangerous waste should be recommended by the use of natural ingredients, i.e., water, ethyl alcohol, and herbs in the beverage. Though, it was found that the waste encompasses heavy metals. The trash having a noteworthy concentration of metals, e.g., Fe, Cu, Zn, Ni, Cr, and Cd are assumed to be dangerous and need treatment before dumping.

The portion of the metal content is subjected to the flavoring herbs and combined usage of the metallic pot yet and another portion to the

manufacturing process. As compared to water, alcohol has a minimum boiling point, therefore, distillers can vaporize the alcohol (largely) by themselves, accumulate the vapors into a tube and use low temperatures to compel the alcohol to condense back into liquor. These standard techniques are easy and work with a low-cost apparatus, but they need full attention and are time-consuming, which turns into less throughput.

2.23.4. Petroleum Products

In the petroleum industry, distillation is the most broadly used separation method.

2.23.4.1. Petrol

Petrol is a liquid state fuel that is kept in the fuel injectors (or rather carburetor on earlier engines) and the fuel tank. It is nebulized along with air before being entered into the combustion chamber. Petroleum is a combination of organic compounds having just two components [hydrogen and carbon]. There is a natural leakage of oil to the earth from cracks in the rocks and fault lines, collecting to make asphalt, tar, and pitch ponds.

The percentage of evaporated fuel is measured by the distillation test on the increase of the temperature. A curve is obtained as the test result under the percentage of evaporated fuel vs standardized conditions of temperature (refer to Figure 2.14) where various portions of the curve allow us to analyze various behaviors of the product (Huss and Westerberg, 1996).

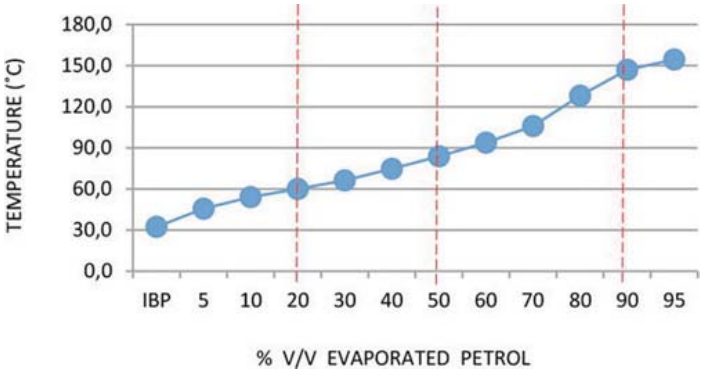

Figure 2.14. Distillation curve of petrol.

Source: https://www.researchgate.net/figure/Distillation-curve-of-petrol_fig1_318075312.

2.23.4.2. Diesel

Any fuel that can be utilized in a diesel engine is called diesel. Diesel is formed by fractional distillation within 392° and 662° Fahrenheit. Comparable to gasoline, there is a higher density of diesel, and it is easier to improve from crude oil. It has a general use in transportation.

2.23.4.3. Asphalt

For the making of roads, asphalt has a common use. It is a colloid of maltenes and asphaltenes that is isolated by the other elements of crude oil through fractional distillation. Asphalt is operated in a de-asphalting unit after collection and then undergoes a process named "blowing" where there is a reaction between them and oxygen to form it harden. Asphalt is normally stocked and carried at about 300° Fahrenheit.

2.23.5. Perfume

Manufacturing of perfume was one of the oldest usages of distilling, which initiated about 3500 B.C. The aroma from different herbs and plants is collected, known as essential oils, that can be drawn out from distillation. But, various aromatic plants are likely to decompose at high temperatures therefore, separation by usual distillation isn't realistic. In those cases, steam is pervaded through the plant material to extract the essential oils by not burning the mixture. After that, the steam is captured and then condensed as done in the usual distillation.

2.23.6. Food Flavorings

Flavorings are materials that improve or change the flavor of food and can be manufactured with distillation. They cover a range of human-made and natural compounds and can include a single chemical; however, they are usually complex mixtures of compounds. The classes of that are nature-identical flavoring substances (found by isolated or synthesis by chemical processes), natural flavoring substances (acquired from animal or plant raw materials), artificial flavoring substances (not recognized in a natural product expected for human consumption, and not the product is treated).

2.24. SUMMARY

A mature separation technology utterly is the distillation and will remain prevailing in the near future. Except for the requirement of high energy, a

cost-effective method for isolating a large amount of material toward high purity products is distillation. While distillation of a normal mixture, the key component having the lowest boiling point distills initially, pursued by compounds having higher boiling points. Distillation can also be applied to break down a solution formed of two or more fractions or liquids (miscible liquids). Moreover, there are various applications of distillation, particularly in the area of bio-engineering.

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Chapter

3

Chromatographic Separation Techniques

CONTENTS

3.1. INTRODUCTION

Chromatography is a significant biophysical technique that is utilized to isolate, identify, and purify the constituents of a mixture with the intention of qualitative and quantitative analysis. Purification of proteins can be done based on characteristics like shape and size, hydrophobic groups existing on the surface, total charge, as well as binding capacity with that of stationary phase (Cuatrecasas et al., 1968). Based on interaction type and molecular characteristics, four separation techniques use mechanisms of ion exchange, partition, surface adsorption, and size exclusion. However, the rest of the chromatography techniques are based on the stationary bed, including column, paper chromatography, and thin layer. Among these, the column chromatography technique is most commonly used for the purification of protein (Porath, 1997; Donald et al., 2006).

The working principle of Chromatography is based on the fact that the molecules in the mixture are applied onto the surface or into the solid, and the stable phase, i.e., fluid stationary phase, is separating from each other while moving with the help of a mobile phase. Factors affecting this separation process comprise partition (liquid-solid), molecular characteristics associated with adsorption (liquid-solid), and affinity or variations in their molecular weights. As a consequence of these differences, some constituents of the mixture stay for a long time in the stationary phase, and move slowly in the chromatography system, while the other constituents pass quickly into the mobile phase, and leave the system more rapidly (Gerberding and Byers, 1998; Harris, 2004).

Based on this methodology, the basis of the chromatography technique comprises three components (Laurence et al., 1989; Das and Dasgupta, 1998):

1. **Stationary Phase:** This phase includes a "solid" phase or "a liquid layer coated or adsorbed on the surface of a solid support."
2. **Mobile Phase:** This involves "liquid" or a "gaseous component."
3. Separated molecules.

The kind of interaction among stationary phase, mobile phase, and substances present in the mixture is the key component influencing the separation of molecules from each other. Chromatography methods established on the basis of partition are most effective on separation, and identification of small molecules such as carbohydrates, amino acids, and fatty acids. Nevertheless, affinity chromatographies (i.e., ion-exchange

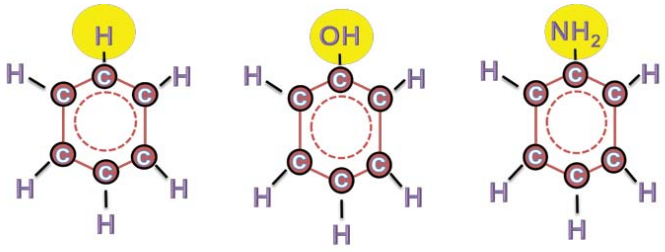
(IE) chromatography) are preferably more effective in the separation of macromolecules like proteins, and nucleic acids. Paper chromatography is utilized for the separation of proteins, and in studies associated with protein synthesis; gas-liquid chromatography is employed for the separation of Esther, alcohol, amino groups, and lipid, and observation of enzymatic interactions, whereas molecular-sieve chromatography is utilized particularly for evaluation of molecular weights of proteins. Agarose-gel chromatography is employed for the purification of viruses, RNA, and DNA particles (Karlsson et al., 1998; Amercham, 2002).

In chromatography, the stationary phase is a solid phase or a liquid phase adsorbed or coated on the surface of a solid phase. Whereas, the mobile phase that flows over the stationary phase is a liquid or gaseous phase. Depending upon the phases of the mobile phase, if the mobile phase is liquid, it is designated as liquid chromatography (LC), and if it is in the gaseous phase, it is termed gas chromatography (GC). GC is utilized for gases, mixtures of different volatile liquids, and solid material. On the other hand, LC is employed specifically for non-volatile and thermally unstable samples (Helmut, 1969; Walls et al., 2011). The purpose of utilizing chromatography as a method of quantitative analysis apart from its separation is to accomplish an adequate separation within an appropriate time interval. Numerous chromatography methods have been employed to that end. Some of them comprise column chromatography, paper chromatography, thin-layer chromatography (TLC), GC, gel permeation chromatography, high-pressure liquid chromatography (HPLC), IE chromatography, and affinity chromatography. Different types of chromatography are listed below (Wilchek and Chaiken, 2000; Determann, 2012):

- Column chromatography;
- Gel-permeation (molecular sieve) chromatography;
- Paper chromatography;
- Hydrophobic interaction chromatography (HIC);
- High-pressure liquid chromatography (HPLC);
- Affinity chromatography;
- Dye-ligand chromatography;
- Ion-exchange chromatography;
- Thin-layer chromatography;
- Gas chromatography;
- Pseudoaffinity chromatography.

3.2. PRINCIPLES OF CHROMATOGRAPHY

Various series of reactions involving intermediates are used to produce the molecules present in a biological system or synthetic chemistry. As deliberated in previous sections, a biological organism has a major fraction of desired product at any instant of time but has other compounds in minute quantities (Regnier, 1983; Firer, 2001). The insignificant species existing in a product are referred to as "impurities" and these compounds are essentially needed to be separated from the desired product for biotechnology applications. How two molecules can essentially be separated from one another? For a better understanding of the answer to this question, the following example of three molecules as given in Figure 3.1 is presented here. These three molecules (benzene, phenol, aniline) are similar to each other but have diverse physical and chemical properties that can be utilized as separation criteria (Sherman et al., 1991; Stoddard et al., 2007).



Name	Benzene	Phenol	Aniline
Molecular formula	C_6H_6	C_6H_6O	$C_6H_5NH_2$
Molar mass (g mol^{-1})	78.11	94.11	93.13
Density	0.8765 g cm^{-3}	1.07 g cm^{-3}	1.0217 g ml^{-1}
Melting point (°C)	5.5	40.5	-6.3
Boiling point (°C)	80.1	181.7	184.13

Figure 3.1. Chemical structure and physical properties of benzene, phenol, and aniline.

Source: https://nptel.ac.in/content/storage2/courses/102103044/pdf/mod5.pdf.

The physical and chemical properties which can be utilized for the separation of molecules are listed below (Glauner, 1988; Donald et al., 2006).

Physical properties include (Amicon, 1989a, b):

- Molecular weight;

- Freezing point;
- Solubility;
- Boiling point (in case both are in liquid phase);
- Density;
- Crystallization.

Chemical properties include (Cutler, 2004; Mahn and Asenjo, 2005):

- Functional Group, for instance, phenol has –OH group whereas aniline has NH_2 group.
- Reactivity toward other reagents to form a complex molecule.

In the current scenario, for instance, you have a mixture comprising compound 1 (benzene) and compound 3 (aniline) as shown above, and you want to purify compound 1 (benzene) instead of compound 3 (aniline). In this condition, you can consider the physical and chemical properties of compound 1(benzene) and isolate it from the mixture (Porath, 1992; Queiroz et al., 2001).

3.2.1. Principle of Separation

By what means a physical or chemical property will permit to separate a specific substance? The mixture of compound 1 (benzene) and compound 3 (aniline) is presented below in Figure 3.2 and assumes if we are considering one of the physical property, i.e., boiling point, as criteria to separate these compounds. By heating the mixture, two phases will exist, one will be in the liquid phase, and the other will be in the vapor phase. The molecules of compound 1 (benzene) and compound 3 (aniline) will be separated between these two liquid and vapor phases and with due course of time as the temp rises to the boiling point of compound 1 (benzene), more amount of compound 1 (benzene) will be accumulated in vapor phase than in liquid phase. While more compound 3 (aniline) will be in a liquid phase. Ultimately at the end of this process, both compounds will be completely separated from each other. The distribution coefficient (Kd) to define the distribution of compound 1 between two phases A and B is as follows (Marinetti, 1962; Okamoto and Yashima, 1998):

$$K_d = \frac{Concentration\ in\ Phase\ A}{Concentrationin\ Phase\ B}$$

Likewise one can also deliberate other physical and chemical parameters as well for separation. Depending upon the physical and chemical parameters, the molecules existing in the mixture will be distributed according to their behavior in each parameter (Hanahan et al., 1957; Rouser et al., 1970).

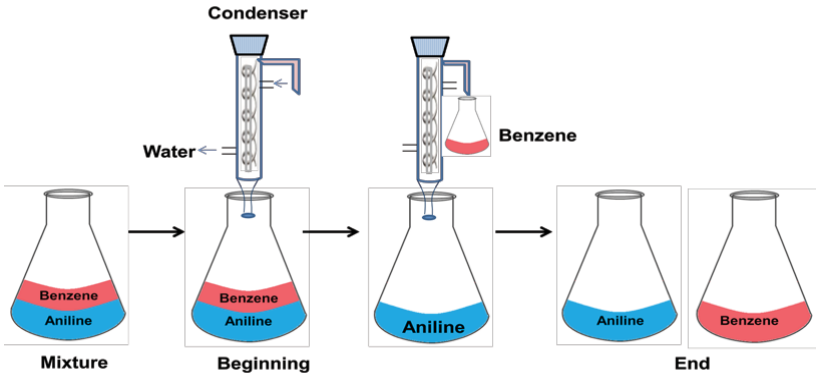


Figure 3.2. Distribution of molecules during distillation.

Source: https://nptel.ac.in/content/storage2/courses/102103044/pdf/mod5.pdf.

3.2.2. Chromatogram

Figure 3.3, shown below, presents the plot of elution volume along with the absorbance, which is termed a chromatogram. The volume or time taken for an analyte to emerge from the column is known as retention volume or time. The chromatogram may consist of separate peaks (A and B) as shown below or peaks (C and D) with overlapping bases, these peaks are designated as fused peaks (Piez et al., 1963; Chen et al., 2006).

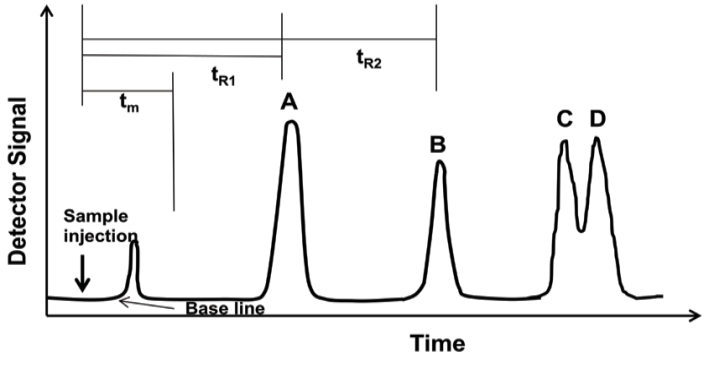


Figure 3.3. A typical chromatogram.

Source: https://xaktly.com/Chromatography.html.

3.2.3. Resolution

The capability of a chromatography column to separate two analyte peaks from one another is entitled as resolution. Resolution is defined as the ratio of the difference in retention time between two peaks and the average of the base of peak width. It is given by:

$Rs = \Delta tR / Wav$

When the value of resolution is 1, i.e., Rs = 1, the separation of two peaks is 97.7%, and therefore a column with Rs value more than 1.5 is considered good. The number of distribution events governs the capability of a column to isolate the two analytes. Therefore, the resolution is directly proportional to the number of distribution events. In column chromatography, each thin plain of the column matrix contributes to the distribution or separation of the molecule. Presume **H** as the height of a distribution plan and **L** as the length of a column, so the number (**N**) of distribution plain in a column is given by:

$N = L/H$

$N = 16 (t_R/W)^2$

$N = 5.54 (t_R/W_{1/2})^2$

Hence, the number of distribution plain in a column is regulating two parameters (Gu et al., 2012):

- As the number of distribution plain will rise, it will permit the analyte to travel for an extended period, as a result, the distance between two peaks will be increased; and
- When the number of distribution plain will go up, the width of the base of the peak will be reduced, consequently more sharp peaks will be obtained. An illustrative example showing how the number of distribution plain influences the base of the peak is shown in Figure 3.4. As the number of distribution is increased, the width of the peak is decreased. Therefore, the number of distribution provides an indirect approach to measure the column efficiency, and a higher N number is anticipated for better separation.

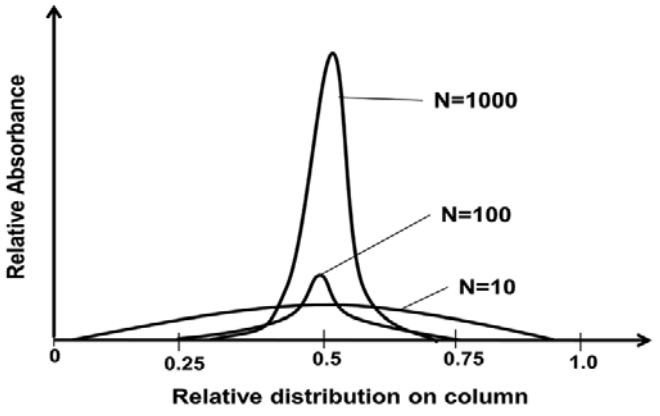


Figure 3.4. Relationship between number of distribution planes (N) and peak width.

Source: https://www.letstalkacademy.com/publication/read/CHROMATOGRAPHY.

3.2.4. Different Components of Chromatography System

The chromatography system comprises various components as illustrated in Figure 3.5. It has the following components:

1. **Reservoir:** One or two reservoirs for mobile phase (buffer).
2. **Pump:** One or two pumps to flow the buffer from the reservoir. Various types of pumps are utilized in chromatography systems, mostly based on the pressure level essentially required to perform chromatography. The selection of a pump is made based on prerequisite pressure to run the mobile phase. Depending upon the pressure level, LC can be categorized into the following types (Ismail and Nielsen, 2010):

i. **Low-Pressure Liquid Chromatography:** Required pressure limit is less than 5 Bar.

ii. **Medium Pressure Liquid Chromatography:** Works on intermediate pressure limit (6–50 bar).

iii. **High-Pressure Liquid Chromatography:** Pressure limit more

than 50–350 bar. A distinctive polysaccharide bead is not suitable to endure elevated pressure during HPLC. Henceforth, in HPLC silica-based beads are suggested. High pressure and reduced size of the silica beads give a higher number of theoretical plates. This provides HPLC superior resolving power to isolate complex biological samples.

3. **Mixer:** It is used to form a linear or step gradient of the buffer required to mix the buffer received from both pumps.
4. **Column:** It comprises glass or steel.
5. **Detector:** It is an online monitoring system to test the elution coming out from the column to investigate the presence of the analyte based on various properties. Different types of detectors are utilized in chromatography like UV-Visible detectors, etc.
6. **Fraction Collection:** Eluent is collected in different fractions by a fraction collector.
7. **Recorder:** The profile of eluent is plotted in the recorder about the measured property in a detector.

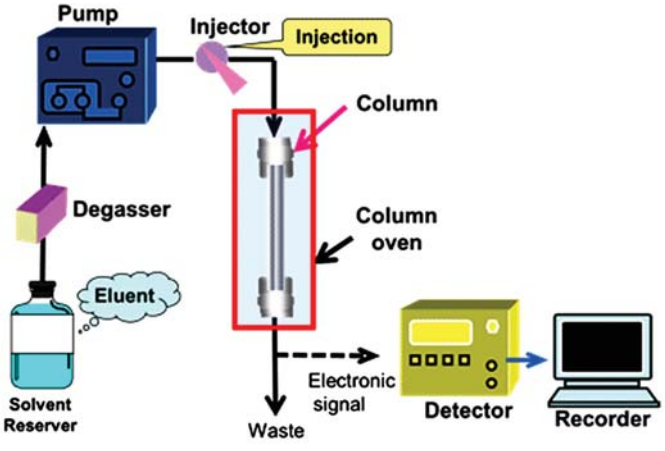


Figure 3.5. Different components of a chromatography system.

Source: https://www.galaklc.com/hplc-system-components/.

3.3. ION-EXCHANGE (IE) CHROMATOGRAPHY

IE chromatography is a resourceful chromatography technique with high resolution to effectively isolate protein from a complex mixture. Additionally, IE chromatography possesses a high loading capacity to handle large sample volumes with very simple chromatography operation (Lederer and Lederer, 1954; Kopaciewicz et al., 1983).

3.3.1. Principle

IE chromatography distributes the analyte molecule based on charge and their affinity to the oppositely charged matrix. Exchange of the analytes bound to the matrix takes place with a competitive counter ion to elute. Net charge, ionic strength, and pH of the buffer determine the interaction between matrix and analyte. For instance, when a positively charged matrix is loaded by a mixture of positively charged analyte (M, M^{+}, M^{-1}, M^{-2}), the neutral or positively charged analyte will not be bonded to the positively charged matrix. However, the negatively charged analyte will bind to the positively charged matrix as a result of relative charge and, therefore, a higher concentration of counterion is needed to elute from the positively charged matrix (Figure 3.6) (Straw, 1985; Knudsen et al., 2001).

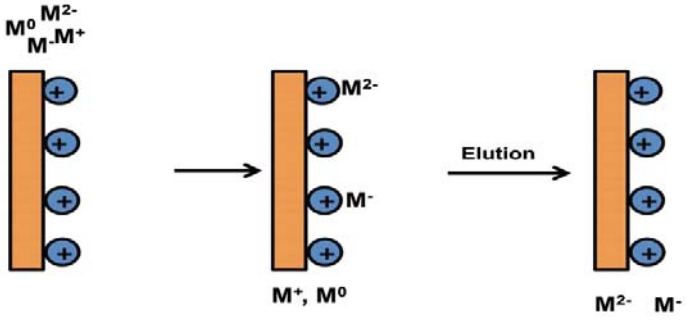


Figure 3.6. Illustration of affinity of analytes (M, M+, M–1, M–2) towards positively charged matrix.

Source: https://nptel.ac.in/content/storage2/courses/102103044/pdf/mod5.pdf.

The matrix utilized in IE chromatography exists in the ionized form with a reversibly bound ion to the matrix. Precipitation of ion present on matrix takes place during the reversible exchange process with the analyte. Resultantly, these processes give rise to two types of IE chromatography

(Zechmeister et al., 1943; Hamilton, 1963): cation exchange chromatography and anion exchange chromatography.

3.3.1.1. Cation Exchange Chromatography

In cation exchange chromatography, a negatively charged functional group is bound to the matrix with an affinity towards positively charged molecules of the analyte. The positively charged analyte binds to the matrix by replacing reversible bound cation. If the mobile phase has a strong cation (such as Na^+), elution of the analyte replaces the positively charged analyte bounded to the matrix (Curtright et al., 1999; Fekete et al., 2015).

3.3.1.2. Anion Exchange Chromatography

Contrary to cation exchange chromatography, in anion-exchange chromatography, the matrix possesses a reversibly bound positively charged functional group with an affinity towards negatively charged molecules of the analyte. The negatively charged analyte binds to the matrix by replacing the reversible bound anion. The existence of strong anion (such as Cl^-) in the mobile phase results in the replacement of matrix-bound negatively charged analyte with the elution of analyte. The most commonly used anion exchangers are shown in Table 3.1 and Figure 3.7 (Koohmaraie, 1990; Atassi et al., 1998).

Table 3.1. List of Selected Ion-Exchange Matrix

SL. No.	Name	Functional Group	Type of Ion Exchanger
1.	Sulphopropyl (SP)	$-OCH_2CH_2CH-_2SO_3H$	Cation Exchanger
2.	Carboxyl methyl (CM)	$-OCH_2COOH$	Cation Exchanger
3.	Sulphonate (S)	$-OCH_2SO_3H$	Cation Exchanger
4.	Quaternary amino-methyl (Q)	$-OCH_2N(CH_3)_3$	Anion Exchanger
5.	diethylaminoethyl (DEAE)	$-OCH_2CH_2NH(C_2H_5)_2$	Anion Exchanger

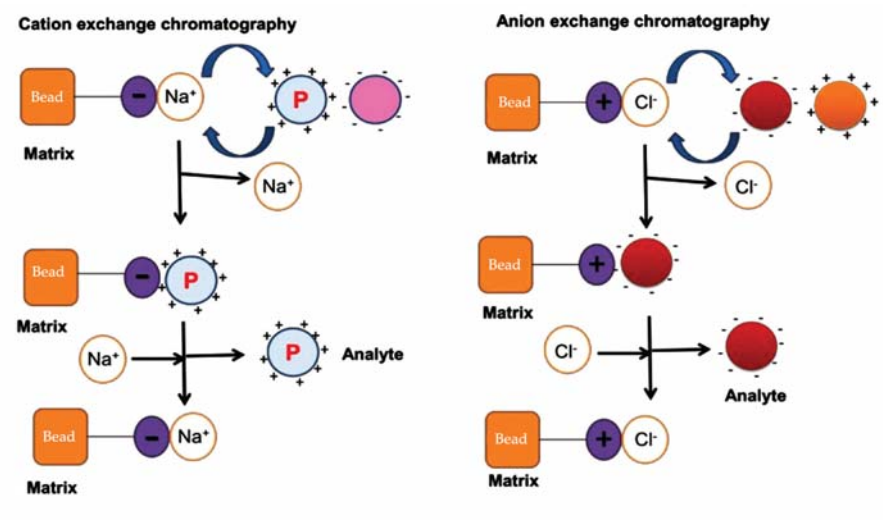


Figure 3.7. Cation and anion exchange chromatography.

Source: https://www.labome.com/method/Protein-Purification.html.

3.3.2. Isoelectric Point and Charge on a Protein

Protein is a polymer comprising amino acids with an ionizable side chain. These amino acid side chain ionizes differentially at a specific pH and gives rise to a net charge (positive/negative) to the protein. At a particular pH, if the net charge on a protein is zero, it is named as Isoelectric point (pI). Below the pI, the protein retains a net positive charge, whereas above the pI value it attains a net negative charge (Hanes and Isherwood, 1949; Pihlasalo et al., 2012).

3.3.3. Choice of an Ion-Exchange (IE) Column Matrix

Before purification and isolation of a substance, an appropriate IE chromatography selection is imperative. Multiple parameters can be considered to choose the right column matrix as explained below (Kantardjieff and Rupp, 2004).

3.3.3.1. pI Value and Net Charge

The pI value will assist in evaluating the net charge at a certain pH on a protein. As deliberated above, a cation exchange chromatography can be utilized below the pI value, and an anion exchange chromatography can be employed above the pI value (Gorbunoff, 1984; Shi et al., 2005).

3.3.3.2. Structural Stability

The 3-D structure of a protein is sustained by van der Waal and electrostatic interaction between charged amino acids, Π-Π interaction between the hydrophobic side chain of amino acids. Consequently, protein structure becomes stable in a narrow range around its pI and a large deviance from it possibly will disturb its 3-D structure (Audain et al., 2016).

3.3.3.3. Enzymatic Activity

Comparable to structural stability, enzymes are also active in a narrow range of pH, and this narrow range could help select an IE chromatography (Figure 3.8) (Cummins et al., 2011).

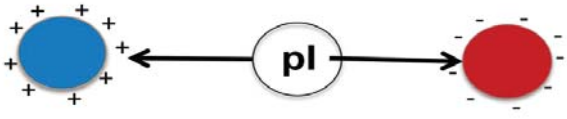


Figure 3.8. Change of charge with respect to the pI.

Source: https://nptel.ac.in/content/storage2/courses/102103044/pdf/mod5.pdf.

3.3.4. Operation of the Technique

The prerequisites include several parameters to be considered to execute IE chromatography (Fang et al., 1984).

3.3.4.1. Column Material and Stationary Phase

To avoid the destruction of biological samples, column material has to be chemically inert. It should permit a free flow of liquid with minimal clogging. Column material should be capable enough to endure the back pressure and avoid compression or expansion during the operation (Arvidsson et al., 2002).

3.3.4.2. Mobile Phase

The property of the mobile phase is significantly influenced by these critical parameters like ionic strength and pH.

3.3.4.3. Sample Preparation

Sample preparation is carried out in the mobile phase and to avoid clogging of the column, it must not contain any suspended particle. Injecting the sample with the help of a syringe is the most appropriate method to apply sample (Farnan and Moreno, 2009).

3.3.4.4. Elution

Elution of analyte from the IE column is carried out in many ways: (1) step-wise gradient; (2) isocratic elution (3); displacement chromatography; (4) affinity elution; and (5) continuous gradient either by salt or pH.

3.3.4.5. Column Regeneration

After the elution of the analyte, the IE chromatography column entails a regeneration step to use next time. A salt solution with an ionic strength of 2 M is used to wash the column to eliminate all non-specifically bound analytes and further to keep all functional groups in an ionized form to bind the fresh analyte (Figure 3.9) (Martin and Synge, 1941; Mallat and Barceló, 1998).

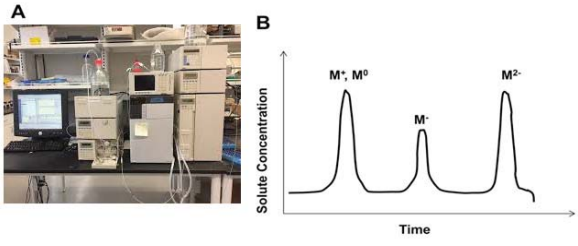


Figure 3.9. Operation of the ion-exchange chromatography. (A) Chromatography system to perform gradient elution of analytes to give an; (B) elution profile.

Source: https://www.researchgate.net/figure/17-The-overall-layout-of-the-HPLC-machine_fig8_339139441.

3.3.5. Applications of Ion-Exchange (IE) Chromatography

3.3.5.1. Protein Purification

Ion-exchange chromatography is extensively used in protein purification. The purification of proteins using Ion-exchange chromatography technique is explained in Section 3.3.

3.3.5.2. Protein-DNA Interaction

Interaction between DNA and a particular protein is studied using an IE column. Negatively charged DNA has a strong affinity towards anion exchange chromatography.

To illustrate the steps involved in DNA-protein interaction, a schematic diagram is shown below (Figure 3.10). In this process, DNA incubates the anion exchange matrix and is permitted to bind tightly. Surplus DNA has washed away from the column. Later, pure protein is passed through the tightly bound beads of DNA, trailed by washing using a buffer to eradicate unbounded proteins.

Moreover, the DNA is eluted from the matrix either with denaturation conditions or by the addition of high salt concentration (Ishii et al., 1977). Now to detect the presence of DNA and protein. Now, these fractions are tested. Eluted protein analysis is carried out in the SDS-PAGE and that of DNA is done in agarose.

As a control, protein without DNA is also added to the matrix to prevent the probability of binding of protein directly to the matrix (Jermyn and Isherwood, 1949). If protein shows an affinity towards DNA, they both will emerge from the column at the same time and should provide the identical pattern in the elution profile. High salt may break the interaction between DNA and protein. In these circumstances, protein emerges first, followed by DNA. Moreover, this IE chromatography approach is yet to be able to answer whether the DNA-protein is interacting or not (Arvidsson et al., 2002).

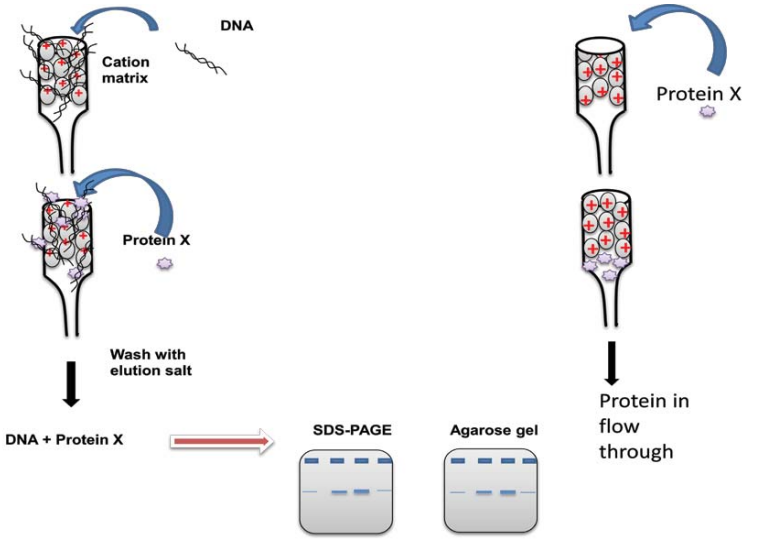


Figure 3.10. DNA-protein interaction.

Source: https://www.letstalkacademy.com/publication/read/CHROMATOGRAPHY.

3.3.5.3. Softening of Water

Groundwater contains several metals such as Mg^{2+}, Ca^{2+}, and other cationic metals. Because of the presence of these metals, this hard water generates problems in industrial settings. IE chromatography is employed to eradicate these metals present in the water through an exchange of matrix-bound Na^{+}. Resultantly, magnesium or calcium present in the hard water show more affinity towards the matrix, and it is replaced with matrix-bound sodium ions converting it to soft water. The schematic representation of water softening is shown in Figure 3.11. A cation exchanger matrix containing bounded sodium is packed in the column and the hard water comprising calcium and magnesium is passed through the column. During this process, the calcium ion present in the solution particularly migrates towards the matrix, whereas sodium ions present on the matrix are transferred to the solution. The matrix can be used to convert hard water into soft water as far as it contains bound sodium ions. When sodium ions are drained, regeneration of the matrix can be done by flowing sodium chloride or sodium hydroxide solution. The calcium/magnesium bound to the matrix emerges in the solution and can easily be dumped into the sewage (Figure 3.12) (Partridge, 1946).

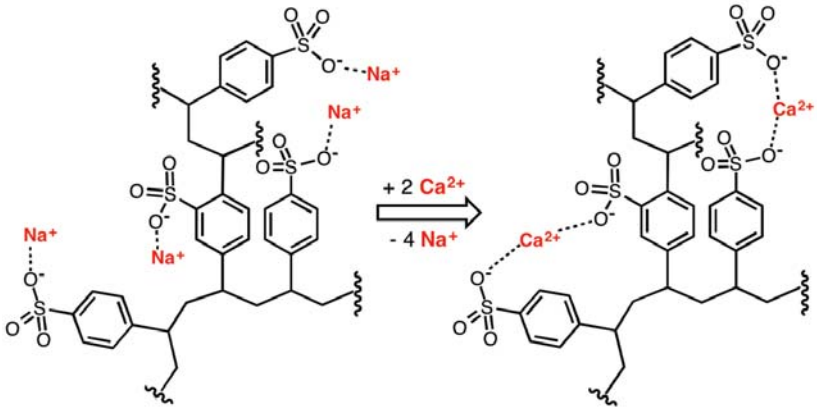


Figure 3.11. Mechanism of metal exchange during water softening.

Source: https://en.wikipedia.org/wiki/Water_softening.

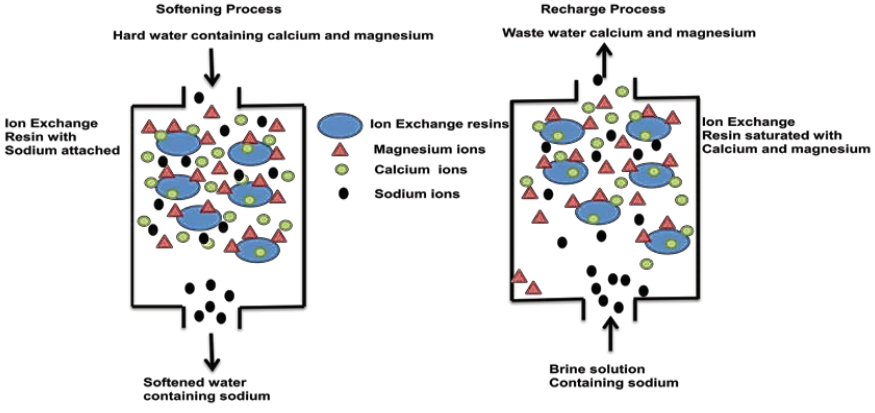


Figure 3.12. Softening of water by a cation exchanger matrix column.

Source: https://www.letstalkacademy.com/publication/read/CHROMATOGRAPHY.

3.3.5.4. Protein Kinase Assay

Phosphate groups are transferred on the substrate molecule by a class of enzymes called Protein kinase. A radioactive substrate (preferential radioactivity on carbon) was incubated with the enzyme protein kinase, non-radioactive ATP, and $MgCl_2$ in the protein kinase assay. A negative

control is also been incorporated where enzyme protein kinase is lacking from the assay mixture. The reaction mixture will be loaded from negative control and experimental on two separate cation exchange chromatography columns to bind unphosphorylated substrate from the reaction mixture, whereas phosphorylated radioactive substrate exists in the flow-through. A liquid scintillation reagent was used to measure the radioactive count of the flow-through (Figure 3.13) (Searle et al., 2018).

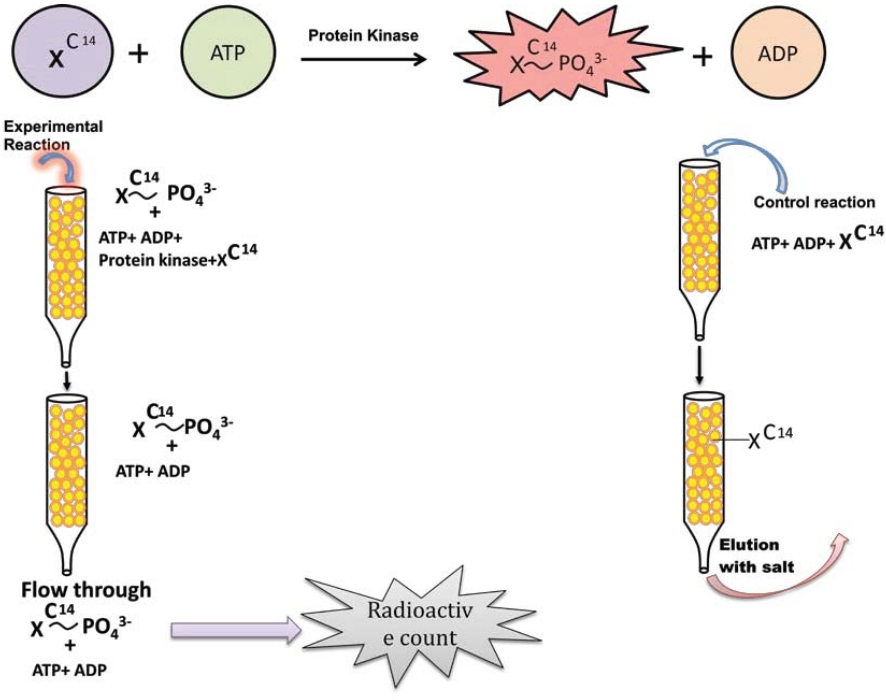


Figure 3.13. Protein kinase assay using ion-exchange chromatography.

Source: https://www.letstalkacademy.com/publication/read/CHROMATOGRAPHY.

3.3.5.5. Purification of Rare Earth Metals From Nuclear Waste

Rare earth metals like uranium or plutonium are also isolated and purified using an IE matrix. Frank Spedding developed the first process to separate uranium in large quantities. The use of IE beads was also found appropriate

to recover uranium from the water coming out of the nuclear power plant (Juza et al., 2000). The IE process results in the binding of uranium to the matrix. The uranium bound bead is transferred to the processing unit for separation of uranium from the beads to form 'yellow cake' and is stored in the drum for additional processing. After the separation of uranium, the IE beads are reused in the IE facility (Ning et al., 2014).

3.3.5.6. Concentrating a Sample

An IE bead can be utilized to bind the analyte from a diluted solution and later sample can be eluted in smaller volume to enhance the concentration (Mazzotti et al., 1997).

3.4. HYDROPHOBIC INTERACTION CHROMATOGRAPHY (HIC)

In this technique, the adsorbents prepared as column material for binding of the ligand in affinity chromatography are utilized. The working of the HIC technique is based upon the hydrophobic interactions between side chains which are bound to the chromatography matrix (Tarantilis et al., 1995; Jiang et al., 2010).

Hydrophobic interaction chromatography (HIC) describes the strong interaction capability of the hydrophobic group bound to the matrix and hydrophobic patches existing on an analyte such as protein.

Protein is comprised of amino acids with a polar and non-polar (aliphatic or aromatic), acidic, and basic side chain. Protein is produced from the ribosome as a linear chain and later it acquires folded 3-D conformation typically guided by the environment of the side chain and the outer medium (Queiroz et al., 2001; Myers et al., 2017).

Folding of protein is assisted by an aqueous local environment in a cell to keep the non-polar side chain within the inner core and polar or charged amino acids on the surface. The major amount of the hydrophobic amino acids are protected from the outer polar environment whereas polar amino acids existing on the surface carries bound water molecule to produce a hydration shell (Figure 3.14) (Haidacher et al., 1996; Ciogli et al., 2018).

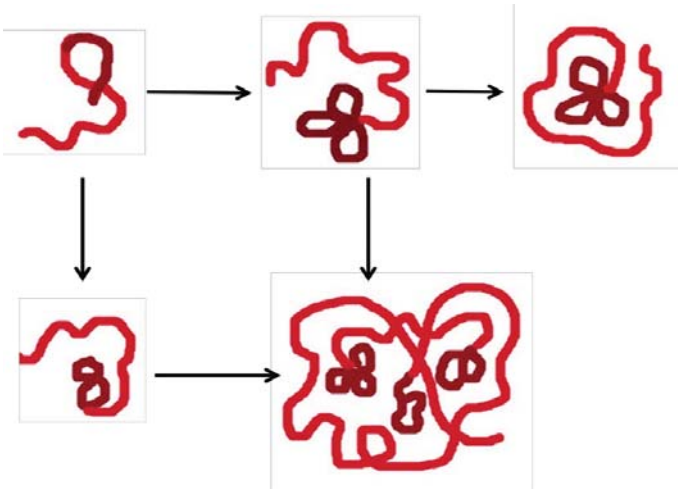


Figure 3.14. Folding of protein in an aqueous environment. Following a series of folding stages, protein adopts a 3-D conformation with hydrophobic patches present in the core.

Source: http://www.bioinfo.org.cn/book/biochemistry/chapt07/bio1.htm.

Adding a low quantity of salt to the protein solution consequences in the displacement of bonded water molecule increasing protein solubility. This effect is termed as "salting-in."

While adding more amount of salt, water molecules shielding protein side chains are entirely displaced with an exposure of hydrophobic patches on the surface of the protein to persuade protein precipitation or reduction in protein solubility.

This effect is named "salting-out." The phenomenon of salting out is restrained so that the addition of salt prompts exposure of hydrophobic patches on protein less causing aggregation or precipitation. The exposure of hydrophobic patches expedites the binding of a protein to the non-polar ligand bound to the matrix. When the salt concentration is reduced, the exposed hydrophobic patches on protein decrease the affinity towards matrix and resultantly it gets eluted (Figure 3.15) (Melander et al., 1984; Welch et al., 2010).

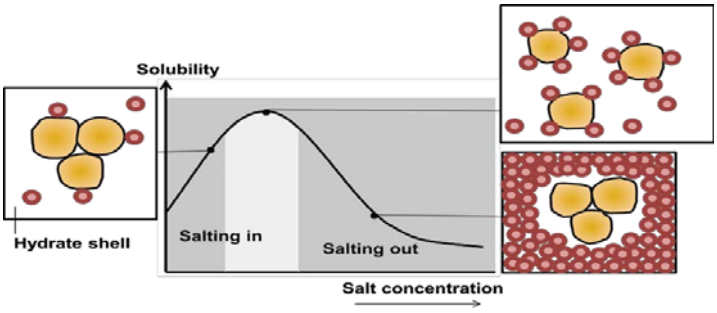


Figure 3.15. Effect of salt on protein, salting in and salting-out effect.

Source: https://fac.ksu.edu.sa/sites/default/files/bch_332_lectures_3_and_4.pdf.

3.4.1. The Choice of HIC Gel

Various commercially available HIC matrix are listed in Table 3.2. Selecting an appropriate HIC matrix is crucial to accomplish the optimum result. The length of the aliphatic linear ligand governs the binding strength of the analyte on a HIC column. Matrix with ligand contained aromatic ring makes supplementary Π-Π interaction resulting in binding analyte more strongly as compared to the same number of carbon aliphatic ligand. Moreover, the presence of Π-Π interaction provides selectivity as well, such as a ring comprising phenylalanine and aromatic ligand. Eventually, ligand density plays a dynamic role in strengthening the analyte bond with the matrix. Therefore, these facts must be deliberated to choose a proper matrix for purification (Figure 3.16) (Fausnaugh and Regnier, 1986; Perkins et al., 1997).

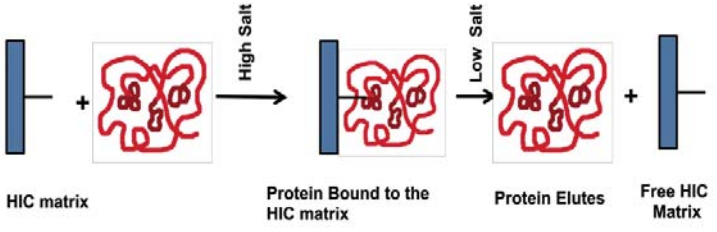


Figure 3.16. Principle of the hydrophobic interaction chromatography.

Source: https://nptel.ac.in/content/storage2/courses/102103044/pdf/mod5.pdf.

Table 3.2. Selected List of Popular HIC Column Matrix

SL. No.	Column Material	Functional Group
1.	Phenyl Sepharose (High Sub)	-Phenyl, high density
2.	Phenyl Sepharose (Low Sub)	-Phenyl, low density
3.	Octyl Sepharose	-Octyl
4.	Capto phenyl sepharose	-Phenyl
5.	Butyl-S-Sepharose	-Butyl

3.4.2. Operation of the Technique

Numerous parameters should be taken into account to execute HIC.

3.4.2.1. Equilibration

HIC column material is contained in a column and equilibrates with a buffer comprising 0.5–1.5 M ammonium sulfate (mobile phase). The salt essentially is below the concentration to promote the salting-out effect (Hjertén et al., 1974).

3.4.2.2. Sample Preparation

Manufacturing of sample is done in the mobile phase, and it should not contain any suspended particle to prevent clogging of the column. The most suggested technique to apply the sample is to use a syringe to inject the sample.

3.4.2.3. Elution

Several methods exist to elute an analyte from the hydrophobic interaction column. (1) altering the polarity of the mobile phase such as alcohol, (2) reducing salt concentration, (3) By a detergent to dislocate the bound protein.

3.4.2.4. Column Regeneration

After the elution of the analyte, the HIC column needs a regeneration step for later use. Around 6 M urea or guanidine hydrochloride is used to wash the

column for removing all non-specifically bound protein. Later, the column is equilibrated with the mobile phase to achieve regeneration of the column. Storing of the column is done using 20% alcohol containing 0.05% sodium azide at 4°C (Lienqueo et al., 2007).

3.5. GEL FILTRATION CHROMATOGRAPHY

In-gel filtration chromatography, the distribution of the protein or analyte is accomplished based on their size by passing through porous beads. In 1955, the first report was published explaining performing a chromatography column using the swollen gel of maize starch to isolate the protein based on their size. 'Porath and Floidin' coined the term "gel filtration" for such chromatography technique in which analytes are separated based on their molecular sizes (Hagel, 1998; Striegel et al., 2009). Since then, the chromatography technique emerged in terms of established different sizes beads to isolate the protein of narrow range, besides performing the technique in the non-aqueous and aqueous mobile phase. The beads utilized in gel filtration chromatography are fabricated by cross-linked material (dextran in Sephadex) to form a 3-D mesh. These 3-D mesh structures swell in the mobile phase to produce pores of multiple sizes. Pores size produced within the gel beads is controlled by the extent of cross-linking (Figure 3.17) (Werner et al., 1994; Gordon et al., 2010).

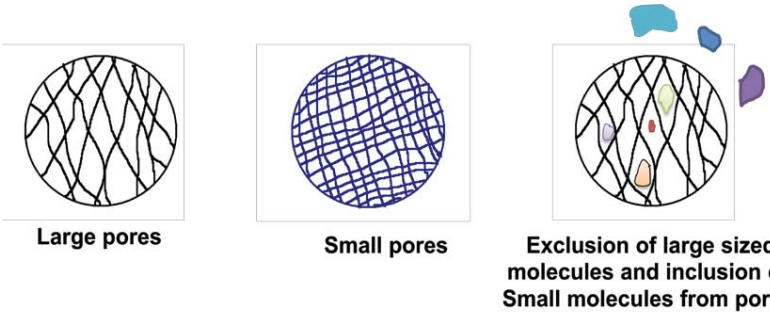


Figure 3.17. Gel filtration matrix having beads with different pore sizes.

Source: http://lib.kstu.kz:8300/tb/books/2019/HiHT/Andreyeva/%D0%9B%D0%B0%D0%B1%D0%BE%D1%80%D0%B0%D1%82%D0%BE%D1%80%D0%BD%D0%B0%D1%8F%20%D1%80%D0%B0%D0%B1%D0%BE%D1%82%D0%B0/lab4.htm.

3.5.1. Principle

The working principle of the chromatography technique is demonstrated in Figure 3.18. The column is packed with porous beads to permit the entry of molecules based on their sizes. The smallest size lies in the inner part of the pore trailed by a gradually increasing size, and at the end, the largest molecule is restrained from entering into the gel. The separation between different sized molecules is carried out with the time taken to travel to come out from the pores. As soon as the mobile phase passes through the column, it carries protein along with it. The small molecules existing in the inner part of the gel face a longer flow of liquid (or time) and travel more to come out, whereas larger molecules travel less distance to emerge out. Resultantly, the large molecule and small molecule are separated from each other. A schematic diagram of gel filtration chromatogram is illustrated in Figure 3.18 (Tepfer and Taylor, 1981; McKenna et al., 2007).

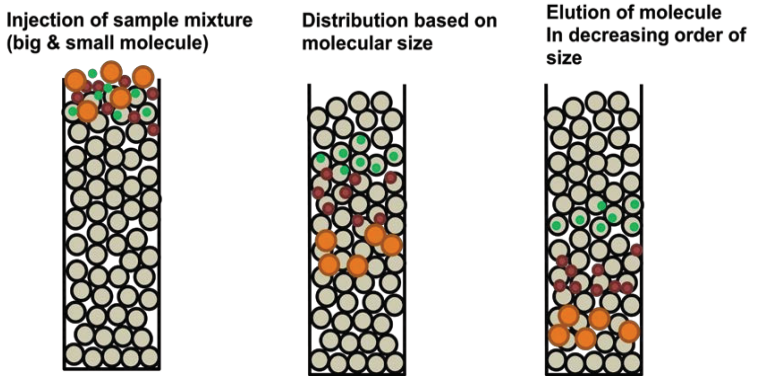


Figure 3.18. Principle of gel filtration chromatography.

Source: https://gojitisiqini.wowinternetdirectory.com/gel-filtration-11146nb.html.

Suppose V_t is the total column volume of a gel and is given by:

$$V_t = V_g + V_i + V_o \qquad (1)$$

where V_g, V_i, V_o is the volume of the gel matrix, pore-volume, and void volume, respectively. Elution volume (V_e) is the volume of mobile phase flow to elute a column from a column. The elution volume is associated with the void volume as well as with the distribution coefficient Kd as shown below:

$V_e = V_o + KdV_i$ (2)

Where Kd represents the ratio of inner volume available for an analyte independent of the column geometry or length. According to the relationship given in Eqn. (3), three different types of analytes are conceivable:

- Analyte with Kd>1, the analyte will be adsorbed to the column matrix.
- Analyte with Kd = 1 or $V_e = V_o + V_i$, entire analytes will be present in the pore of the column.
- Analyte with Kd = 0, or $V_e = V_o$, these analytes will be completely excluded from the column (Figure 3.19).

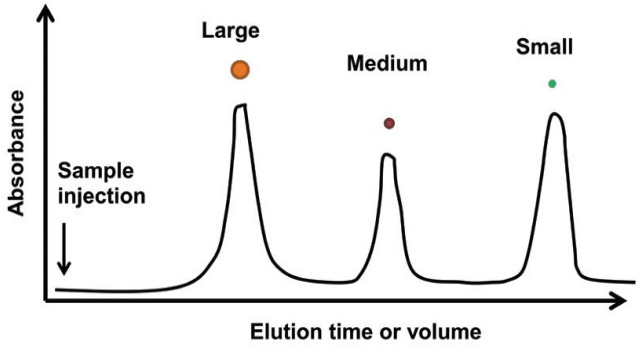


Figure 3.19. A typical gel filtration chromatogram.

Source: https://nptel.ac.in/content/storage2/courses/102103044/pdf/mod5.pdf.

3.5.2. Choice of Matrix for Gel Filtration Chromatography

The selection of columns is determined by the range of molecular weight as well as the pressure limit of the operating equipment.

3.5.3. Operation of the Chromatography

3.5.3.1. Column Packing

The column material is permitted to swell in the mobile phase. It is then poured into the glass tube, and the beads are allowed to settle without entrapping air bubbles within the column. After settling the matrix to give a column, it can be evaluated for the existence of an air channel and well-

packed with the help of flowing an analyte with Kd = 1. It is anticipated that the elution volume (Ve) in such a case should be $V_o + V_i$.

3.5.3.2. Sample Preparation

Manufacturing of sample is done in the mobile phase, and it should not contain any suspended particle to prevent clogging of the column. The most suggested technique to apply the sample is to use a syringe to inject the sample (Duong-Ly and Gabelli, 2014).

3.5.3.3. Elution

No gradient of salt is used in the gel filtration column to elute the sample from the column. However, to elute the molecules from the column, the flow of the mobile phase is utilized.

3.5.3.4. Column Regeneration

Gel filtration column is cleaned after analysis of analyte by washing with the salt-containing mobile phase to eliminate all non-specifically adsorb protein to the matrix. Later, the column is equilibrated with the mobile phase to achieve regeneration of the column. Storing of the column is done using 20% alcohol containing 0.05% sodium azide at 4°C (Table 3.3).

Table 3.3. List of Popular Gel Filtration Matrix

SL. No.	Name of the Matrix	Fractionation Range (Daltons)
1.	Sephadex G10	Up to 700
2.	Sephadex G25	1000–5000
3.	Sephadex G50	1500–30,000
4.	Sephadex G100	4000–150,000
5.	Sephadex G200	5000–600,000
6.	Sepharose 4B	60,000–20,000,000
7.	Sepharose 6B	10,000–4,000,000

3.5.4. Determination of Native Molecular Weight of a Protein Using Gel Filtration Chromatography

Protein size and weight is related to the morphology or shape of the molecule and the relationship between the radius of gyration (R_g) and molecular weight (M) is as follows-

$Rg \propto M^a$

where; "a" is constant and it depends upon the shape of the molecule; a = 0.33 for spherical molecules; a = 0.5 for coils, and a = 1 for Rod.

A set of known molecular weights of standard protein can be run on a gel filtration column, and elution volume can be calculated by using a chromatogram. A separate run with the analyte will provide elution volume for an unknown sample. With the help of the following formula, the Kd value for the test analyte and all standard proteins can be calculated.

$\mathbf{Kd} = \boldsymbol{V^e – V^o / V^i}$

A plot of Kd versus log mol wt. is shown in Figure 3.20(B), and it will permit the calculation of the molecular weight of the unknown analyte.

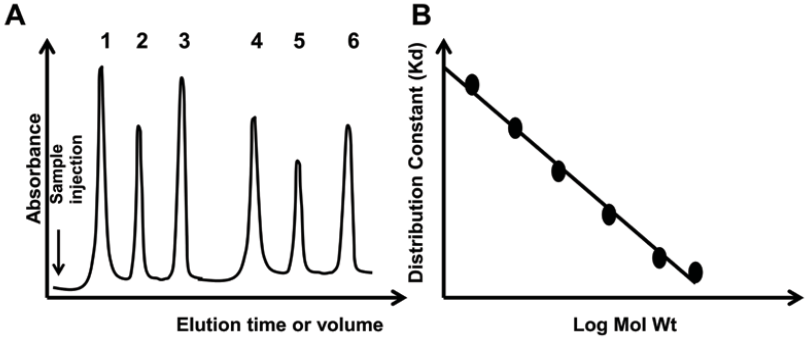


Figure 3.20. Determination of molecular weight by gel filtration chromatography. (A) Gel filtration chromatogram with the standard proteins (1–6); (B) relationship between distribution constant (Kd) and log molecular weight.

Source: http://lib.kstu.kz:8300/tb/books/2019/HiHT/Andreyeva/%D0%9B%D0%B0%D0%B1%D0%BE%D1%80%D0%B0%D1%82%D0%BE%D1%80%D0%BD%D0%B0%D1%8F%20%D1%80%D0%B0%D0%B1%D0%BE%D1%82%D0%B0/lab4.htm.

3.5.4.1. Oligomeric Status of the Protein

After determination of native molecular weight by using gel filtration in conjugation with the SDS-PAGE, it can be used to conclude the oligomeric status of the protein.

Oligomeric Status = Molecular weight (gel filtration)/Molecular weight SDS-PAGE

3.5.4.2. Studying Protein Folding

Different types of amino acid residues link by the peptide bond to form a protein. When the peptide chain emerges from the ribosome, it bends into the 3-D conformation directed by the external environment, amino acid sequence, and other factors.

Protein structure has multi-level organization; Primary structure (sequence of the protein), secondary (α-helix, β-sheet, and turn), tertiary, and quaternary structure.

When an increased concentration of denaturing agents (such as urea) is used to incubate the protein, it results in unfolding the native structure of protein into the unfolded extended conformation following various stages (Queiroz et al., 2001).

Different protein conformation forms in the course of the unfolding pathway have the distinctive hydrodynamic surface area, and it can be used to track protein folding-unfolding stages using gel filtration chromatography. Different concentrations of urea (0–8 M) are used to incubate protein for 8–10 hrs. at 37°C.

The incubation mixture is analyzed while equilibrating the gel filtration column with the buffer comprising urea (similar to incubation mixture). An increase in the concentration of denaturing agents results in the unfolding of protein with an escalation in hydrodynamic surface area.

Consequently, the protein peak is shifted towards the left. Protein unfolds completely at the highest concentration of denaturant and typically appears in the void volume (Jungbauer et al., 2005).

3.5.4.3. Studying Protein-Ligand Interaction

Gel filtration chromatography splits the molecules based on their size. Ligand binding to the protein persuades conformational variations, which ultimately results in a change in shape or size. Additionally, ligand possesses

a small size whereas protein-ligand complex has a bigger size and may appear at a distinctive place in the column. In step 1, equilibration of gel filtration column using a buffer is carried out, and elution profile of ligand is noted down.

Later, the column is equilibrated with the buffer comprising the ligand molecule. With the increase in the concentration of ligand, the protein starts binding with ligand and ultimately forms a larger complex with the increased hydrodynamic surface area (Hage, 1999; Haverick et al., 2014). Resultantly, the protein peak is shifted towards the left.

Furthermore, As the concentration of ligand increases with a stable amount of the protein, the free ligand will appear in the chromatogram. Amount of protein,

the concentration at which free ligand appeared, and the elution data can be utilized to evaluate the stoichiometric ratio of ligand/protein as well as the equilibrium constant (Figures 3.21 and 3.22).

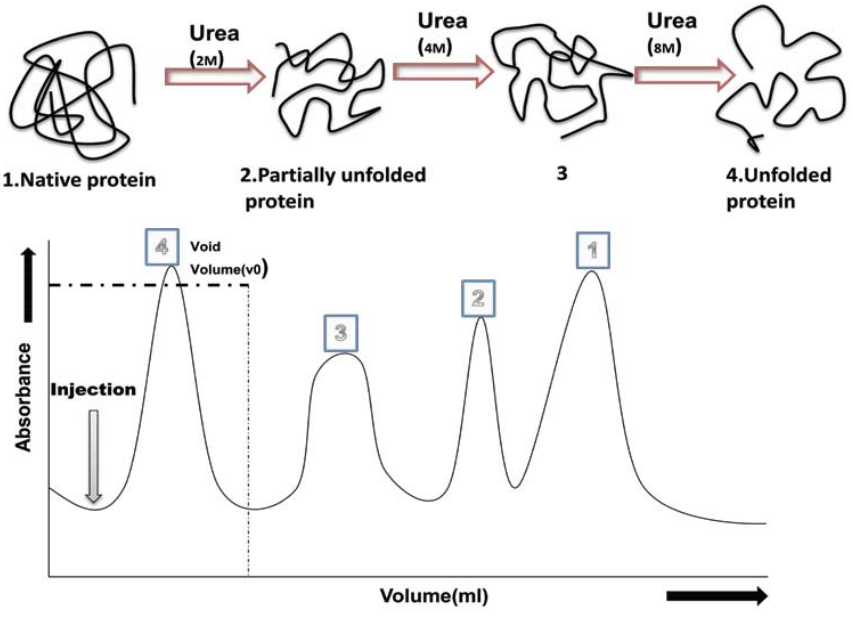


Figure 3.21. Protein folding stages by gel filtration chromatography.

Source: https://nptel.ac.in/content/storage2/courses/102103044/pdf/mod5.pdf.

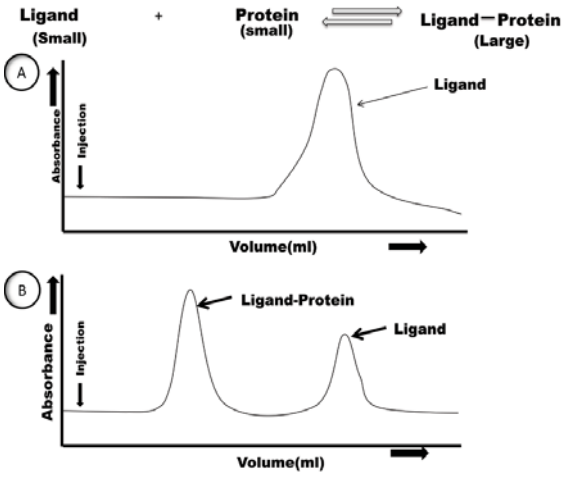


Figure 3.22. Studying protein-ligand interaction by gel filtration chromatography.

Source: https://www.letstalkacademy.com/publication/read/CHROMATOGRAPHY.

3.5.4.4. Desalting

Desalting or elimination of the small molecule from the protein is significant for activity assay and additional downstream processes. Equilibration of gel filtration column is done using buffer or water before loading of sample for desalting. After the run salt and protein are eluted separately as a peak (Figure 3.23) (Mahn et al., 2004).

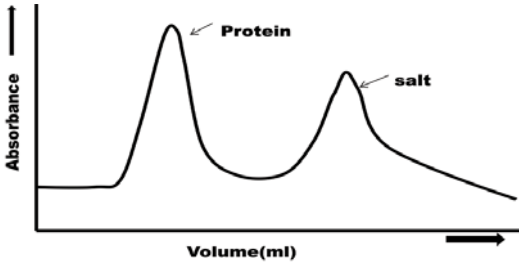


Figure 3.23. Desalting of a sample by gel filtration chromatography.

Source: https://www.letstalkacademy.com/publication/read/CHROMATOGRAPHY.

3.6. AFFINITY CHROMATOGRAPHY

The chromatography techniques discussed yet were manipulating various kinds of interactions between the matrix and the groups existing on the analyte, but these chromatography techniques are not explicit towards a particular analyte. The generalized chromatography methods need a higher sample volume to isolate the specific molecule. In the current section, another chromatography technique, i.e., affinity chromatography will be discussed in which chromatography matrix is specific for a molecule of interest or a group of protein (Cuatrecasas et al., 1968; Hage, 1999).

3.6.1. Principle

The working principle of affinity chromatography comprises mutual recognition forces between a receptor and ligand. The foremost determinants, liable to provide specificity are electrostatic, hydrogen bonding, shape complementarity, and Van der Waal interactions between the groups existing on the ligand-receptor pair. Ligand-receptor complex (RL) is formed by mutual interaction between a ligand (L) and receptor (R) with a dissociation constant Kd as shown below (Porath et al., 1975).

$R + L \leftrightharpoons RL$

Kd = [R][L]/[RL]

Dissociation constant Kd is specific to the receptor-ligand pair as well as the number of interactions between them. The receptor present on the matrix reacts with the ligand which is present on different molecules when a crude mixture passes through an affinity column. The mutual collision between receptor and ligands tests the affinity between them, and accordingly, the best choice binds to the receptor leaving all other molecules unbound and appear inflow through. Later, during washing, weakly bound molecules on the matrix are removed. Subsequently, a counter ligand is utilized to elute the bound molecules through a competition between the counter ligand and the matrix-bound molecules (Deutsch and Mertz, 1970).

3.6.2. Advantages of Affinity Chromatography

3.6.2.1. Specificity

Affinity chromatography is explicitly for analytes as compared to other purification techniques that utilize molecular charge, size, isoelectric point or hydrophobic patches, etc.

3.6.2.2. Purification Yield

Affinity purification provides a very high-quality purification fold with a high yield as compared to other purification techniques. For instance, in a particular affinity purification, more than 90% recovery is conceivable.

3.6.2.3. Reproducible

Affinity purification is reproducible and provides consistent outcomes from one purification to another as far as it is independent of the occurrence of polluting species.

3.6.2.4. Easy to Perform

Affinity purification is a robust technique depending on the force prevailing ligand-receptor complex formation. Nevertheless, no column packing, no special purification system, and sample preparation are required for affinity purification as compared to other techniques (Figure 3.24).

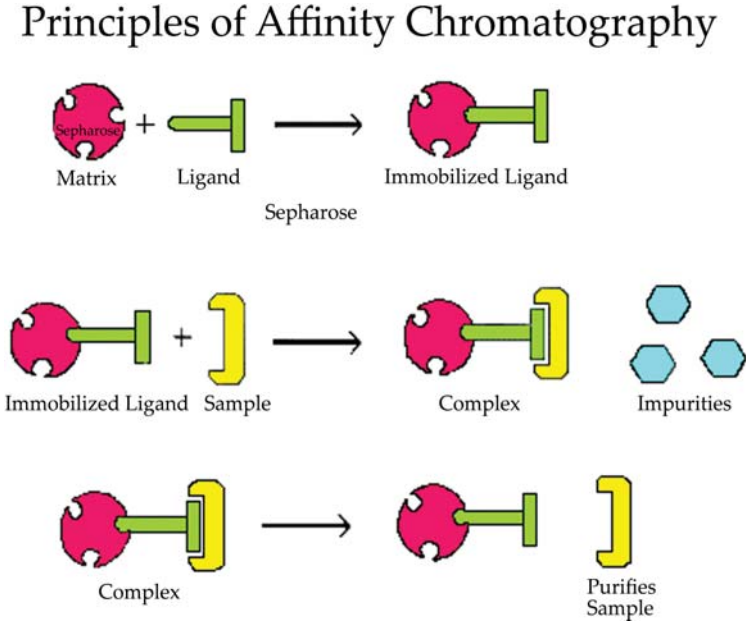


Figure 3.24. Principle of affinity chromatography.

Source: http://upendrats.blogspot.com/2013/06/affinitiy-chromatography.html.

3.6.3. Different Types of Affinity Chromatography

Affinity chromatography is further distributed into different types depending upon the nature of the receptor existing on the matrix to bind the tag present on the analyte molecule. Various types of affinity chromatography are as follows-

3.6.3.1. Bio-Affinity Chromatography

In bio-affinity chromatography, biomolecules are utilized as receptors present on the matrix to exploit the biological affinity phenomenon such as antibody-antigen. Additionally, enzyme-inhibitor or enzyme-substrate also belong to this class.

3.6.3.2. Pseudo-Affinity Chromatography

In pseudo-affinity chromatography, a non-biological molecule is used as a receptor present on the matrix to exploit the purification and separation of biomolecules. Following are the two examples belonging to this class:

1. **Dye-Affinity Chromatography:** In this technique, the matrix is coupled to the reactive dye and the matrix-bound dye has an affinity towards a particular enzyme. For instance, Cibacron Blue F3G-A dye coupled to the dextran matrix possesses a strong affinity towards dehydrogenases.
2. **Metal-Affinity Chromatography:** In this technique, transition metals like Ni^{2+}, Fe^{2+}, or Zn^{2+} are coupled to the matrix and the matrix-bound metal produces a multidentate complex with protein carrying poly-his tag (6x His). The affinity of the protein for matrix-bound metal is different and these differences are exploited in metal-affinity chromatography to purify the protein.

3.6.3.3. Covalent Chromatography

This type of chromatography technique is quite different from other techniques in which binding of an analyte to the matrix is irreversible as it implicates the formation of a covalent bond between functional groups existing on matrix and analyte. Thiol group (SH) existing on the neighboring residues of protein forms disulfide bond after the oxidation-reduction environment, disulfide reversible is broken back to a free thiol group. The matrix in covalent chromatography contains immobilized thio group which produces covalent linkage with the free thiol group comprising protein

present in the mixture. After washing to remove non-specifically bound protein, a mobile phase comprising a compound with a reducing thio group is passed to elute the bound protein. The thio group-containing compound existing in mobile phase breakdowns the disulfide bond between protein and matrix thio group to discharge the protein in the mobile phase (Sherma and Fried, 2003).

3.6.4. Choice of Matrix for Affinity Chromatography

Various types of the matrix for affinity chromatography for purification of protein are given in Table 3.4. The selection of an appropriate matrix depends on the affinity tag existing on the recombinant protein formed after genetic engineering (Figure 3.25).

Table 3.4. Matrix Containing Receptor for Ligand Present on Protein

SL. No.	Receptor	Affinity Towards Protein-Ligand
1.	Lectin	Glycoprotein
2.	Poly-A	Poly U mRNA
3.	Heparin	DNA binding site
4.	Concanavalin A	Glycoprotein
5.	Cibacron Blue F3GA	NAD^+ Containing dehydrogenase
6.	Lysine	rRNA
7.	Avidin	Biotin-containing enzymes
8.	5'AMP 2'5'-ADP	NAD^+-dependent dehydrogenase $NADP^+$-dependent dehydrogenase
9.	Protein A and Protein G	Immunoglobulin

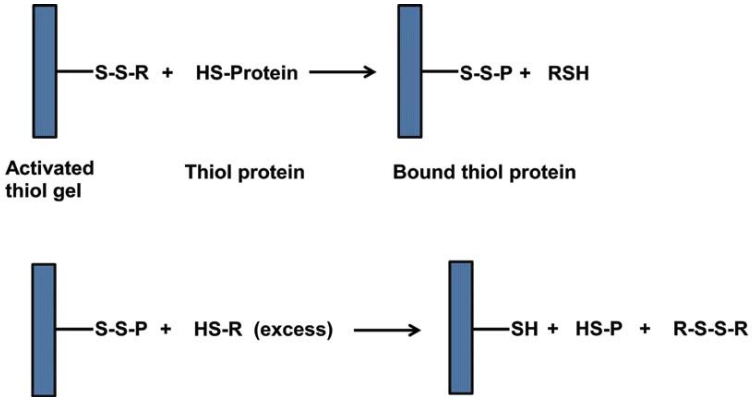


Figure 3.25. Principle of covalent chromatography.

Source: https://www.letstalkacademy.com/publication/read/CHROMATOGRAPHY.

3.6.4.1. Generation of Receptor

The receptor molecule located on the matrix can be created either by isolation from the crude extract, genetic engineering, or in the case of antibody, it is formed in the mouse/rabbit model and purifies.

3.6.4.2. Coupling of the Receptor

When the receptor molecule is accessible, it can be coupled to the matrix by using the following steps. (1) Matrix activation (2) covalent coupling using reactive group on the ligand. (3) deactivation of the leftover active group on the matrix.

3.6.4.3. CNBr Mediated Receptor Coupling

CNBr mediated receptor coupling is quite appropriate for protein/peptide to the polysaccharide matrix such as dextran or agarose. CNBr chemically reacts with polysaccharide at pH 11–12 to produce reactive cyanate ester with matrix or less reactive cyclic imidocarbonate group. Cyanogen ester reacts with the amine group on the receptor to create an isourea derivative

under alkaline conditions. Cyanate ester yielded more with agarose however imidacarbonate is formed more with dextran as a matrix. To couple the receptor for affinity purification, protein or peptide ligand with a free amino group is added to the activated matrix (Figure 3.26) (Sherma and Fried, 2003).

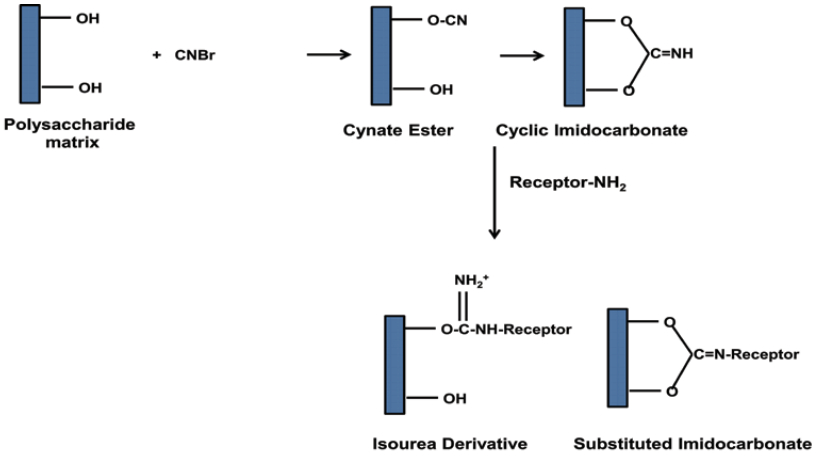


Figure 3.26. CNBr mediated coupling of receptor to the matrix.

Source: https://nptel.ac.in/content/storage2/courses/102103044/pdf/mod5.pdf.

3.6.4.4. Epichlorohydrin Mediated Receptor Coupling

Polysaccharide matrix is activated by epichlorohydrin by adding an oxirane group along with a 3-carbon alcohol group (propanol) spacer arm. The activated matrix reacts with the receptors comprising the primary amine or thiol group. The coupling of receptors to the matrix is carried out by a thioester or a secondary amine linkage. Coupling can also be done by hydroxyl group-containing receptor molecule as well as by an ether linkage.

3.6.4.5. Carbodiimides Mediated Receptor Coupling

Isourea ester is formed by the reaction of carboiimides with the matrix containing the carboxyl group. The activated matrix is then permitted to react with the receptor molecule carrying carboxyl or free amino group. Secondary amine linkage is utilized to couple the receptors to the matrix (Adams et al., 1980).

3.6.5. Operation of the Affinity Chromatography

Various steps involved in affinity chromatography are given in subsections.

3.6.5.1. Equilibration

To reduce the non-specific interaction of the protein with the analyte, affinity column material packed in a column is equilibrated with a buffer comprising high salt (0.5 M NaCl) concentration.

3.6.5.2. Sample Preparation

Sample preparation is carried out in the mobile phase and to avoid clogging of the column, it must not contain any suspended particle. Injecting the sample with the help of a syringe is the most appropriate method to apply sample.

3.6.5.3. Elution

There are several ways to elute an analyte from the affinity column. (1) altering the pH polarity of the mobile phase, (2) by increasing counter ligand concentration, (3) By using a detergent or chaotropic salt to partly denature the receptor for reducing the affinity for bound ligand.

3.6.5.4. Column Regeneration

After the elution of the analyte, the affinity column needs a regeneration step for later use. Around 6 M urea or guanidine hydrochloride is used to wash the column for removing all non-specifically bound protein. Later, the column is equilibrated with the mobile phase to achieve regeneration of the column. Storing of the column is done using 20% alcohol containing 0.05% sodium azide at 4°C (Dixon and Summers, 1985).

3.6.6. Applications of Affinity Chromatography

3.6.6.1. GST-Based Purification

For xenobiotic detoxification, glutathione S-transferase (GST) uses glutathione as a substrate to catalyze conjugation reactions. Purification of recombinant fusion protein contains GST as a tag is done using glutathione coupled matrix. GST fusion protein is created by recombining protein of interest with the GST coding sequence located in the expression vector

(either before or after the coding sequence of the protein of interest). It is over-expressed, transformed, and the bacterial lysate comprising fusion protein is purified, by utilizing an affinity column. The sample is loaded on the affinity column already equilibrated with the buffer having high salt (0.5 M NaCl). To eliminate unbound protein equilibration buffer is used for washing, and then the fusion protein is eluted with a diverse concentration of glutathione dissolved in the equilibration buffer. Further, to remove the GST tag from the protein of interest, purified fusion is treated with the thrombin. The mixture comprising free GST tag and the protein can be further purified by utilizing the affinity column again as the tag will bind to the matrix and protein will emerge in the unbound fraction (Figure 3.27) (Kim et al., 2013).

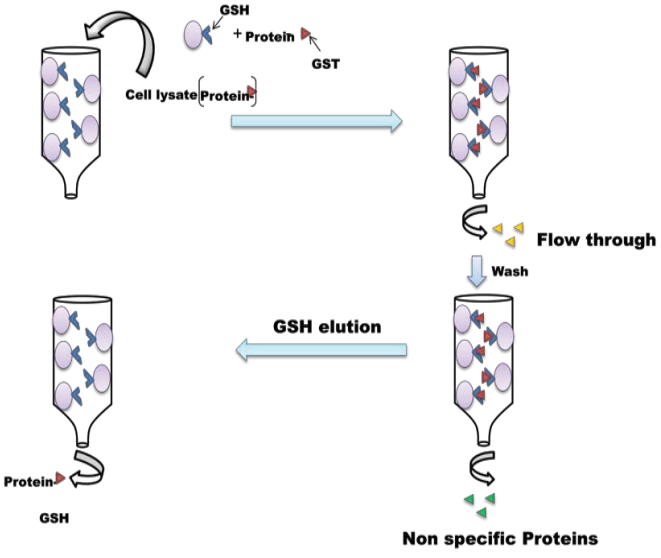


Figure 3.27. GST purification process.

Source: https://www.biologicscorp.com/blog/recombinant-protein-purification-definition/.

3.6.6.2. Protein-Protein Interaction

Protein-protein interaction can be explored using numerous techniques or methodologies. An affinity column can also be utilized to eliminate the interacting partner of a specific protein. In this method, the matrix is incubated with the pure protein-1 and later washed to confirm strong binding. The remaining sites located on the bead are blocked using an unrelated cell

lysate or a non-specific protein such as BSA. For removing unbounded proteins, cell lysate or the pure protein-2 is passed to the protein-1 having beads, and washing is done to serve the purpose. Protein-1 is eluted from the matrix by the addition of a high concentration of ligand, or with denaturation condition. The eluted protein is then examined in the SDS-PAGE or SDS-PAGE followed by the western blotting to distinguish protein 1 or protein 2. As a control, protein-2 or cell lysate is also added to the matrix without protein-1 to prevent the prospect of protein-2 binding directly to the matrix (Figure 3.28) (Depountis et al., 2001).

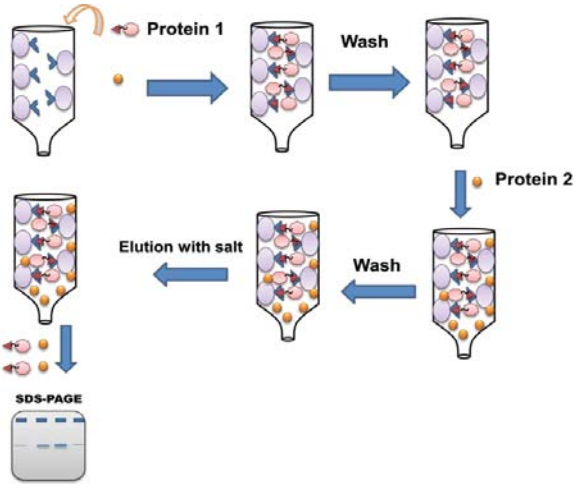


Figure 3.28. Protein-protein interaction studies with affinity chromatography.

Source: https://www.letstalkacademy.com/publication/read/CHROMATOGRAPHY.

3.6.6.3. Enzymatic Assay

Affinity chromatography can be employed to perform enzymatic assay such as protease assay. In this assay, peptides having diverse amino acid sequences bound with the terminal residue to the affinity bead are incubated with the protease assay for an optimum time. The enzyme reacts to the attached peptide thereby releasing the free portion into the supernatant. The supernatant is recuperated from the reaction mixture and can be analyzed in a MALDI to comprehend the sequence of amino acid from the molecular weight. Analysis of a set of reactions possibly will permit to envisage the protease recognition and cutting site (Woo, 1970).

3.6.6.4. Clinical Diagnosis

Receptor located on the matrix delivers an inimitable tool to segregate, identify, and characterize biomolecules from the crude mixture. For instance, in diabetic patients, a matrix comprising boronic acid is utilized to detach and evaluate glycosylated hemoglobin from the blood. Similarly, an affinity matrix comprising boronic acid followed by the reverse phase chromatography can be used to identify ribonucleoside in patient urine (Hage, 1999).

3.6.6.5. Immuno-Purification

The avidin-biotin system is employed for capturing and separating cytokines from immune cells. Biotinylation of antibodies permits immobilization of antibodies in the right orientation on the glass beads coated with streptomycin. To bind cytokines, lymphocyte lysate is transferred to the column packed with the glass beads comprising antibodies (Keren et al., 2008; Grosser and van Dam, 2017). Afterward, these cytokines are eluted by washing with a buffer of decreasing pH or by chaotropic ions. Due to the strong affinity of avidin-biotin as well as inert behavior towards these chemical treatments, antibodies remain in contact to the column (Figure 3.29).

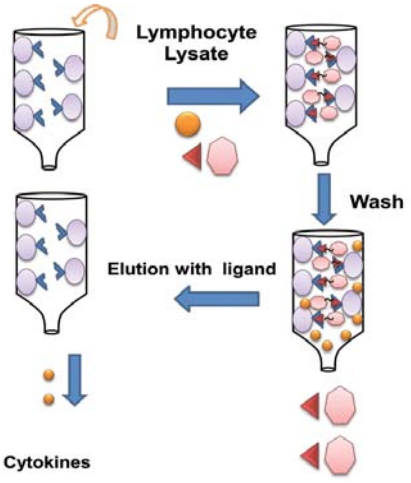


Figure 3.29. Immuno-purification with an affinity column.

Source: https://www.letstalkacademy.com/publication/read/CHROMATOGRAPHY.

3.7. THIN LAYER CHROMATOGRAPHY

The TLC technique is an analytical chromatography technique to isolate and examine complex biological or non-biological samples into their ingredients. It is prevalent for observing the progress of a chemical reaction or assessment of a constituent in a mixture. It is also one of the most common techniques for evaluating the purity of a sample. In this technique, the silica or alumina is coated onto a glass or aluminum foil as a thin layer as a stationary phase, and then the sample is permitted to run in the existence of a mobile phase (solvent). As compared to other chromatography techniques, the mobile phase in this case runs from bottom to top by diffusion process (mobile phase runs from top to bottom by gravity or pump generally in most of the chromatography techniques). As the sample runs accompanying the mobile phase, it is disseminated into the solvent phase and stationary phase (Poole, 2003). The interaction of the sample with the stationary phase hinders the mobility of the molecule, whereas the mobile phase entails an effective force on the sample (Stoffyn and Jeanloz, 1954). If the force triggered by the mobile phase is F_m and the hindrance force by the stationary phase is F_s, then the effective force on the molecule will be ($F_m - F_s$) by which it will move. The molecule immobilizes on the silica gel (where, $F_m = F_s$) and multiple factors will control the position which are given below (Skipski et al., 1964):

- The thickness of the stationary phase;
- nature or composition of the mobile phase;
- Functional group located on the stationary phase;
- Nature or functional group located on the molecule or analyte.

If the distance traveled by a molecule on TLC plate is denoted by D_m and the distance traveled by the solvent is denoted by D_s, the retardation factor (R_f) of a molecule is given by:

R_f = Distance traveled by the substance (D_m)/Distance traveled by the solvent (D_s)

where; R_f value is characteristic to the molecule as far as the solvent system and TLC plate remain unchanged. It will be helpful to identify the substance in a crude mixture (Figure 3.30).

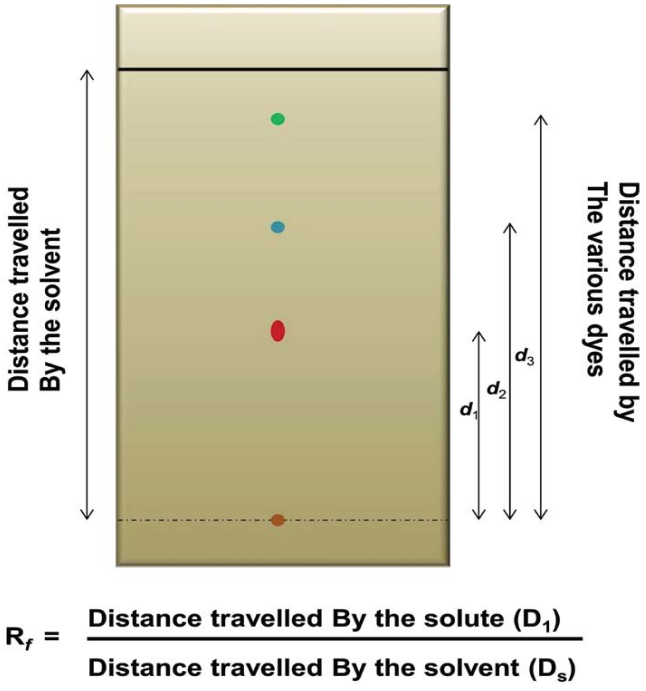


Figure 3.30. Principle of thin-layer chromatography.

Source: https://pharmaxchange.info/2012/11/thin-layer-chromatography-tlc-principle-with-animation/.

R_f = Distance traveled by the solute (D_1)/Distance traveled by the solvent (D_s)

3.7.1. Operation of the Technique

Numerous steps are obligatory to execute a thin layer chromatography to analyze complex samples. These preliminary and operative steps are as follows:

3.7.2. Thin Layer Chromatography Chamber

A thin layer chromatography chamber either cylindrical or rectangular is manufactured from transparent and non-reactive material such as glass. A thick glass sheet is used to cover the top of the chamber, and the joints are made impenetrable using a high vacuum grease to evade loss of solvent vapors. Whatman filter paper is used to cover all three sides of the chamber

to make the chamber uniformly equilibrate. A solvent system is filled in the chamber to humidify the chamber with the solvent vapors. Further, it is imperative for the unvarying running of the solvent front during TLC (Minnikin et al., 1980).

3.7.3. Preparation of TLC Plate

A slurry of silica prepared in water is spread on the glass or alumina sheet as a thin layer and is allowed to dry. Later, the dried thin layer is baked at 110°C for 1 hr. in an oven and then the plate is ready for TLC. The thickness of the layer varies, i.e., thin layer (~ 0.1–0.25 mm) is used for analytical applications whereas thick layer (0.4–2.1 mm) is utilized for preparative or bio-assay purposes (Figure 3.31) (Meek, 1980).

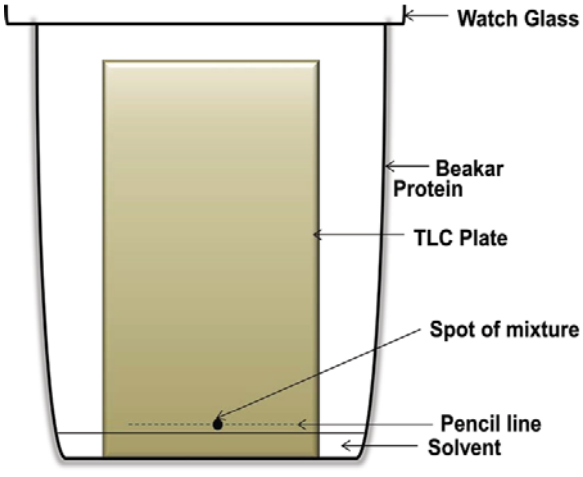


Figure 3.31. Thin-layer chromatography chamber.

Source: https://www.chemguide.co.uk/analysis/chromatography/thinlayer.html.

3.7.3.1. Spotting

The events involved in spotting are shown in Figure 3.32. A line is drawn using a pencil a little away from the bottom. The sample is drawn into the capillary tube or in a pipette. The capillary tube is touched onto the silica plate, and the sample is permitted to dispense. Suitable volume must be taken before applying depending upon the thickness of the layer of silica. The spot is dried in air or by using a hairdryer (Jeanes et al., 1951).

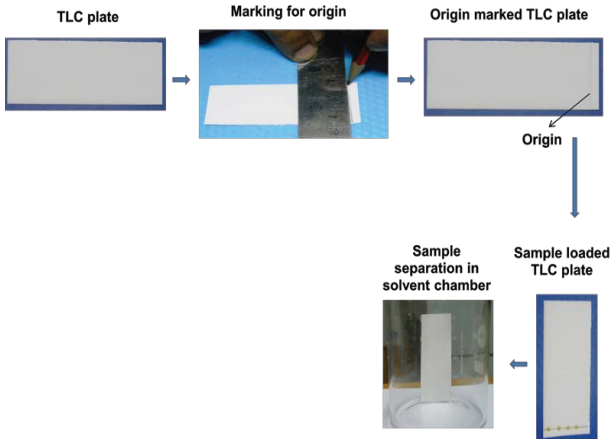


Figure 3.32. Events in spotting during thin-layer chromatography.

Source: https://vidyamitra.inflibnet.ac.indata-server/eacharya-documents/570485508ae36c9272b0afc9_INFIEP_226/48/ET/226-48-ET-V1-S1__mod5-lec_32.pdf.

3.7.3.2. Running of the TLC

After drying the spot, it is positioned in the TLC chamber above the solvent level. The solvent front is permissible to move till the end of the plate.

3.7.3.3. Analysis of the Chromatography Plate

The plate is then allowed to cool and air dried out of the chamber. If the compound is colored, a spot is formed and it requires no additional staining. Two methods (staining and non-staining) are utilized to develop a chromatogram.

3.7.3.4. Staining Procedure

In the staining procedure, a staining reagent is sprayed on a TLC plate to stain the functional group located in the compound. For instance, Forx. Ninhydrin is utilized to stain amino acids.

3.7.3.5. Non-Staining Procedure

In the non-staining procedure, the spot can be detected by succeeding methods:

1. **Autoradiography:** TLC plate is placed along with the X-ray film for 48–72 hrs. (exposure time varies with the type and concentration of radioactivity) and then X-ray film is processed.
2. **Fluorescence:** Various heterocyclic compounds provide fluorescence in UV because of the conjugated double bond system. Therefore, the TLC plate can be visualized in a UV chamber (Figure 3.33) to detect the spots on the TLC plate.

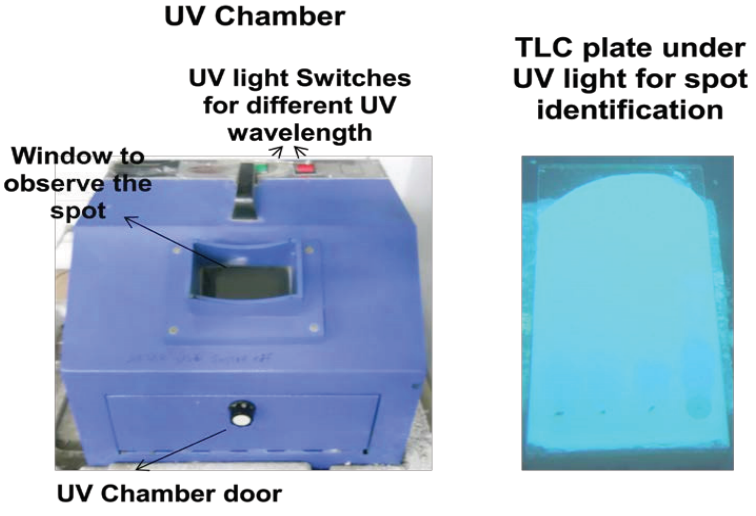


Figure 3.33. *UV-chamber and UV illuminated TLC plate.*

Source: https://vidyamitra.inflibnet.ac.indata-server/eacharya-documents/570485508ae36c9272b0afc9_INFIEP_226/48/ET/226-48-ET-V1-S1__mod5-lec_32.pdf.

3.7.4. Technical Troubles with Thin Layer Chromatography

3.7.4.1. Tailing Effect

Generally, the sample produces a circular spot on the TLC plate. This is because of the uniform movement of the solvent front throughout the plate. Nevertheless, in few cases, a compound forms a spot with a long trail or rocket shape spot (Figure 3.34). The reasons governing these shapes are given in Figure 3.34 (Benson et al., 1950).

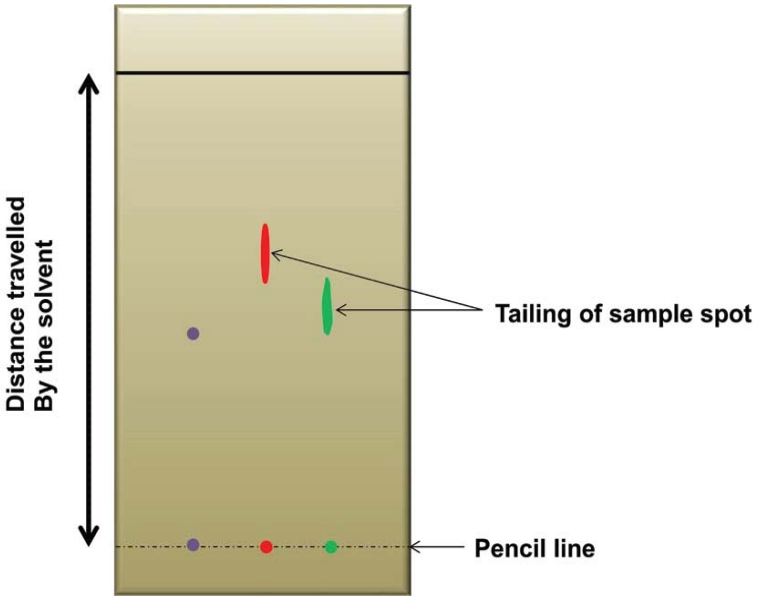


Figure 3.34. Tailing effect in thin layer chromatography.

Source: http://lib.kstu.kz:8300/tb/books/2019/HiHT/Andreyeva/%D0%9B%D0%B0%D0%B1%D0%BE%D1%80%D0%B0%D1%82%D0%BE%D1%80%D0%BD%D0%B0%D1%8F%20%D1%80%D0%B0%D0%B1%D0%BE%D1%82%D0%B0/lab6.htm.

3.7.4.2. Over-Loading

If the sample is loaded greater than the loading capacity of the TLC plate, a spot with a trail or rocket shape spot is appeared. This issue can be resolved by using a diluted sample.

3.7.4.3. Fluctuation in Temp or Opening of the Chamber

Fluctuation in temperature or solvent saturation in the chamber (due to opening of the chamber during running) disrupts the flow of solvent front and subsequently causes a spot with a trail. It can be eluded by sustaining a uniform temperature and by minimizing the opening of the chamber during operation.

3.7.4.4. No Movement of the Sample

In some cases, sample movement is restricted from the spot after the completion of the run. These complications are recurrent with high molecular weight substances like protein or chemicals having numerous functional groups. In this scenario, variation in polarity or pH of the solvent system can assist to bring the compound into the solvent front to facilitate it to run on a silica plate to get resolved.

3.7.4.5. Movement Is Too Fast

In some cases, the movement of a compound is so vigorous to gives time to interact with the matrix to resolve into individual compounds. In such a case, modification in the polarity of the solvent system could be discovered to impede the running of the sample (Comfurius and Zwaal, 1977; Levison, 2003).

3.7.5. Applications of Thin Layer Chromatography

- Approximation of biomolecules;
- Identification of impurities in a sample;
- Quality analysis of the compound;
- Bio-assay;
- Progress of the chemical reaction;
- Composition analysis of biomolecules/synthetic preparation.

3.8. COLUMN CHROMATOGRAPHY

Proteins have different characteristic features as net charge, shape, stationary phase used, size, and binding capacity, each of these characters feature can be decontaminated using chromatographic techniques. Amongst these techniques, column chromatography is extensively employed (Bartlett, 1959; Guiochon et al., 1983).

This technique is employed to purify biomolecules. Initially, on a column (stationary phase), the sample to be isolated is loaded, then washing is done using buffer (mobile phase) as shown in Figure 3.35. Flow is ensured inside column material located on fiberglass support. The samples are accumulated at the bottom of the device in a time-, and volume-dependent manner (Roth and Hampaĭ, 1973; Revathy et al., 2011).

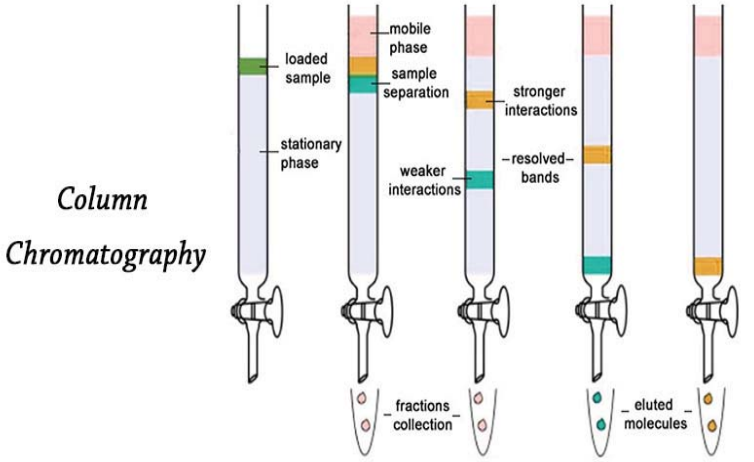


Figure 3.35. Column chromatography process.

Source: https://microbenotes.com/column-chromatography/.

3.9. PAPER CHROMATOGRAPHY

In paper chromatography, support material comprises a cellulose layer highly saturated with water. In this method, a thick filter paper containing the support, and water drops are settled in the pores of filter paper made up of the stationary "liquid phase." The mobile phase comprises a suitable fluid placed in a developing tank. Hence, paper chromatography can be termed as "liquid-liquid" chromatography (Toennies and Kolb, 1951; Saeman et al., 1954).

3.10. GAS CHROMATOGRAPHY (GC)

In GC, the stationary phase is a column placed in the device and carries a liquid stationary phase which is adsorbed onto the surface of non-reactive, i.e., inert solid. GC is also termed "gas-liquid" chromatography. Its carrier phase comprises gases like He or N_2. The mobile phase is an inert gas that is passed through the column under high pressure. The sample to be examined is vaporized and goes into a gaseous mobile phase (Santos and Galceran, 2002). The constituents enclosed in the sample are distributed between the mobile phase and stationary phase on the solid support. GC is a simple, highly sensitive, multifaceted, and rapidly applied method for high-quality

separation of very minute molecules. It is employed for the separation of very little amounts of analytes (Cohen and Karasek, 1970); Marriott et al., 2012; McNair et al., 2019).

3.11. DYE-LIGAND CHROMATOGRAPHY

This technique was developed based on the ability of several enzymes to bind purine nucleotides for Cibacron Blue F3GA dye (Bond and Van Wart, 1984; Neale et al., 1986). Negatively charged planar ring structure has a resemblance to the structure of NAD. This analogy has been demonstrated by binding of Cibacron Blue F3GA dye to adenine, ribose binding locations, or sites of NAD. The dye acts as an analog of ADP-ribose. The binding capability of this type of adsorbents is 10–20-fold durable than that of the affinity of other adsorbents. Under suitable pH conditions, the adsorbed proteins are isolated from the column by elution with high-ionic strength solutions as well as using the IE property of adsorbent (Subramanian and Ross, 1984; Wang et al., 2012).

3.12. HIGH-PRESSURE LIQUID CHROMATOGRAPHY (HPLC)

By employing this technique, one can perform functional, and structural analysis, as well as purification of several molecules within a short interval of time. This method provides perfect results of separation, and identification of carbohydrates, lipids, amino acids, steroids, nucleic acids, proteins, and other biologically active molecules. In HPLC, the mobile phase is passed through columns under 10–400 atmospheric pressure at a high (0.1–5 cm/sec) flow rate. While employing this technique, the use of tiny particles and high pressure on the rate of solvent flow upsurges the separation power of HPLC and the analysis is concluded within a short interval of time (Hancock et al., 1978; Li et al., 2015).

Important components of an HPLC device are high-pressure pump, solvent depot, detector, commercially prepared column, and recorder. The duration of separation of particles is controlled with the help of a computerized system, and material is accrued (Felton, 1969; Chen et al., 2012).

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Chapter

4

Advanced Solvent Extraction Techniques and Processes

CONTENTS

4.1. INTRODUCTION

Conventional solvent extraction is a proven technology for the isolation of solutes from comparatively concentrated feeds found in the industrially produced chemicals and metals by hydrometallurgy. Dilute streams, instead, are quite challenging. A very large value of distribution ratio is required to treat these streams using the conventional liquid-liquid extraction method; otherwise, the organic phase volume would become excessively high from environmental and safety concerns (Hennion, 1999; Gilbert-Lypez et al., 2009).

In the last decades, the established innovative solvent extraction technologies tried to address these challenges. The potential of these technologies to enhance the outcome of conventional solvent extraction is explored in this chapter consistent with modern trends in the principles and solvent development (Figure 4.1) (Aulakh et al., 2005; Sбnchez-Rojas et al., 2009).

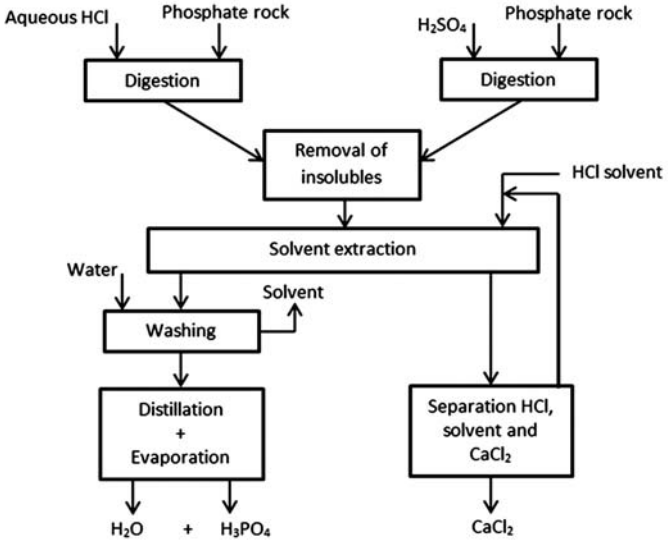

Figure 4.1. Diagram of the solvent extraction process.

Source: https://www.researchgate.net/figure/Diagram-of-solvent-extraction-process_fig3_318397493.

The preparation of treated solutions endures a key role in the development of safer, faster, and more environmentally friendly techniques for extraction,

leaching, and extract cleaning as observed in the last two decades. The primary driving force for developing these techniques is the process of reduction of organic solvent consumption. Some approaches include miniaturization (e.g., headspace solid-phase microextraction (SPME), stir bar sorptive extraction, SBSE, HS-SPME), adsorption of solutes on solids (e.g., matrix solid-phase dispersion, solid-phase extraction, MSPD, SPE), and enhanced solvent-extraction (e.g., pressurized liquid extraction, microwave-assisted extraction, MAE, PLE, etc.), which at present perform a vital role in the handling of a sample in analytical labs. Deep considerations have also been paid for using alternate solvents, primarily supercritical fluids, and more lately, ionic liquids (ILs). Majority of these state-of-the-art techniques are established initially by analytical chemists for preparation of samples and determination necessities, but modern and future propensities in extraction-based technologies use and develop them (Du et al., 2010; Rodriguez et al., 2010).

Some innovative technologies developed in the last decades will be deliberated in the following sections.

4.2. EQUIPMENT DESIGN AND SCALE-UP CONSIDERATIONS

Lab-scale solvent extraction studies are generally carried out using test tubes, a procedure that is inexpensive but more arduous, time-consuming, and typically produces more dispersed data than using Lewis-type cells or semi-continuously stirred baffled beakers. Highly precise distribution data can be acquired resourcefully through mechanical means by continuous-flow centrifugal separators, or physically by utilizing distinctive phase separating filter devices—few of these techniques are described. Even though these techniques are intended for improvement, new, and more progressive methods are expected to appear shortly (Hauthal, 2001; Buszewski and Studzinska, 2008).

Particular reagents with exotic structures are expensive to create, which needs the experimentalist to utilize slight amounts to prepare these reagents. It indicates a desire of shifting from “milli-experiments” (e.g., mixing 10 mL of each phase) to micro experiments (mixing 50 μL of each phase), which is now transpiring, to even smaller sizes (volumes and amounts)—the nano experiments. Development in this field entails a high degree of creativity (Anklam et al., 1998).

Though single-stage laboratory techniques deliver the first step toward multistage industrial procedures, such process development typically obliges small-scale multistage and pilot-plant scale equipment. Numerous exceptional designs exist, and we consider additional fundamental developments (Del Valle and Aguilera, 1999).

The industrial application of solvent extraction is an established technique, and it is now possible to transfer from laboratory experiments to a new extraction system for complete industrial exercise with slight technological risk.

A sufficient variety of large-scale types of equipment is available to manage most of the problems that come across in this application, even though much of the equipment remains relatively massive. Efforts to miniaturize, such as, by employing centrifugal forces to mix and isolate different phases, yet need further developments (Ravento et al., 2002).

Various industrial processes start with a leaching stage, yielding a slurry that must be clarified earlier than solvent extraction. The solid-liquid separation is an expensive step.

The solvent extraction of unclarified liquids (solvent-in-pulp) has been suggested to eliminate solid-liquid separation. Large revenue and abridged energy cost have made this process attractive, but many complications remain unresolved: equipment design optimization, loss of solutes and extractants to the solid phase, effluent removal, etc., (Hildebrand and Scott, 1958).

Regeneration of the extractant is a critical step in the industrial solvent extraction technique. This can be accomplished through numerous ways, e.g., by evaporation, distillation, or stripping (back-extraction). Even though distillation and evaporation do not differentiate between solutes (the diluent is merely removed by heating),

stripping, by a cautious choice of strip solution and conditions, can be made extremely selective. Otherwise, all the solutes can be stripped and then exposed to a selective extraction by varying the extractant. There are many opportunities, and it may be valuable to explore new pathways (Valcárcel and Tena, 1997).

Membrane extraction is comparatively a new technique for solvent extraction, in which a solute is transported from one aqueous phase to another through a membrane holding an extractant dissolved in a diluent. This resourceful scheme has only been explored to some extent, though

it proposes great potential forthcoming, e.g., for cleaning of wastewater (Bungert et al., 1998).

The step from laboratory experiments through pilot plants to industrial-scale necessitates serious deliberations of all the points here; practical exposure is priceless to avoid inaccuracies and excess expenditures (Erkey, 2000).

4.3. SUPERCRITICAL FLUID EXTRACTION (SFE)

Solvent extraction processes commonly require ambient temperature and pressure to run. Higher pressures and elevated extraction temperatures are required when equilibrium or mass transfer conditions are more advantageous at higher temperatures and pressure.

In the case of distillation, the difference in vapor pressures of the constituents to be separated is considered, whereas in solvent extraction, differences in intermolecular interactions in the liquid phase are used (Teja and Eckert, 2000). The combination of both distillation and solvent extraction to some extent gives rise to another separation technique known as supercritical fluid extraction (SFE).

Because of having various physicochemical characteristics, SFE delivers numerous operational benefits over conventional extraction methods. Since, because of having low viscosity and high diffusivity, supercritical fluids possess improved transport properties than other liquids, which make them diffuse easily through solid materials, thereby providing faster extraction yields.

One of the foremost features of a supercritical fluid is the prospect of altering the density of the fluid by varying its temperature and/or its pressure. As density is interrelated to solubility, solvent strength of the fluid can be adjusted by changing the extraction pressure (Tonthubthimthong et al., 2004). Similarly, supercritical fluid possesses other advantages as compared to other extraction methods, such as consumption of solvents usually acknowledged as safe, higher efficiency of the extraction process (increased yields with lower extraction times), and the prospect of direct coupling with analytical chromatographic methods like gas chromatography (GC) or supercritical fluid chromatography (SFC) (Figure 4.2).

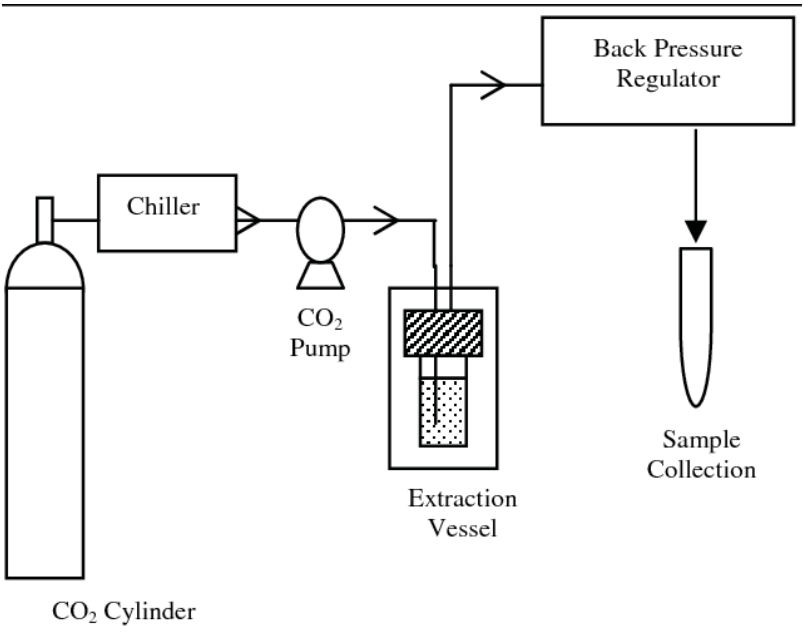

Figure 4.2. Flow diagram of supercritical fluid extraction system.

Source: https://www.researchgate.net/figure/Flow-diagram-of-supercritical-fluid-extraction-system_fig1_26439786.

By increasing temperature at constant pressure, solvent density decreases, resulting in lowering solubility. Conversely, an increase in temperature leads to an upsurge in vapor pressure. At elevated pressures, the reliance of density on temperature is lesser compared to that on vapor pressure, resulting in improved solubility. At low pressure, the density effects are more dominating at increased temperatures, triggering a reduction in solubility (Figure 4.3) (Fernández-Ronco et al., 2010).

Figure 4.1 shows the characteristic curve for various mixtures depicting limited mutual solubility of the components. From the figure, it is pertinent to derive abridged, general principles from the figure in the supercritical area for extraction processes, demonstrated schematically in Figure 4.2 (Perakis et al., 2010).

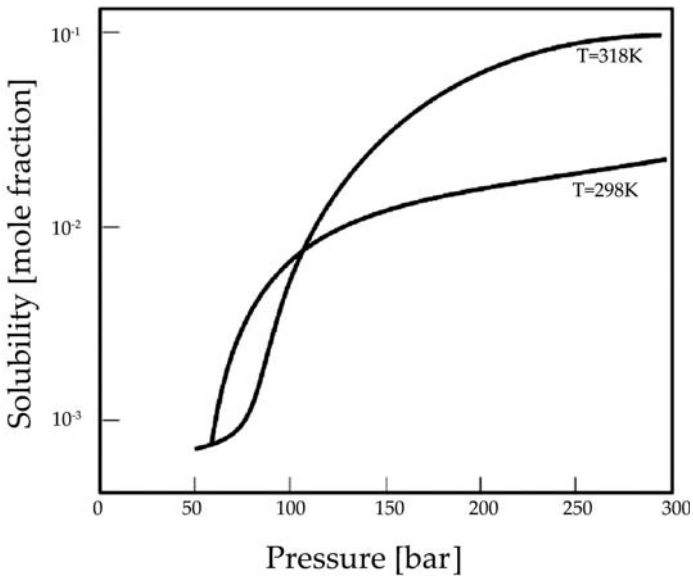

Figure 4.3. Solubility of naphthalene in dense ethylene as a function of pressure at two different temperatures: The solubility enhancement in the vicinity of the critical point, as well as the crossover effect, is evident.

Source: https://www.semanticscholar.org/paper/Solubility-of-Solids-and-Liquids-in-Supercritical-Aim-Fermeglia/1d8ded2348b676fc74f821f2bbab32406b5781a4/figure/0.

Figure 4.2 demonstrates the principle for extraction and solvent retrieval by changing pressure. The point E1 shown in Figure 4.1 denotes the state in the extractor (30 MPa). The extract phase is passed via an expansion valve at a reduced pressure of 8 MPa (point S1 in Figure 4.1). Resultantly, naphthalene starts to precipitate in the separator. Later, the solvent is recompressed and reverted to the extractor (Klesper et al., 1962).

In Figure 4.2, naphthalene is extracted at a pressure of 6 MPa and temperature 285 K (point E2 shown in Figure 4.1). The extract phase is passed through a heat exchanger at an increased temperature of 315 K (point S2 shown in Figure 4.1). Additionally, naphthalene also precipitates in the separator. The solvent is passed through one more heat exchanger to reduce the temperature and is then reverted to the extractor (Griffiths, 1988).

As shown in Figure 4.2, the extracted substances can also be retrieved through adsorption (e.g., on activated carbon). Varying the density of the solvent avoiding passing through any phase borders makes the supercritical area more exciting because of separation. It can be achieved by gradually decreasing the pressure, e.g., to fractionate a mixture of substances (Figure 4.4) (Harris, 2002).

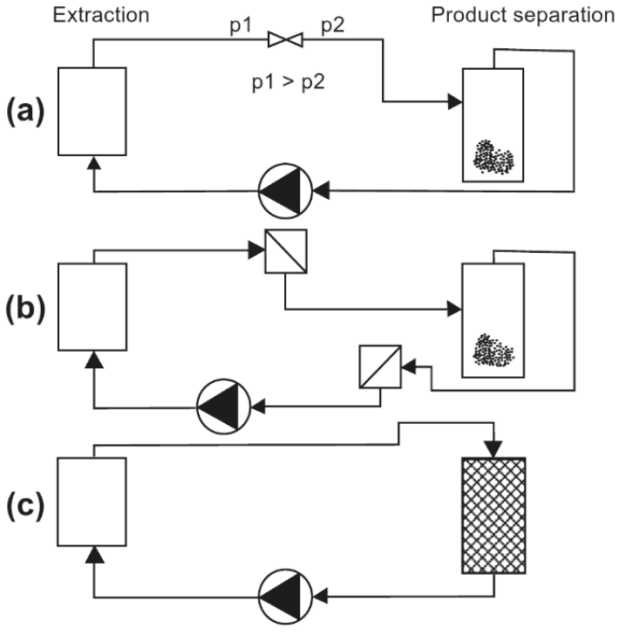

Figure 4.4. Extraction and separation through reducing the pressure; (b) extraction and separation through a temperature change; and (c) extraction and separation through adsorption.

Source: https://www.sciencedirect.com/book/9780444537782/solvent-extraction.

A range of compounds can be consumed as supercritical fluids (Table 4.1). The main focus has been given to supercritical carbon dioxide solvent because of having traits like low cost, nontoxicity, environmentally friendly, low critical temperature (31.3°C) as well as moderate critical pressure (72.9 atm). Carbon dioxide is a nonpolar solvent and dissolves primarily nonpolar solutes (Taylor, 2009). The solubility and selectivity can be modified by the addition of small amounts of polar solvents, called entrainers (e.g., water or ethanol).

Carbon dioxide exists in the gas phase at room temperature, therefore when the extraction is finalized, and the system decompressed, a significant amount of CO_2 eliminated without residues, producing a solvent-free extract. When carbon dioxide usage is high as in industry, the operation can be organized to recycle it. Nevertheless, because of the low polarity of supercritical CO_2, where solubility parameter, *d*, that provides a capacity of the solvent polarity, has minimal effect on extracting considerable polar compounds from natural matrices. To overwhelmed this problem, modifiers (also known as entrainers or cosolvents) are usually used. These modifiers are highly polar, and by adding small amounts, they can yield significant changes in the solvent properties of neat supercritical CO_2 (Welch et al., 2005; Taylor, 2009).

4.3.1. Applications of SFE

Several vegetable matrices as a natural source have been utilized for compressed fluid extraction. Natural antioxidant compounds have been obtained by processing aromatic plants, legumes, spices, and fruit beverages, like natural orange juice, etc. Till now, more than 60 SFE extraction plants have been explored which are operating around the world. In SFE much more interest has been given to carbon dioxide to utilize it to extract various natural products from solid and liquid materials. Examples of bulk use of carbon dioxide are processes in the pharmaceutical industry, food, decaffeinating coffee beans, cosmetics, and oil and hop extraction. For extraction of solutes from aqueous solutions, several processes have been vetted in feasibility tests that use carbon dioxide as a solvent. SFE carbon dioxide extraction of metal ions from aqueous solutions has been studied enormously. A partial list contains acetic acid, dioxane, ethanol, acetone, and ethylene glycol which are used as a solute. The reason behind these efforts is to achieve potentially low energy costs as compared to distillation and the environmental benefits of using carbon dioxide (Bolanos et al., 2004; Toribio et al., 2006).

The design of an SFE system is designed simple or highly complex depending upon the particular requirements. It is conceivable to discriminate between analytical systems and pilot- or industrial-scale systems. An analytical system is used for sample preparation preceding, e.g., a chromatographic analysis. Several configurations exist depending upon the degree of automation (Figure 4.5) (Pinkston et al., 2006; Ramirez et al., 2007).

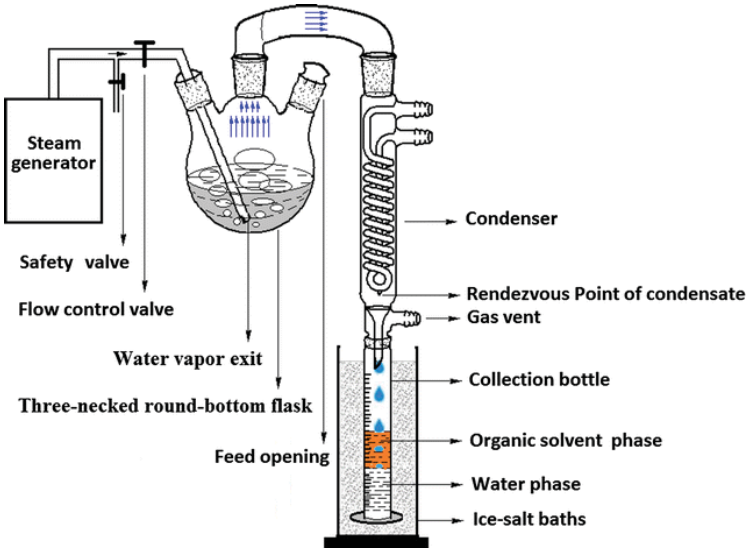

Figure 4.5. The diagram of steam distillation/drop-by-drop extraction device.

Source: https://bmcchem.biomedcentral.com/articles/10.1186/s13065-017-0329-6.

For extraction in grams, pilot systems are utilized, whereas, industrial-scale is used for extracting in kilograms. Generally, the system on a pilot-scale plant (Figure 4.3) comprises a solvent pump, which distributes the fluid all over the system, a modifier pump if necessary, an extraction cell or extraction column, depending on the system configuration (i.e., for solids or liquids), and one or more separators (also known as fractionation cells) for collection of the extract and the solvent is depressurized. Similarly, the extraction cell or column and the separators are generally fitted with independent control of temperature and pressure to carry out fractionations of the extracted compounds by stepwise depressurization. As a result, different compounds can be acquired within each separator, based on their differential solubility in the supercritical fluid. Moreover, a specially designed refrigerated system can also be installed to trap highly volatile compounds, as well as a reprocessing system to recycle the fluid employed (Luque et al., 1999; Miller and Hawthorne, 2000). As mentioned earlier, the major difference between pilot plants to process a solid or a liquid sample lies in the use of an extraction cell or an extraction column. Solid processing is carried out in batch in a discontinuous or semicontinuous mode, whereas liquid processing is done under countercurrent conditions in a continuous

process. In process of liquid-sample extractions, the supercritical fluid (CO_2) moves upwards, whereas the sample feed, whether introduced from top or bottom in the system, moves in a downward direction with the help of gravity (Li et al., 2000). Other systems that are inspected include the separation of biocides from edible oils and isolation of different components in vegetable oils. The utilization of SFE in different applications are studied in detail in different literature references (Luque de Castro and Jiménez, 1998).

4.3.2. Supercritical Fluid Chromatography (SFC)

While using supercritical fluids in mobile phases in chromatography should act as constituent carriers similar to the mobile phases in GC and should dissolve these constituents as mobile phases do in LC. This chromatographic technique, called supercritical fluid chromatography (SFC), was explored by Klesper et al. (1962) and proved as a bridge between GC and LC. However, the technique became prevalent by introducing capillary columns. Key elements of those open tubular columns SFC instruments comprise the capillary columns, pump, restrictor, GC-like oven, and flame ionization detector (Jiménez et al., 1997). Open tubular SFC was principally applied in the petrochemical industry because of the lipophilic character of supercritical CO_2. Nevertheless, the technique possessed severe limitations owing to its poor reproducibility and limited application range (Figure 4.6) (Hawthorne et al., 1994; Yang et al., 1995).

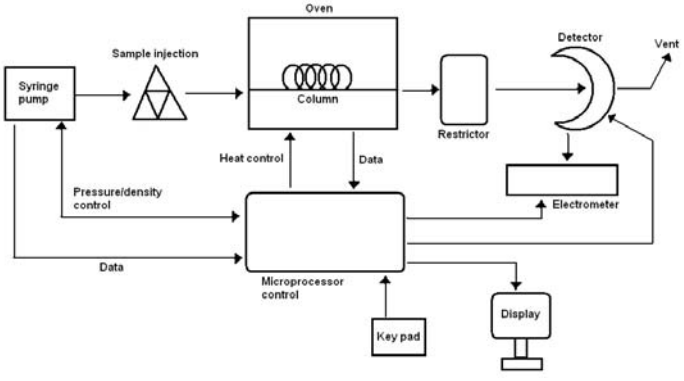

Figure 4.6. Scheme of a supercritical fluid chromatography instrument.

Source: https://chem.libretexts.org/Bookshelves/Analytical_Chemistry/Book%3A_Physical_Methods_in_Chemistry_and_Nano_Science_(Barron)/03%3A_

Principles_of_Gas_Chromatography/3.03%3A_Basic_Principles_of_Supercritical_Fluid_Chromatography_and_Supercrtical_Fluid_Extraction.

On the other hand, the packed column SFC technique has widespread applications in major sectors because of having components similar to those of LC instruments comprising packed columns, injectors, digital back pressure controllers, UV detectors, and organic modifiers for increasing the solvating tendency and decreasing the retention time of polar solutes (Basile et al., 1998). The main reasons behind the last revitalization of the technique are: steady flow rates, automatically controlled modifier addition, the elimination of back-pressure regulation problems, automation, and new stationary phase incorporation, the increased claim for environment-friendly methods that exterminate or significantly decrease the use of organic solvents, sample injection, and the hyphenation of packed column SFC to MS. Currently, it is extensively acknowledged that SFC is a sort of normal-phase LC, deprived of many of the problems related to normal-phase LC (Kipp et al., 1998).

The use of packed columns in SFC is emphasized currently, although numerous studies appear every year which use open tubular columns. Packed column SFC applications comprise chiral separations, 26 mass spectrometric detections of pharmaceutical compounds, 27 and natural product applications with preparative separations (Fernández et al., 2000).

In brief, SFC constitutes a green alternative, i.e., CO_2 as a mobile phase and occasionally altered amounts of green organic modifiers, like ethanol or methanol, to lessen the organic solvent usage in analytical chemistry (Gámiz-Gracia et al., 1999).

4.3.3. Advantages and Disadvantages of Supercritical Extraction

The foremost disadvantage of SFE is to use of expensive process equipment to generate the elevated pressure. The critical pressure requirements are lower than the pressures used in many high-pressure processes in the petrochemical industry these days. Table 4.1 gives information regarding critical data for commonly used solvents in high-pressure extraction. It demonstrates the satisfactory mass transport properties that can be attained in the supercritical area in consequence of low viscosity and high diffusivity, compared with the liquid phase. The separation properties in SFE are determined by the selection of solvents and solutes (Jiménez et al., 1999).

4.1. Critical Properties of Several Solvents Used in Supercritical Fluid Extraction (SFE)

Solvent	**Critical property**			
	Temperature (°C)	**Pressure (atm)**	**Density (g/mL)**	**Solubility parameter δ_{SFC} ($cal^{-1/2}$ $cm^{-3/2}$)**
Ethene	10.1	50.5	0.2	5.8
Water	101.1	217.6	0.322	13.5
Methanol	-34.4	79.9	0.272	8.9
Carbon dioxide	31.2	72.9	0.47	7.5
Ethane	32.4	48.2	0.2	5.8
Nitrous oxide	36.7	71.7	0.46	7.2
Sulfur hexafluoride	45.8	37.7	0.73	5.5
n-Butane	-139.9	36	0.221	5.2
n-Pentane	-76.5	33.3	0.237	

4.4. SUBCRITICAL WATER EXTRACTION (SWE)

In subcritical water extraction (SWE), hot water under pressure (critical point of water, 22.4 MPa, and 374°C) is used for extraction. This useful tool is recently emerged to substitute the traditional extraction methods. SWE is an environment-friendly technique that delivers higher extraction yields from solid samples. SWE is employed using hot water (from 100 to 374°C,) under high pressure (from 10 to 60 bar) to sustain the liquid phase of water (Figure 4.7) (Jiménez and Luque de Castro, 1999).

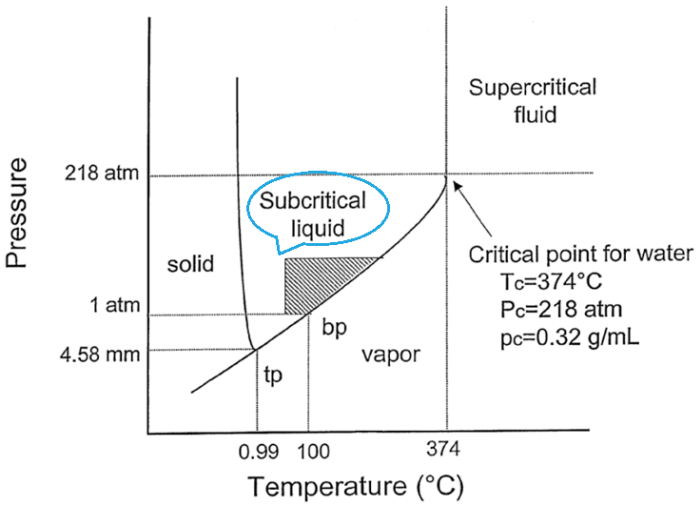

Figure 4.7. Phase diagram of water as a function of temperature and pressure.

Source: https://www.intechopen.com/books/mass-transfer-advances-in-sustainable-energy-and-environment-oriented-numerical-modeling/subcritical-water-extraction.

4.4.1. Extraction Technique

Imperative factor to deliberate in this sort of extraction procedure is the changeability of the dielectric constant with temperature. Water is a polar solvent having a dielectric constant close to 80 at room temperature. But, the value of the dielectric constant significantly drops to 27 while increasing temperature up to 250°C and keeping water at its liquid state under appropriate pressure. This dielectric constant value is comparable to that of ethanol, i.e., 30 (Chalchat et al., 1991).

At elevated temperatures (beyond the boiling point of water: 100–374°C), pressurized steam is required proficient transfer through the material. A user-friendly experimental device is required for SWE. Essentially, the arrangement comprises a water reservoir attached to a high-pressure pump to transfer solvent into the system, a heating oven, where the extraction cell is sited, and extraction occurs, and a restrictor or valve to sustain the pressure. Extracts are accumulated in a container located at the end of the extraction system. Additionally, for rapid cooling of the resulting extract, a coolant device can also be attached to the system (De-Pooter et al., 1995; Soto et al., 2001).

Even though this technique has been employed mainly as a batch process, studies also reported some continuous techniques as well as online coupling of an SWE system to an HPLC equipment through a solid phase trapping (Verma et al., 1990).

4.4.2. Extraction From Plants Using SWE

The SWE is evolving as a prevailing substitute for the extraction of solid substances. It has been utilized for the extraction of pollutants comprising a wide range of polarities from environmental samples as well as for the extraction of pesticides and polycyclic aromatic hydrocarbons from soils (Kubatova et al., 2001). Basile et al. (1998) suggested utilizing SWE as a very prevalent alternate system as compared to conventional and supercritical CO_2 extraction methods for the separation of necessary oils. Studies revealed that the suggested SWE method is rapid, economical, and more operative than hydrodistillation (Ibañez et al., 2003). In the meantime, this method has shown its widespread utilization in the field of essences compared to other conventional techniques such as steam distillation and solvent extraction, which possess some renowned disadvantages: extended extraction time, low extraction proficiency, and large amounts of toxic solvent waste. It also has the benefit of being selective (Kubatova et al., 2001).

SWE has been employed extensively for the extraction of various compounds from numerous vegetable matrices. Similarly, rosemary (*Rosmarinus officinalis* L.) is one of the materials studied in detail using SWE. De-Pooter et al. (1995) carried out a study on the extraction of antioxidant compounds of rosemary using SWE at various temperatures. Different temperatures, ranging from 25°C to 200°C, were tested to explore the extraction selectivity toward antioxidant compounds. The effect of water temperature was evident by the extraction yield, which was further improved at higher extraction temperatures. Researchers proved that the “rosmanol” was the main and most polar compound extracted at low temperatures (25°C). However, it was observed that the extraction performed at 200°C, decreased the ability of water to dissolve the most polar compounds, instead, a high concentration of other compounds, like carnosic acid, was obtained. Antioxidant extraction by SWE could be acquired similar to those achieved by using supercritical carbon dioxide (Ozel et al., 2003; Moreda-Piñeiro et al., 2006).

Generally, the use of SWE delivers numerous advantages over conventional extraction techniques (i.e., organic solvents, hydrodistillation,

and solid-liquid extraction). These provide rapid extraction at low operational temperatures, consequently evading losses and degradation of volatile and thermolabile compounds. It is beneficial of being selective because, through kinetic experiments, manipulation of the extract composition is conceivable in certain working conditions. Additional advantages of the technique are the optimum quality of the extracts (for essential oils), reduced costs of the extracting agent, and environmental compatibility (Lou et al., 1997).

The extraction of essential oil from *Thymbra spicata* was explored. The impacts of several factors, for instance, pressure (20, 60, and 90 bar), temperature (100, 125, 150, and 175°C), and flow rate (1, 2, and 3 mL/min) were studied in detail. It was revealed that the optimal extraction yields (3.7%) were achieved at 150°C and 60 bar, with a flow rate of 2 mL/min for 30 min. The essential oils of *T. spicata* were found to prevent the mycelial development of several fungi species (Guillemin et al., 1981; Alonso-Rodríguez et al., 2006).

4.4.3. Pressurized Liquid Extraction (Also Known as Accelerated Liquid Extraction (ALE))

PLE technique is more or less similar to SWE—the difference lies only in the use of solvents. PLE is utilizing organic solvents or diluted organic acids including acetic and formic acids, at elevated temperature and pressure without attaining the critical point. For instance, the PLE system testing intended for pretreatment of biological material for extraction and trace element leaching is explained below (Smith et al., 1999; Su et al., 2005).

This system comprises a stainless-steel extraction cell for the placement of samples and where electronically controlled heaters and pumps are used to regulate the programed parameters like temperature, static extraction time, pressure, and extraction steps. When the sample is loaded in the extraction cell, the solvent is added, and the required temperature and pressure parameters are selected to heat and pressurized the extraction cell. Subsequently, a static extraction period follows when the sample releases solutes to the solvent. After completion of the static step, the valve is untied and the solvent is moved to the collection container. Residues of the solvent are expelled from the sample to the collection vial using a suitable gas. By increasing the pressure on the sample cell (from 4 to 20 MPa), the solvent can be kept in a liquid phase even at comparatively high extraction temperatures (up to 200°C). This increases productivity over classic extractions by reducing extraction times and decreasing solvent volumes

(Coym and Dorsey, 2004; Smith, 2006). PLE procedures are deliberated to be comparatively straightforward because of having limited parameters to be optimized, thus decreasing the time dedicated to the development of the extraction procedure (Ingelse et al., 1998). The use of PLE to support trace metals acid leaching could preferably provide several advantages while compared to mechanical shaking, ultrasound (water bath or probe), or microwave-assisted acid leaching techniques (Pawlowski and Poole, 1999).

The following are the advantages of the technique (Teutenberg et al., 2001; Kondo and Yang, 2003):

- Weak organic acids can be used in place of strong concentrated mineral acids, usually needed for leaching. Besides, small volumes of such acids are used, which infers low toxic wastes.
- Short leaching or extraction times are adequate to conclude the extraction, which further escalates the sample output.
- PLE offers a high degree of automation and therefore no supplementary filtration step is required after acid leaching assisted PLE.
- Ever since diluted organic acids could be utilized to leach the metals, chemical species integrity is assured, which makes PLE to be useful for studying organometallic speciation (Hatti-Kaul, 2000).

To extract or leach entire elements PLE may be completely exploited in ores leaching and extraction evading concentrated mineral acids use. While applying PLE conditions, diluted weak carboxylic acids can react with the matrix, and can proficiently leach major trace elements. Variables that affect the carboxylic acid can be found by testing the PLE process using multivariate approaches compromising acid leaching and extraction conditions (Sanagi and See, 2005).

4.4.4. Superheated Water Chromatography (SHWC)

In reversed-phase liquid chromatography (RPLC), water is utilized to decrease the elution strength of the organic modifiers, like acetonitrile, methanol, and tetrahydrofuran. Though, the same enhanced solvation strength defined in the preceding section also empowers superheated water to be used as a mobile phase in LC. This approach of separation has been termed superheated water chromatography (SHWC), pressurized water chromatography, or subcritical water chromatography (Wilkes and Zaworotko, 1992).

The detailed study of separation about the use of hot water as the mobile phase offers an introduction to the technique and designates the huge potential of the technique governing greening analytical chemistry (Rogers and Seddon, 2002). The equipment required is similar to that used in conventional LC, with the addition of a high-temperature oven and a method of regulating the backpressure of the column (Figure 4.7). An additional advantage of SHWC includes the use of water with wide-ranging detectors. It is well-suited with refractive index, UV, and fluorescence, electrochemical, evaporative light scattering, and mass spectroscopy (MS) detection. Furthermore, it also possesses compatibility with flame ionization detection, being convenient for revealing analytes lacking chromophores, like amino acids, aliphatic alcohols, and carbohydrates (Rogers et al., 1999, 2000).

Nevertheless, the limited accessibility of commercial systems appears to be the foremost cause for the shortage of widespread implementation of the method. The selectivity variations on heating water are not much effective compared to adding an organic modifier, like methanol, acetonitrile, or 2-propanol. Though, high-temperature water could deliver a complementary selectivity which makes it most suitable for polar analytes. A 3.5°C increase in water temperature relates to a 1% increase in methanol and a 5–8°C rise in temperature corresponds to a 1% increase in acetonitrile, by employing a series of aromatic analytes (Nakashima et al., 2003; Cornmell et al., 2008).

SHWC has been effectively applied in the pharmaceutical industry, where the polarity of analytes is mostly compatible with an aqueous eluent. For example, separation of anticancer drugs is accomplished by using polystyrene-divinylbenzene column with buffered superheated water as the mobile phase. The temperature range was maintained between ambient temperature up to 160°C, and the pH value of water was adjusted to 11.5 and 3.5 using phosphate buffer. The aggregate elution time was below 13 min. Another study successfully executed the separation of alkyl aryl ketones and barbiturates by using water-rich and superheated water at elevated temperature (100–200°C) as the eluent (Visser et al., 2001a, b). SHWC may be explored to provide benefits in the environmental and food industries.

4.5. AQUEOUS TWO-PHASE SYSTEMS

Under certain circumstances, polymer unsuitability in aqueous solutions can result in the creation of two phases with high water content. With such kind of system, it is likely to separate delicate biological molecules, such as proteins, without denaturation, which could be faced by using an ordinary

aqueous-organic solvent system (Visser et al., 2002; Liu et al., 2005). Biotechnological processes, on the whole, work at small concentrations of reactants and products. One cause of this is the high sensitivity of biocatalysts to product inhibition. The reaction may probably stop after the conversion of a small fraction of the substrate into the product. In such conditions, continuous extractive processes are highly desirable in which the product is uninterruptedly removed (Fadeev and Meagher, 2001). The combination of bioconversion and extraction is termed extractive bioconversion. The unfolding of enzymes may occur when exposed to the interface between the two solvents having considerable differences in surface tension and dielectric constants such as water and common organic solvents. This issue can be resolved through extractive bioconversions in aqueous two-phase systems. A series of innovative methods envisioned to deliver practical strategies for the area of separations in two-phase systems have been studied (Vidal et al., 2007).

4.6. EXTRACTION PROCESSES WITH IONIC LIQUIDS (ILS)

Ionic liquids (ILs) are a new class of extractants of pronounced interest deliberated as potential "green solvents." Essentially, zero vapor pressure and thermal stability of ILs mark them attractive solvents for numerous applications. ILs are achieving high significance as novel solvents in chemistry, though they are not new, e.g., $[EtNH_3]^+ NO_3^-$, was discovered in 1914. They are also recognized as nonaqueous ILs, molten salts, room-temperature (RT) ILs (not all ILs are RTILs), liquid organic salts, or fused salts. Ethyl methylimidazolium tetrafluoroborate as one of the new IL, is getting much consideration being used as a novel medium for homogeneous catalysis, as reported in 1992. The use of ILs to separate analytes in analytical chemistry, is valuable because of having some exceptional properties like high thermal stability, negligible vapor pressure, tunable viscosity, and miscibility with water and organic solvents, along with good extractability for several organic compounds and metal ions (Cull et al., 2000). Being nonvolatile and nonflammable, make them environment-friendly solvents for "clean processes" and "green chemistry," and good substitutes for traditional volatile and flammable organic solvents. However, high viscosity at ambient temperature could be their less favorable property (Huddleston et al., 1998; Banerjee et al., 2008).

4.6.1. ILs in Separation Techniques

The use of ILs as extractants for the extraction of metal ions, such as lanthanides, and actinides, are of particular industrial attention. Furthermore, the extraction of Cd^{2+}, Co^{2+}, Ni^{2+}, Fe^{3+}, Na^{+}, Cs^{+}, Sr^{2+}, $Hg^{2+,}$ and Cd^{2+} from wastewater by using task-specific ILs has been effectively achieved with high yield (Marták and Schlosser, 2003). Likewise, ILs have also been used for the extraction of multiple organic compounds including carbohydrates, biofuels, substituted benzene derivatives, and erythromycin from aqueous solutions.

Experiments comprising the use of ILs for solvent extraction of organic acids revealed promising results in the extraction of organic acids, particularly lactic acid. In the extraction of lactic acid, butyric acid, and phenol, fairly higher distribution coefficients were observed for solvents with tested developmental ionic liquid IL-A compared to the solvents comprising tertiary amines. The value of the distribution coefficient of lactic acid obtained is up to 30 at reduced acid concentrations. IL-A extractant creates undissociated lactic acid/IL-A complexes with ratios of 1:1 and 2:1 (lactate anions are not extracted).

In the pertraction of LA through the supported liquid membrane (SLM), the entire mass-transfer coefficient rises with decreasing concentration of lactic acid in the aqueous phase, which directly relates to the increasing value of its distribution coefficient. Amplified concentration of the carrier IL-A showed no effect on the value of the mass-transfer coefficient in pertraction of lactic acid differing from the improved value of the distribution coefficient. This may specify that either the slower kinetics of the interfacial reaction in the decomposition of the complex plays a vital role or the increased viscosity of the membrane is responsible for this (Marták and Schlosser, 2004a, b).

Separation of taurine (2-aminoethanesulfonic acid) and sodium sulfate is accomplished by leaching a solid mixture by ILs as observed elsewhere. Dialkylimidazolium chloride IL was developed as a leaching agent and organic solvent including ethanol as a precipitating agent, showed promising results. Selective separation of taurine from a solid mixture comprising a large amount of sodium sulfate could yield 67–98.5% within a single separation step (Gu et al., 2004).

Transport of amines and neutral organic substances through liquid membranes having IL has also been investigated in references. Similarly, pertraction of organic acids through liquid membranes aided by enzymatic reactions on L-L interfaces by using ILs as a liquid membrane was also explored. Modifications in ILs for attaining better partitioning of particular

solutes and adequate viscosity of IL may bring about remarkable results (Branco et al., 2002; Fortunato et al., 2002). Extraction of formaldehyde from mushrooms by LPME using ILs has also been explored. Furthermore, the approach has also been implemented to the screening of chlorobenzenes, phenols, dichlorodiphenyltrichloroethane, and its metabolites, and chlorophenols in water samples. But, this SDME procedure is irreconcilable with GC due to the nonvolatile and nonflammable nature of the IL (Miyako et al., 2003a, b).

4.6.2. Ionic Liquids (ILs) in Analytical Separations

Scientists are paying more attention to exploring applications of ILs in separation and analysis. Numerous reports comprise the use of ILs as running electrolytes in capillary electrophoresis (CE). Furthermore, ILs possess many promising characteristics, such as nonvolatility, nonflammability, high viscosity, high solubility for numerous compounds, and polarity, making them distinctive stationary phases in GC, however, both the topics are beyond the scope of this chapter and are not addressed in detail. Alternatively, ILs cannot be used as a mobile phase because of high viscosity, instead, these can only be used as beneficial additives in mobile phases. For detailed information work of Marsza and Kaliszan (2007) on the use of ILs in LC is referred (Wood and Stephens, 2010).

4.7. LIQUID MEMBRANE AND NONDISPERSIVE, MEMBRANE-BASED SOLVENT EXTRACTION TECHNIQUES

Membrane separation is a comparatively new and emerging field of supramolecular chemistry. The liquid membrane process is a three-phase system comprising two phases of identical nature with varying compositions (aqueous-aqueous, gas-gas, organic-organic) separated by the third phase of different nature as well as insoluble with the other two phases. This central phase is designated as the liquid membrane (Bekou et al., 2003).

Liquid membrane separation techniques comprise three major configurations which are extensively explored due to their potential industrial use (Liu et al., 2005, b):

- Emulsion liquid membrane (ELM), or surfactant liquid membrane;
- Supported liquid membrane (SLM); and
- Bulk liquid membrane (BLM).

In the ELM configuration, the liquid membrane is made by dispersing emulsion of the stripping phase in an organic phase containing an emulsifying agent. In the SLM process, the liquid membrane phase infuses into the solid support containing pores of micron size and located between the two bulk phases. The liquid membrane becomes stable by capillary forces or through bonding. Two types of support configurations, i.e., hollow fiber or flat sheet membrane modules, are usually used.

BLM involves three bulk phases—two phases of an identical nature with varying composition and the third immiscible phase is located between them. Separation of phases is done by using hollow fiber or flat sheet membrane supports or may have no support. Numerous different BLM techniques are published, based on membrane-based nondispersive selective solvent extraction attached to permselective diffusion of solute-extractant complexes and selective stripping of the solute in one continuous dynamic process (Peng et al., 2005).

Nondispersive solvent extraction belongs to one of the configurations of the BLM. In "non-dispersive solvent extraction," phases are intacted through porous membrane instead of generating a drop dispersion of one phase in the other phase (Ye et al., 2006, 2007).

Two dissimilar arrangements exist for this process. One comprises two modules—one for extraction and the other for stripping—having close similarity with conventional solvent extraction. The other configuration utilizes one three-liquid phase module in which all the three phases flow through. In the hollow fiber module, the liquid membrane phase lies in the shell, and the feed and the stripping phase get passed through the lumen of various fibers in the module.

The solution to be extracted is provided to the lumen side of a microporous hollow fiber membrane module, with extracting organic solvent being fed to the shell side. The selected membrane is especially wetted by one of the phases (organic phase), and the pressure is controlled in such a way as to avoid capillary penetration of the aqueous phase into the membrane. As follows, the solvent-water interface is stabilized at the boundary of membrane-aqueous solution, and extraction of the particular component into the organic phase occurs through transmembrane diffusion and convection. The solute-enriched organic phase is then transported to a second membrane unit where the solute is shifted into an aqueous solution in which it has eminent solubility—the solute-depleted organic is then recycled to the first unit (Liu et al., 2005c).

This technique partakes significant advantages over conventional solvent extraction—it considerably eradicates emulsification, and requirement of phase separation equipment such as centrifugal separators; it delivers a stable, large interfacial area for interphase mass transfer; and it avoids contamination of the organic phase and extracted substance from solid impurities in the feed. Certainly, if hollow fiber membranes of large lumen diameter are utilized, processing of whole fermentation broths holding high concentrations of suspended biomass is possible, which may result in "continuous extractive fermentation," in which the product-bearing whole broth is transferred to the extraction unit, and the product-depleted broth (containing viable cells) is reverted to the fermenter (Jiang et al., 2003).

4.8. SUPRAMOLECULAR-BASED EXTRACTION TECHNIQUES

During the last few decades, numerous innovative separation techniques were established based on supramolecular extractants. Some examples of these techniques are shown below, which retain many perspectives in the future developments of separation technologies (Breitbach and Armstrong, 2008).

4.8.1. Nano- and Microtechnological Extraction

Nanometer-sized amphiphilic aggregates, made utilizing a self-assembly process, remain growing by the addition of surfactant, and this is the second self-assembly process in supramolecular solvent development. This process cannot occur without the previous one being in place. Aggregate growth will continue till a separate, amphiphilic-rich, liquid phase is created. The phenomenon of liquid-liquid phase separation, usually taking place in colloidal solutions, is called coacervation. These are micrometer-sized aggregates or reversed micelles (Marszałł MP and Kaliszan, 2007).

Ordered structures of molecules in supramolecular solvents comprise a hydrophilic and a hydrophobic part. Resultantly, these structures contain different polarity regions that deliver different interactions for solutes. The type of interaction may be adjusted varying the hydrophobic part of the polar group of the amphiphile and, in theory, one may design the most suitable aggregate for a specific application because of the ubiquitous nature and synthetic chemistry of amphiphiles.

The type of polar compounds which can be extracted by supramolecular extractants depends upon the nature of polar groups located in the ordered structures governing these polar compounds. Until now, frequently used polar groups in analytical applications include carboxylic acids, sulfates, polyethylene oxides, carboxylates, sulfonates, and ammonium and pyridinium ions. Binding interactions intricated during the extraction of polar compounds mostly contain ionic, hydrogen bonding, π-cation, and π-π. Hydrogen bonding is an exceedingly effective preservation mechanism for polar compounds. Instead, when the surfactant comprises a benzene ring, electrophilic interactions are delivered through delocalized electrons in π-orbitals. These delocalized electrons interact with conjugated groups such as aromatic rings or double/triple bonds. The foremost property of supramolecular compounds is their ability to extract amphiphilic compounds (e.g., surfactants, drugs, pesticides, etc.), through the development of mixed aggregates with the amphiphiles resulting in the ordered aggregates. Both hydrophobic and polar interactions manage the development of mixed aggregates. These nanometer- and micrometer-sized aggregates or micelles are innovative extractants for several separation techniques (Michaels, 1990).

While using liquid-phase microextraction (LPME), the solvent is employed in a hollow fiber. Analytes are moved from the donor phase (aqueous) to a receptor phase via an organic phase immobilized in pores of the hollow fiber. In a two-phase mode, the solvent in the pores of the fiber is similar to that in the fiber. In a three-phase mode, the solvent located in the pores of fiber varies from that present in the fiber. Extraction of polar analytes is carried out using a three-phase model, while the two-phase model is used for extracting nonpolar and/ or semi-polar analytes. The amount of solvent in the fiber is about 10–25 L. The advantage of LPME over SDME is the existence of the fiber supporting organic solvent by decelerating the evaporation and dissolution of solvent.

4.8.2. Surfactant-Based Extraction

Alternate extraction methods, based on the usage of surfactant solutions, are suggested to lessen the environmental influence of analytical approaches. Aqueous solutions of some surfactants have also been used in micellar extraction (ME) as well as in cloud point extraction (CPE). ME is based on the fact that the Micellar aggregates, which are capable to solubilize various compounds and have a size that inhibits them from crossing ultrafiltration (UF) membranes, form the basis of the ME technique. The CPE technique

is based on the cloud point effect revealed by the aqueous micellar solution of some surfactants while heating or cooling below or above a certain temperature (Figure 4.8) (Chou and Bell, 2007).

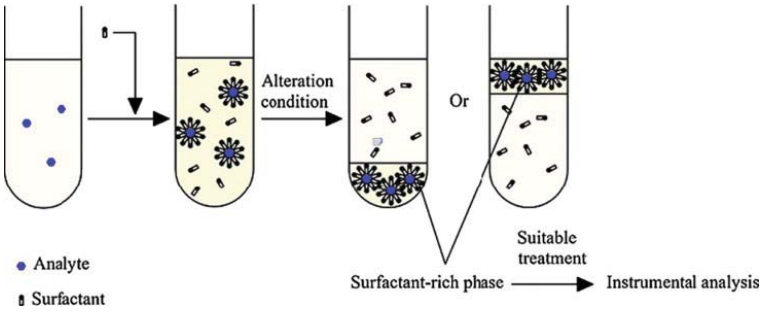

Figure 4.8. Surfactant based extraction process.

Source: https://www.sciencedirect.com/science/article/abs/pii/S0165993611000951.

CPE is also recognized as a micelle-mediated extraction technique, extensively used for the extraction and preconcentration of organic acids, trace metal analysis, and hydrophobic proteins. Though, in recent times, CPE has been employed for the extraction of organic molecules excluding biomolecules. CPE has been effectively exploited for the extraction and preconcentration of PAHs as a primary step to determine them by liquid chromatography (LC) using nonionic surfactants and anionic surfactants, like SDS, sodium dodecylbenzene sulfonic acid (SDBSA), and sodium dodecanesulfonic acid (SDSA). Similarly, various nonionic surfactants have been utilized in the preconcentration of PCBs and dibenzofurans (PCDFs) from seawater. The existence of organophosphorus pesticides, such as paraoxon, methyl, and ethyl parathion, and fenitrothion, has been determined in river water samples by using CPE with the nonionic surfactant Triton X-114 before their separation by LC. The use of the nonionic surfactant poly(oxyethylene glycol) monooctyl ether headed for better extraction yield even for polar species, such as chlorinated phenols and anilines, with recoveries of 88–99% (Seike and Oishi, 2003).

4.8.3. Solvent Extraction with Microemulsions

Some examples of microemulsion application in solvent extraction of metal ions and biological compounds are given below for reference. The effect of extraction parameters is deliberated shortly.

4.8.3.1. Metal Ion Extraction with Microemulsion

Relative to conventional extraction, the organic phase is replaced by a W/O microemulsion containing the reactant in metal ion extraction. Two possible ways are given for the formation of a W/O microemulsion in the solvent phase (Li Guan-Shan, 2008):

- The extractant creates reverse micelles in the organic phase resulting in the development of a microemulsion when this phase is contacted with the aqueous one; and
- The extractant does not make reverse micelles under the conditions of the process, in such case, a surfactant, and occasionally a cosurfactant too must be added to the organic phase to create a reverse micellar phase. In this situation, the reverse micelles are ordinarily mixed, i.e., contain reactant and the additives in the micellar shell.

Only case (2) shows a comparison between conventional and ME. The few comparisons reported in the literature on the metal extraction efficiency of microemulsions comprising an extractant with that of the extractant on its own are, at first sight, inconsistent. In certain circumstances, microemulsions yield both synergism and extraction rate improvement about the single surfactant, whereas in others they significantly decrease the metal distribution coefficient and the extraction rate, or leave them unaffected. Extraction with di(2-Ethylhexyl)phosphoric acid (DEHPA) is a relevant, interesting example. DEHPA does not produce microemulsions in aliphatic solvents at pH 4 or below; however, it forms microemulsions by adding a surfactant and a cosurfactant, e.g., sodium dodecylbenzene sulfonate and *n*-butanol. Enhancements in the extraction of trivalent and tetravalent metals of the conventional system with a DEHPA microemulsion were reported. At extraction of Al(III) using similar microemulsion enhancements were also obtain but not at the extraction of Zn(II), which was considerably reduced concerning the conventional DEHPA system. The researchers tried to elucidate this phenomenon of the microemulsion by the different interfacial behavior of their complexes—the aluminum complex being more hydrophilic have greater desorption energy as compared to the zinc complex, making the interface its desired place, whereas the zinc complex has a high solubility in the organic phase (Drioli et al., 2002). This proposal remains to be confirmed. The same trend of microemulsion in the extraction of Bi(III) and Zn(II) was observed increased yield in the extraction of Bi(III) only, but not of Zn(II).

Microemulsion extraction is an intricate process comprising three phases: two interfaces and an unknown phase morphology. Performance prediction of the microemulsion extraction process is quite challenging, especially while handling the solutes of diverse properties. Different studies revealed that as a result of the emulsifying additives effect, this method does not always improve metal extraction, and may impede the performance. Therefore, the author does not see foresee its contribution towards future development.

4.8.3.2. Extraction of Biological Molecules

Extraction parameters affecting the distribution coefficient and kinetics of biological molecules like proteins and amino acids extraction by microemulsion have been extensively explored as compared to that of inorganic solutes. This is primarily because of the different sizes and structures of these molecules as well as their characteristics are highly prone to solvents and surfactants.

System parameters such as ionic strength, pH, salt type, temperature, surfactant, and cosurfactant concentration, solvent structure and type, and some others define the partitioning of biomolecules between the aqueous and the reverse micelle phases. Moreover, protein size and hydrophobicity are also key characteristics that determine its distribution in the reverse micelle phase (Bartsch et al., 1996).

The solubility of a protein is affected by pH by varying the charge distribution over the protein surface. A protein attains a net positive charge when the pH values are below its isoelectric point (pI), or point of zero net charge, while if the pH values are above its pI, the protein will stay negatively charged. Therefore, if electrostatic interactions are the prevailing factor, solubilization can be achieved if anionic surfactants have low pH values than the isoelectric point (pI) of the protein. The opposite effect would be expected in the case of cationic surfactants.

Protein distribution is also influenced by the ionic strength of the aqueous solution in contact with a reverse micelle phase in many ways. The first effect is through alteration of electrostatic interactions between the protein surface and the surfactant head groups governed by the modified electrical double layers nearby the protein surface and the charged inner micelle wall. The second effect is to “salt out” the protein from the micelle phase as a result of the increased susceptibility of the ionic species to transfer to the micelle water pool, decrease the size of the reverse micelle, and so dislocate the protein (Kislik, 2010).

An increase in the concentration of surfactant increases the number of micelles instead of any significant change in size, thereby improves the capability of the reverse micelle phase to solubilize proteins. Besides, an increase in the surfactant concentration rises the solubilization of protein in the reverse micelle phase. At low "minimal" concentrations (6–20 mmol/dm^3 AOT), reverse micelles could be extremely discriminating in separating identical proteins from fermentation broths (Watanabe and Tanaka, 1978). 95% recovery inactivity could be accomplished.

Transfer of a-chymotrypsin in chloroform-tri octyl methylammonium chloride (TOMAC) system is affected by the temperature. Therefore, almost 50% higher transfer yield was comprehended when the temperature was increased from 25 to 40°C. However, transfer of glucagon was not noticed at room temperature, but the transfer of glucagon may take place at 37°C (Watanabe and Tanaka, 1978).

Evaluation of back-extraction of proteins encapsulated in AOT reverse micelles was performed by the addition of a counterionic surfactant, such as TOMAC or DTAB, to the reverse micelles.128 This innovative backward transfer technique provided optimum backward extraction yields compared to the other traditional methods. The back-extraction process using TOMAC as a counterionic surfactant was 100 times faster compared to the back-extraction with the conventional method, as well as three times faster than forwarding extraction. The 1:1 complexes of TOMAC and AOT present in the solvent phase could be proficiently eliminated using adsorption onto montmorillonite to facilitate the reuse of organic solvent (Bordier, 1981).

4.8.3.3. Potential Applications of Micellar Extraction (ME)

Reverse micelle extraction has also been employed to study the series of binary and ternary protein mixtures. Cytochrome c and lysozyme were quantitatively removed from a ternary protein with ribonuclease A. Separation of ribonuclease A and concanavalin A mixture indicated no interaction among the proteins.

Some studies also demonstrated the recovery of an extracellular alkaline protease using microemulsion extraction from a whole fermentation broth. In a three-stage cascade, a purification factor of 6 and yields of 56% were accomplished. The grouping of a cascade with a higher aqueous/organic ratio, and by using true cross-flow designs, could show promising results for purification without dilution.

Extraction of an a-amylase broth of Bacillus licheniformis by utilizing a CTAB/isooctane/5% octanol reverse micelle system revealed that in a two-step extraction the concentration of protein was declined by a factor of 10 with a purification factor of 8.9, and a maximum yield of 89% a-amylase activity.

Extraction yield of 98% of lysozyme from egg white was accomplished by adding multiple demulsifiers and which improved yields significantly (Boeckelen and Niessner, 1993). Numerous studies carried out to investigate the extraction of intracellular enzymes with reverse micelles have been published recently. CTAB in octane with hexanol was used as cosurfactant which rapidly separated enzyme from whole cells into the water, and additionally, it was capable to lyse promptly and accommodate the pool of surfactant aggregates. In another study, a periplasmic enzyme called cytochrome c553 was extracted from the periplasmic fraction by utilizing reverse micelles. The purity level attained in a single separation step was comparable to that accomplished using extensive column chromatography. Hence, These outcomes indicate that reverse micelles can be effectively utilized for the extraction of intracellular proteins (Boeckelen and Niessner, 1993).

4.8.3.4. Scale-Up Considerations

Extractors established for traditional liquid-liquid extraction are also appropriate for this application. Spray columns, Graesser contactor (raining bucket), are effectively verified, and an integrated system of separation and back-extraction is established. To upgrade the process of extraction of biomolecular compounds using reverse micelles to an industrial-sized unit, still needs to be explored (Casero et al., 1999).

4.8.4. Micellar Liquid Chromatography (MLC)

Micellar liquid chromatography (MLC) is an RPLC, in which the mobile phase comprises an aqueous solution of surfactant above its critical micellar concentration (CMC).140 Given green analytical chemistry, the replacement of organic solvents by surfactants as mobile phases in LC is extremely appreciated. MLC is mainly based on the solubility changes by varying the surfactant concentration above the CMC, which entails the increase of the concentration of amphiphilic aggregates or micelles in the solution, keeping constant the number of monomers of surfactant in the mobile phase. A detailed description of the Retention mechanisms in MLC has been studied

in detailed.141 Main features of MLC include removal of organic solvents and offers the quickest way to perform gradient elutions with an appropriate phase equilibrium (Eiguren-Fernandez et al., 1997).

Because of two drawbacks, the use of MLC was limited when compared to conventional RPLC. The first is the unnecessary retention witnessed for hydrophobic compounds because of the weak eluting power of micellar mobile phases and the second is the minimum efficiency due to the confrontation to analyte mass transfer and/or enhanced flow anisotropy. In MLC routine applications, a small amount of an organic solvent is added to the micellar solution to accomplish retention in practical time windows as well as to increase peak efficiency and resolution.144 In 1983, to improve the efficiency of the separations, the addition of short-chain alcohol, 1-propanol or butanol, to the micellar eluent was suggested. Propanol is the most utilized alcohol additive, trailed by butanol, pentanol, and acetonitrile. From an environmental perspective, high percentages of organic solvent are not recommended. Furthermore, high amounts of organic modifiers would weaken or terminate the micelle role along with the complete removal of the surfactant molecules adsorbed in the stationary phase. To preserve the reliability of micelles, the concentrations of organic solvents is kept low, i.e., about 10% for butanol, 15% for propanol and acetonitrile, and 6% for pentanol.

MLC has shown to be a beneficial technique in the control of various groups of drugs in pharmaceutical preparations, such as diuretics, antihistamines, and phenethylamines, and benzodiazepines using mobile phases comprising SDS and alcohol, like butanol, propanol, or pentanol (Eiguren-Fernandez et al., 1999). Additionally, SDS and an organic modifier have also been employed as mobile phases to regulate antioxidants in foods, for instance, synthetic antioxidants in meat, sulfonamides in milk, phenolic antioxidants in oils, and amines in wine.

4.9. SOLID-LIQUID EXTRACTION SYSTEMS

Most techniques discussed in different sections of this chapter are established and employed in analytical applications, but the author deliberates that these techniques are quite useful for the development of commercial technologies, including pharmaceutical, food, and environmental industries (Figure 4.9) (Eiguren-Fernandez et al., 1999).

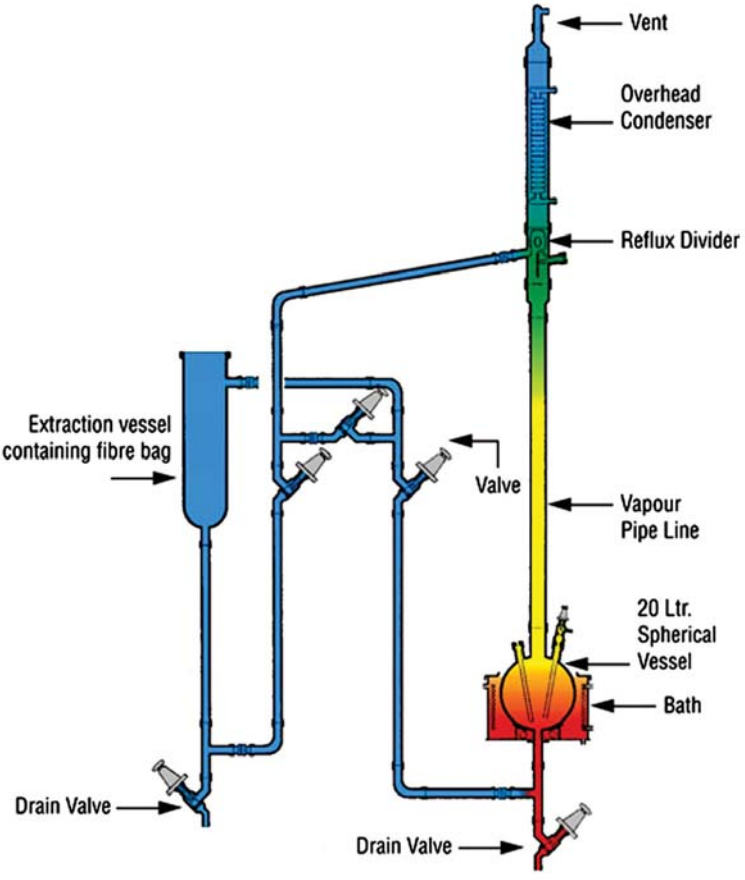

Figure 4.9. Solid-liquid extraction system.

Source: http://www.goelprocess.com/solid_liquid_extraction_unit.html.

4.9.1. Solid-Phase Extraction

SPE is presently the foremost extraction technique for separating or enriching solutes from water solutions. The separation process comprises partition of organic compounds dissolved in water located in between a solid sorbent and an aqueous phase, because of which solutes are selectively confined or trapped on this sorbent. The trapping process is directed by the following mechanisms (Garcia-Pinto et al., 1995):

- Van der Waals forces, π-electron interactions, hydrogen bonds, hydrophobic effects, and cation- and anion-exchange processes result in adsorption of the solutes dissolved in water, on the surface of the sorbent (solid phase).
- Dissolution of a solute in some organic solid sorbents.
- Partitioning of solutes between a sample and liquid sorbent (liquid stationary phase) using thin-film coated solid support. SPE techniques can be employed in several modes (Garcia-Pinto et al., 1995).

4.9.2. Solvent-Impregnated Resins

SPE columns are the most commonly used methods for cleaning. Different column packings including Florisil, C8, C18 reversed-phase, silica, charcoal, and aluminum oxide are commercially available. These surfaces are chemically modified according to the requirement for the adsorption of particular solute or impurities. In conclusion, the feed solution is transferred to a preconditioned SPE column, and the separated solute is retained on the column and is washed to eliminate the impurities, then eluted through a solvent wash. In most cases, the eluate is evaporated till dried and reconstituted in a specific solvent or buffer using a particular analytical technique as required. A comprehensive review of a variety of SPE column packings is discussed in detail by different researchers (Garcia-Pinto et al., 1995). Moreover, the drawback of using such columns for the trichothecenes infers that these toxins often vary depending upon the polarity and solubility, resulting in compromised recovery. Therefore, to resolve this issue, a commercial company (e.g., Varian (now Agilent Technologies, Inc., USA)) introduced a Bond Elut Mycotoxin cartridge designed for the instantaneous cleanup of type A and B trichothecenes as well as zearalenone—another important Fusarium mycotoxin food impurity. This will result in a meaningful reduction in the cost of the technique besides making it more rapid and straightforward.

Another well-known method for the production of trichothecenes is the use of MycoSep columns (Romer Lab, Inc.). Three types of columns are used, dependent on the nature of samples to be analyzed and also the amount of required resultant extract for the analytical procedure, which includes:

- MycoSep 113 Trich, used for analytical procedures needing insignificant amounts of extracts;

- MycoSep/MultiSep 227 Trich^{+}, pertinent to complex food and feed matrices;
- MycoSep/MultiSep 225 Trich, appropriate for grain and simple sample matrices.

These columns contain different adsorbents (e.g., charcoal, and ion-exchange (IE) resins). A plastic tube having the desired adsorbent is packed between filter discs and comprises a rubber flange at the bottom end of the tube, containing a porous frit and a one-way valve. IE columns, Immunoaffinity columns (IACs), and the more conventional liquid-liquid partitioning.158 Antibodies get attached to chemically inert support and will explicitly bind the required solute while permitting inquisitive components to pass through the column. In other SPE formats, rinsing, preconditioning, and elution of the toxin are required and the extract must be an aqueous solution comprising negligible or no organic solvent unfavorably affecting the antibody in addition to the antibody-antigen-binding event. Specific interaction between the antibody and the analyte is the major benefit of this approach, but, in the case of nonspecific interactions in antibody-based methods, because of cross-reactivities with other trichothecenes, may adversely affect the attained results (Frankewich and Hinze, 1994).

4.9.3. Solid-Phase Microextraction (SPME)

Solid-phase microextraction (SPME) is a comparatively new technique, introduced by Pawliszyn and Lord in the early 1990s. It can easily be controlled electronically and has been extensively employed for the preparation of samples for chromatographic analysis, with a rapid rise in applications ever since the first fibers were commercially available in 1993. The applications are limited by the characteristic properties of commercially available fibers, include organic compound determination (i.e., alkylbenzenes, polycyclic aromatic hydrocarbons, volatile organic compounds, and pesticides as target analytes). This technique is based on the partitioning of an analyte located between a sample and a fused-silica fiber coated with a stationary phase, comprising a liquid polymer or a solid polymer distributed in the liquid polymer. Later, the analyte is desorbed from the fused-silica fiber coated with stationary phase directly into a suitable separation and detection system (Frankewich and Hinze, 1994).

4.10. EXTRACTION OF GAS IN ANALYTICAL APPLICATIONS

Gas extraction is employed in static or dynamic headspace modes. The static headspace (gas-phase extraction) technique comprises the transfer of the part headspace or gas phase through a gastight syringe or by transfer line into a GC machine over the sample in a condensed phase. Dynamic headspace is the collection of the analytes in a trap as a result of the flow of gas-phase above or through the sample, and then the trapped analytes by desorption into GC for their analysis.

Headspace techniques are free of solvent use since inert gas is utilized as an extracting agent. The other advantage includes the injection of volatiles into the GC instrument. This is of great importance as nonvolatile compounds could decline the quality of the column. Similarly, a new column needs material as well as energy inputs; by applying gas extraction, this problem is easily resolved (Brejza and Perez de Ortiz, 2000).

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Chapter

5

Mechanical Separation Techniques

CONTENTS

5.1. INTRODUCTION

We can divide mechanical separations into four groups that are sedimentation, filtration, sieving, and centrifugal separation. In sedimentation, a solid and a liquid, or two immiscible liquids having different densities are separated. This is done by allowing them to reach an equilibrium state under the influence of gravity, as compared to lighter material, the heavier material fall. This can be a time taking process. To speed it up, centrifugal forces are applied as they increase the sedimentation rate; often known as centrifugal separation (Cui and Forssberg, 2003; Perazzolo et al., 2015). The separating process of solids from liquids is called filtration. It is done by flowing the mixture through small fine pores, due to the small size of pores, the solid particles are stopped while allowing the liquid to pass. For classifying the solid particles, sieving is used. It interposes a barrier that does not allow the larger elements to pass (Zhang and Forssberg, 1997).

The process of the mechanical separation of particles involves the use of external forces. These external forces can either be directly restraining forces, for example, filtration and/or sieving, or these forces can be indirect, for example, the process that takes place in impingement filters. Gravitational or centrifugal action can be used as negative restraining forces as they move the particles from the fluid they are present in (Wang et al., 2018). Hence the act of separation of these particles relies on both the nature of the force and the characteristics possessed by the particles. Particle characteristics such as the density, size, and shape of the particle and the density and viscosity of the fluid act as determining factors in the process of mechanical separation of the particles. The characteristics play a key role in how these particles and fluids interact with these forces to set up relative motion between the fluid, particles, and particles having different properties. Using these relative motions, the fluid and particles gather up and can be collected in different regions, such as the following (Dinuccio et al., 2008; Santini et al., 2012):

- Different-sized sieves used on the sieve set;
- The filtrate tank and filter plate components on a filter press;
- The centrifuge's outlet stream;
- The top air outlet and the base discharge valve on a cyclone.

For the mechanical separations looked at, the forces considered were gravity, gravity in combination with other forces, pressure forces that employ the use of forces to push the fluid away from the particles, the total restraint of solid particles, used when the fluid is not required, and lastly centrifugal

forces. In many of these separation techniques, the particle velocities play a key role.

5.2. THE PARTICLE'S VELOCITY WHILE MOVING IN A FLUID

With a constant force, such as gravitational force, acting on the particles present in the fluid, the particles gain acceleration before reaching a uniform maximum velocity known as the terminal velocity. The terminal velocity is determined by the properties of the fluid and the characteristic density, size, and shape of the particles (Adachi et al., 1978; Dawson, 1990).

Two integral forces are acting on the particles as they move through the liquid. One of these is the external force that causes the particles to move, while the other is the drag force caused by the friction in the liquid and providing resistance to the motion (Khan et al., 1987; Lallemand and Luo, 2003). The total force acting upon the particle is obtained by subtracting the resistive force, a product of the external force, from the applied force, such that:

$$F_s = Va\,(\varrho_p - \varrho_f)$$

where; F_s is equated as the total external accelerating force acting upon the particle; V represents particle volume; a denotes resulting acceleration due to the applied force; ϱ_p denotes particle density and lastly; ϱ_f represents the fluid density (Cunningham, 1910; Sarvar et al., 2015).

The drag/resistive force acting upon the particle is equated to the product of the flowing liquid's velocity pressure divided by the particle's projected area, given as follows:

$$F_d = C\varrho_f v^2 A/2$$

where; C denotes the drag coefficient; ϱ_f is representing fluid density; v is the particle velocity; and A represents the particle's projected area perpendicular to the motion's direction (Lee et al., 2013).

If considering a particle spherical in shape such that $A = \pi D^2/4$ and $V = \pi D^3/6$, with D denoting particle diameter, and comparing F_d and $F_{s,}$ for which terminal velocity v_m is achieved, we get:

$$(\pi D^3/6) \times a\,(\varrho_p - \varrho_f) = C\varrho_f v_m^{\,2}\,\pi D^2/8$$

Theoretically, in the case of a streamlined motion for sphere particles, the drag coefficient is found using the following:

$$C = 24/(\mathrm{Re}) = 24\,\mu\,/Dv_m\pi_f$$

Now placing this in place for the value of C and reordering the equation, we obtain the following formula for terminal velocity:

$$v_m = D^2 a(\varrho_p - \varrho_f) / 18\mu \quad (1)$$

This relation represents the principal equation for the particle movements in a liquid.

5.3. SEDIMENTATION

Employing the use of gravitational forces to separate fluids and particle matter is known as sedimentation. In this method, the particles are generally solid compounds, but may also be liquid drops in certain cases. The fluid in either situation could be in a liquid or gaseous state. In the food industry, the process of sedimentation is mostly used to separate crystals from their parent fluid, dirt, and rubble present in the raw material being brought in, and the product particles or dust present in the airflow (Kynch, 1952; Xiao et al., 2017). During the sedimentation process, due to the gravity acting upon them, the particles fall from rest. Hence for sedimentation, Eqn. (1) takes after the recognizable shape of Stokes' Law **(**Garcia, 2008**)**:

$$v_m = D^2 g(\varrho_p - \varrho_f)/18\ \mu \quad (2)$$

Observe that Eqn. (2) relies on the use of consistent units throughout, as it is not dimensionless. If following the SI system, D equates to m, g represents ms^{-2}, ϱ is taken as kgm^{-3} with μ equal to Nsm^{-2}, with v_m in ms^{-1}. The diameter of particles is measured in micrometers (microns) = 10^{-6} m and symbol being μ m (Goldberg, 1953).

However, Stoke's Law is only applicable to spherical shaped particle and streamline motion. Concerning spherical particles, the streamline flow must be (Re) ≤ 2. Generally, most practical situations fall under the domain of streamline flow or approximate to it. For situations where greater values of Reynolds number occur, further cases could be looked into. Stokes Law is limited to situations where an individual particle's motion is independent of the motion of surrounding particles or where settling of the particles is free (Bizzo et al., 2014).

5.3.1. Gravitational Sedimentation of Particles in a Liquid

For fluids with lesser density than the density of the solid particles, the particles will settle at the base. In cases with low concentration, Stoke's Law

may be applicable, however, in most practical occurrences, this is seldomly the case with higher concentrations being more common (Behr et al., 2015).

For a uniform suspension in a cylinder, if the particles can settle then distinct regions appear as the settling process propagates. The cleared fluid forms the topmost layer. The next layer to be found has an approximate consistent composition as the velocity for differently sized particles is uniformly constant. The bottom-most layer at the cylinder base is composed of larger particles increasing in size as we go lower; this is known as the sedimentation region and has no constant composition. In the instance that the spectrum of particle sizes occurring in the liquid is wide, then the constant composition region may not form at all with the liquid layer atop only the variable composition region (Durner et al., 2017).

For instance, with a continuous thickener, as the settling process continues and the cleared fluid is removed from the top and the sedimentation deposits are removed from the bottom, the same regions occur. To find the least area required to form a continuous thickener, equate the sedimentation rate for the concerned region to the rising fluid's counterflow velocity. For this, the relationship obtained is (Bauer et al., 2020):

$v_u = (F - L)(dw/dt)/A\varrho$

Here, v_u represents the liquid's flow upward velocity; F denotes liquid to solid ratio of their masses within the feed; L is used to represent liquid to solid mass ratio for the underflow liquid; dw/dt stands for the solids in the feed mass rate; ϱ characterizes the liquid density; and A is used to represent the settling region within the tank.

Considering that the particles have settling velocity, then $v_u = v$, hence:

$$A = (F - L)(dw/dt)/v\varrho \quad (3)$$

This same analysis is also applicable for droplets of a non-miscible as it is for solid particles. With the relative motion present between the liquid and the particles, some of the particles may even rise.

Equipment designed to separate solid particles from liquids using gravitational sedimentation are known as sedimentation equipment. The equipment is designed to offer sufficient time for the process of sedimentation to occur and allow for the removal of the overflow and sediment deposits without interfering with the process of sedimentation. Ideally, continuous flow from the equipment will help in keeping the flow velocities low enough to not disturb the sediment (Springston et al., 1987). Vessels of different

shapes and adequate cross-section are used to keep the flow velocity low. They are accompanied by slow speed, pumps, and scraper conveyors that allow for the removal of the sediments. In the instance that cylindrical vertical tanks are employed, mostly the scrapers revolve around the axis located in the tank's center, and the overflow is directed around the tank's periphery, as represented in Figure 5.1 (Goldberg, 1953).

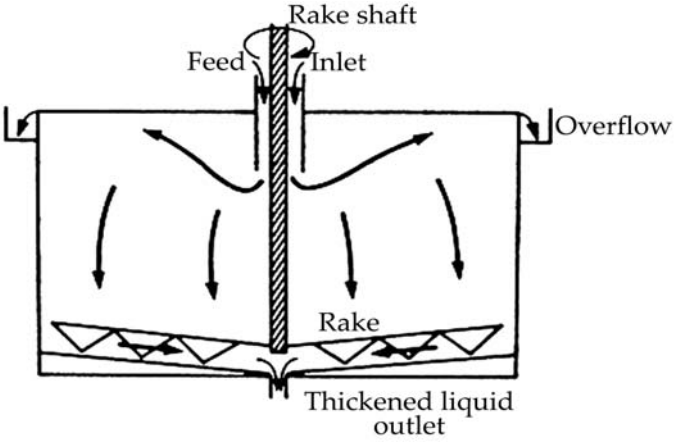


Figure 5.1. Continuous-sedimentation plant.

Source: https://nzifst.org.nz/resources/unitoperations/documents/UnitopsCh10.pdf.

5.3.2. Flotation

In situations where sedimentation of fine particles is not possible, the particles can be, using air bubbles, floated, or brought to the surface, this process is known as floatation. The technique is dependent on the relative capability of adherence of water and air to the surface of the particle. In this process, the water adhering to the particle surface is displaced by the air. The replaced air then provides sufficient buoyancy to allow for both the particle and the air bubble to rise to the surface of the fluid.

Due to being dependent on the interaction of surface forces, drastic changes may occur if for the presence of even minuscule amounts of surface-active agents. Using appropriate agents, the process of floatation can be aided. For example, in certain situations, air bubbles may stick to the particle surface, forming a froth. This phenomenon occurs in vessels that

use mechanical agitators, which agitatedly mix the air or liquid mix. The froth formed is then collected in troughs.

Majorly used to concentrate minerals, floatation is also used in the food industry to separate fat droplets or particles present in the water. Mixing the air into the water using pressure creates froth. With the sudden release of this pressure, the air comes up to the surface in the form of air bubbles. These air bubbles are accompanied by the fine fat particles and are removed by the surface scrapers (Garcia, 2008).

5.3.3. Sedimentation of Particles Present in a Gas

Significant use of the sedimentation of solid is done in spray dryers. In the dryer, the material is decomposed into minute particles with an approximate diameter of 100mm. These particles descend through heated air, drying up during the process. The settling area required for the particles is calculated in the same manner as is done for sedimentation. However, two disadvantages accompany this process: the long duration of contact among the heated air and particles, risking the destruction of products that may be sensitive to heat, additionally, chambers with large areas are necessary.

5.3.4. Settling Under Combined Forces

Certain situations may call for the use of multiple forces to bring about the mechanical separation of the particles and fluid. For example, it is feasible to apply centrifugal forces to impact the low velocity of the particles that occur when using gravity as the only external force. The biggest application of this is found in the cyclone separator. The phenomenon is also used in certain powder classifiers, for example, the ring dryers and rotary mechanical classifiers.

5.3.5. Cyclones

Cyclones are mostly employed to separate particles of an approximate or greater than 10mm diameter from streams of air. Additionally, they can be employed for the separation of liquid droplets present in the gasses or for separating particles from fluids. Cyclone is a vertical cylindrical settling chamber, arranged to allow for the air spirals, going around the cylinder, to generate centrifugal forces. The centrifugal forces push the particles onto the outside walls. With the added centrifugal forces to the gravitational forces, the settlement rates are accelerated. In the cyclone, the provided spiral paths allow for adequate time for separation. An example of a cyclone is shown in Figure 5.2(a).

Using Stoke's Law, it can be observed that particles terminal velocity is dependent on the acting force. For centrifugal separator, as in a cyclone the acting force on a particle moving around the cyclone periphery is:

$$F_c = (mv^2)/r \quad (4)$$

where, F_c represents a centrifugal force that acts upon the particle; m denotes particle mass; v represents particle tangential velocity; and r is used to represent cyclone radius (Renkin, 1954).

From the equation, it can be seen that a decrease in the radius of the cyclone results in an increase in the force being applied to the particle. Hence, for the removal of minute particles cyclones with small diameters prove to be the most efficient. However, capital costs incurred to use small-diameter cyclones to allow adequate output in addition to the drop in pressure, both act as restrictions to the usage of the principal (McCarthy et al., 2002; Bourne et al., 2017).

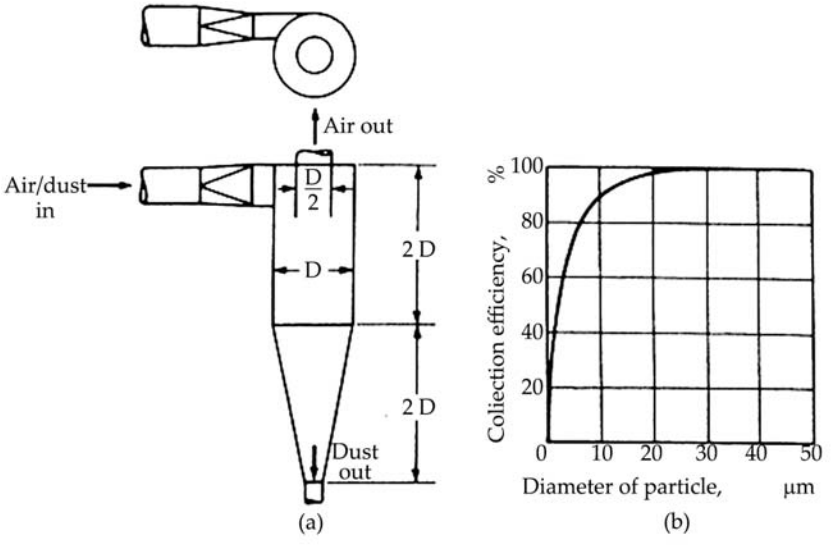


Figure 5.2. Cyclone separator: (a) equipment; (b) dust collection's efficiency.

Source: https://web.fs.uni-lj.si/lvts/wp-content/uploads/2018/05/CyclonePDF.pdf.

Experience and similar to the above-shown proportions have led to the eventual evolution of the optimal cyclone shape as shown in Figure 5.2(a) and has proven to be effective. The even double-helical flow that is produced

is crucial for the cyclone to operate efficiently. Any form of disruption to the generated flow or anything that may hinder the flow from following its pattern can have significant adverse impacts on the cyclone efficiency. Such as, it is integral that the airflow must be fed at a tangent from the top. Any lids or narrowing baffles must be evaded at the air outlets (Bugge et al., 2012).

In Figure 5.2(b), the efficiency of the cyclone used to collect dust is shown. Owing to the complex flow, the particle size cut is not distinct. It can be observed that out of the retained particles in the cyclone, the number of particles with a diameter less than approximately 10mm is lesser. Useful for the separation of droplets of liquid from gasses or particles from liquids/gasses, cyclones have emerged to be highly effective (Foley, 2006; Luo et al., 2013).

5.4. CENTRIFUGAL SEPARATIONS

In certain instances, the sedimentation process for a solid and liquid, or non-miscible liquids can take very long periods. The reason being that the process depends on gravitational forces, for example, different components having a similar effect or due to forces that create interactions among the particles. In some situations, even if the process of sedimentation is to take place, the layers formed are not distinct enough or are merged. An example case is that if milk was to be allowed to stand for one whole day, undisturbed, then a layer of fat atop layer milk would appear (Behr et al., 2015). Despite the clean separation, the method is more suitable for a kitchen at a farm and not for a factory. Combining the centrifugal action and the forces of gravity, as in a cyclone, results can be achieved at a much faster pace. However, in industrial-scale centrifuges, the force of gravity is negligible relative to the centrifugal force being applied, hence can be ignored in the separation analysis (Iritani et al., 2010; Durner et al., 2017).

For a particle rotating in a circular path, the centrifugal force acting the particle can be obtained by using the following relation:

$$Fc = mr\omega^2 \quad (5)$$

where; F_c stands for the centrifugal force keeping the particle in a circular motion; r represents path radius; m denotes particle mass; and ω (omega) represents the particle's angular velocity.

On the other hand, as $\omega = v/r$, where v denotes the particle's tangential velocity:

$$F_c = (mv^2)/r \quad (6)$$

Revolutions per minute are used to represent rotational speeds, thus Eqn. (6) may also be represented using $\omega = 2\pi N/60$; it is divided by 60 to make it s^{-1}:

$$F_c = mr\,(2\pi N/60)^2 = 0.011\,mrN^2 \quad (7)$$

where; N represents speed of rotation as revolutions per minute.

Comparing to the gravitational force (F_g) acting on the particle, represented using $F_g = mg$, it is observed that centrifugal acceleration, equated to $0.011rN^2$, has substituted acceleration due to gravity, equated to g. For comparison, centrifugal forces are generally represented as multiple "g" (Shirato et al., 1971; Goldrick et al., 2017).

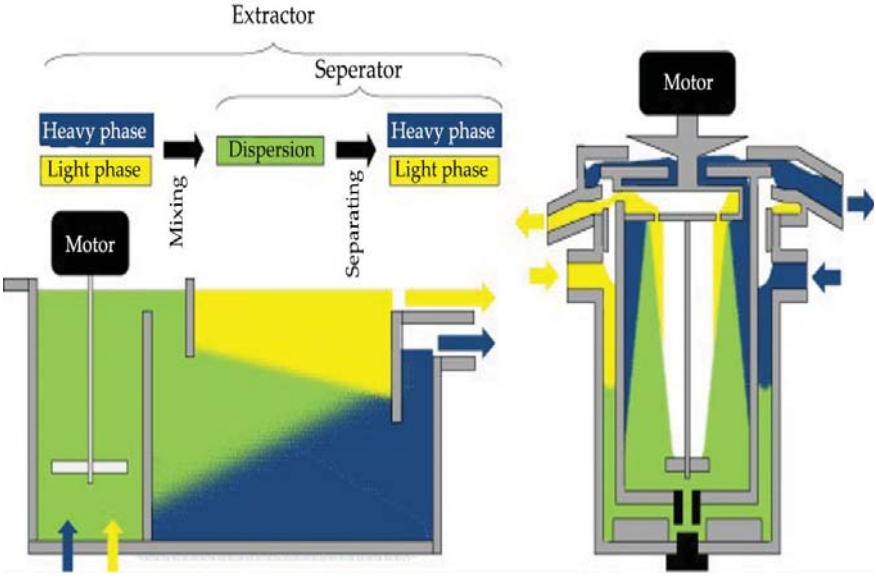


Figure 5.3. Liquid separation mechanism in a centrifuge.

Source:https://www.sciencedirect.com/science/article/abs/pii/S0263876217304586.

The particle mass, rotational speed and radius are all integral to the centrifugal force. Keeping the rotational speed and radius constant, the

particle's weight becomes the sole determining factor. With a direct relationship existing, meaning, the heavier the particle, the greater the centrifugal forces enacting on it. Take for example the centrifugal liquid separator. In it two fluids, with one having a relative higher density than the other, are placed in the centrifuge. The heavier fluid has greater centrifugal force per volume affecting it, as the cylinder is rotated vertically, about the principal center axis. The greater centrifugal force moves the denser liquid to the periphery of the centrifuge. The outer heavier fluid pushes the lighter fluid towards the center. This principle is shown in the diagram in Figure 5.3 (Tiller, 1958; Bauer et al., 2020).

5.4.1. Rate of Separation

To calculate a streamline flow's particles steady-state velocity after being acted upon by the centrifugal force is given as, from Eqn. (1),

$$v_m = D^2 a\,(\varrho_p - \varrho_f)\,/18\mu \qquad (8)$$

In the case that streamlines flow was to occur in the centrifuge, taking from both Eqns. (6) and (7), as *a* represents tangential acceleration:

$F_c = ma$

$F_c/m = a = r\,(2\pi N/60)^2$

so that,

$v_m = D^2\, r(2\pi N/60)^2(\varrho_p - \varrho_f)\,/18\,\mu$

$v_m = D^2 N^2 r\,(\varrho_p - \varrho_f)/l640\,\mu$

5.4.2. Liquid Separation

In the food sector, the separation of a liquid from a liquid, both of which are non-miscible yet finely diffused, for example, emulsion, are separated using a centrifuge. Case in concern, the separation of skimmed milk and cream from simple milk (an emulsion) in the dairy domain. Let us look closely at the two-phase operation of the centrifuge. The milk (an emulsion) is being constantly fed to the machine (Di Bernardo and Cleasby, 1980; Mahdi and Holdich, 2013). The machine is generally taken as giant bowl-shaped equipment rotating about a vertical principal axis. The separated skim milk and cream are collected from their respective discharge valves. At any given

point in time, there will be a surface layer separating the skim milk from the cream (Springston et al., 1987; Bozhko and Tynjälä, 2005).

For the above shown thin cylinder in Figure 5.4(a): the thickness is represented as dr and height as b, to calculate differential centrifugal force over dr use Eqn. (5):

$$dF_c = (dm)r\omega^2$$

where; dF_c represents the differential force applied across the cylinder wall; dm denotes differential cylinder's mass; ω represents cylinder's angular velocity; and r represents cylinder radius. Yet,

$$dm = 2\pi rb\varrho dr$$

where; ϱ represents liquid density; and b represents the cylinder's height.

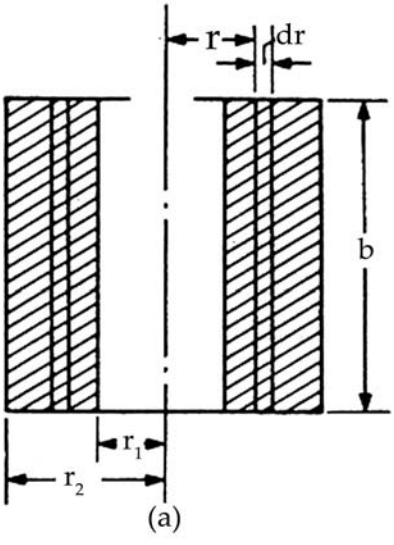
Hence,

$$dF_c = (2\pi rb\varrho dr)r\omega^2$$

Area across being acted upon by the force dF_c is found as $2\pi rb$, such that:

$$dP = dF_c / 2\pi rb = \varrho\omega^2 r dr$$

where; dP denotes the differential pressure over the differential cylinder wall.



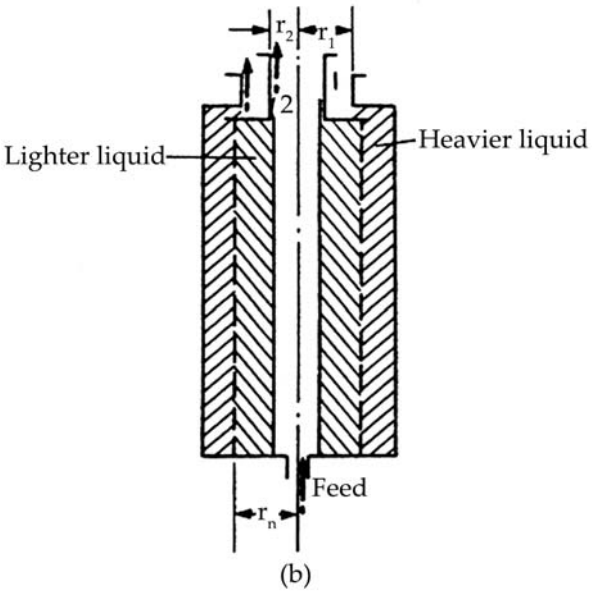


Figure 5.4. Centrifuging liquids: (a) thin liquid cylinder; (b) continuous liquid centrifuge.

Source: https://slideplayer.com/slide/12906359/.

In a centrifuge, finding the differential pressure between r_1 and r_2 radius, the equation for the dP may be integrated, allowing the pressures at radius r_1 and r_2 *to* be P_1 and P_2, making:

$$P_2 - P_1 = \varrho\omega^2 (r_2^2 - r_1^2)/2 \qquad (9)$$

Then Eqn. (9) characterizes radial variation in the pressure over the centrifuge.

Now, let us take a look at Figure 5.4(b), which shows a bowl in a continuous liquid vertical centrifuge. In it the feed is fed to the centrifuge close to the axis, the denser fluid, *A*, is removed using opening 1 at the top while the less dense liquid, *B*, is removed at the opening 2. Considering r_1 as the radius for the discharge pipe used to remove the heavier fluid while r_2 denotes the radius for the discharge pipe used for the less dense fluid (Qiu and Wu, 2012). At another random radius r_n, the surface separation between the two fluids will exist. To make the system achieve and maintain hydrostatic balance, the pressures across all constituents found at radius r_n

has to be consistent, hence if applying Eqn. (9) to calculate pressures of every constituent found at the radius r_n, and making the two equal, we obtain (Yim and Kwon, 1997; Di Carlo et al., 2008):

$$\varrho A\omega^2 (rn^2 - r1^2)/2 = \varrho B\omega 2(rn2 - r2\ 2)/2$$

$$\varrho A(rn^2 - r1^2) = \varrho B(rn2 - r2\ 2)$$

$$r_n^2 = (\varrho_A r_1^2 - \varrho_B r_2^2)/ (\varrho_A - \varrho_B) \quad (10)$$

where; ϱ_A represents the heavier fluid's density; and ϱ_B denotes the lighter fluid's density.

From the Eqn. (10) if the discharge radius for the denser fluid is decreased, the neutral zone radius also decreases. However, if the neutral zone closes in on the centra axis, the centrifugal force being applied is relatively less than the one experienced by the denser fluid. This phenomenon is applied when, for example, in the separation of milk and cream and we want to collect the maximum amount of cream; hence, we keep the neutral radius small. The feed inlet in this type of centrifuge is kept within the neutral zone to disturb the system the least. Hence the relationship is employed to locate the feed inlet as well as the product outlets within the centrifuge to allow for maximum separation (Porath and Flodin, 1959; Choi et al., 2007).

5.4.3. Centrifuge Equipment

Figure 5.4(a) represents the least complex form of centrifuge that constitutes a vertical axis about which the bowl rotates. Liquids and solids, or liquids are fed to this, and using the centrifugal force, the particles or denser liquids move to the outside of the bowl as the less dense constituents move to the center (Woo, 1970; Depountis et al., 2001).

Considering the feed is just fluid, then the collection pipes can be arranged in a suitable fashion to attain the separation of the lighter and heavier components. Different arrangements can be used to get the maximum efficient collection that creates the least amount of disturbance in the system's flow pattern. To get a better understanding of how these collection arrangements work, it is better to imagine the centrifuge action being synonymous with gravity settling. Using a variety of overflows and weirs, that perform in a similar way as they do in a tank, although the centrifugal force is much greater than gravity (Jafari et al., 2015).

For centrifuges that perform liquid and liquid separation, the conical plates, which provide improved separation and an even flow, are arranged as shown in Figure 5.5(a).

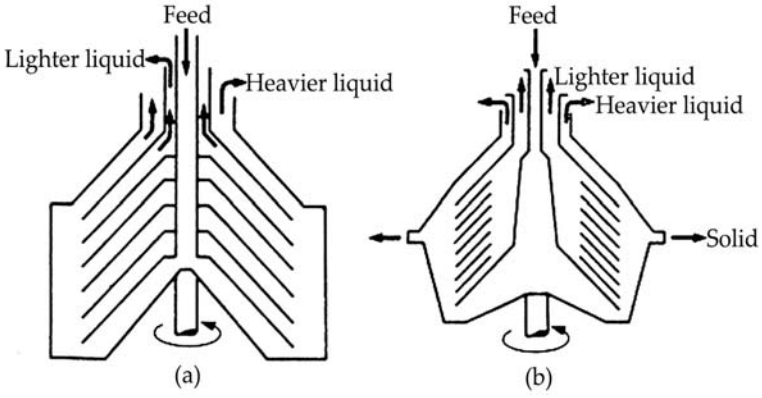


Figure 5.5. Liquid centrifuges: (a) conical bowl; (b) nozzle.

Source: https://www.coursehero.com/file/14660474/Chapter-7-Mechanical-separation-processes/.

While the fluid phases can be extracted easily from the centrifuge, solid particles pose a greater problem. For the solid/liquid separation, using stationary plows is not feasible as they interrupt the flow pattern greatly, hence impacting the reliant centrifuge action that performs separation (Adams et al., 1980; Stanier and White, 2013). The first proposed method to handle the removal of solids is to install nozzles in the centrifuge's circumference as shown in 5.5(b).

Whereas liquid phases can easily be removed from a centrifuge, solids present much more of a problem. In liquid/solid separation, stationary plows cannot be used as these create too much disturbance of the flow pattern on which the centrifuge depends for its separation. One method of handling solids is to provide nozzles on the circumference of the centrifuge bowl as illustrated in Figure 5.5(b). The nozzles can either be opened at fixed intervals, to collect the solids accompanied by the heavier liquid or can be kept open continuously, relying on their placement and size to allow for maximum solid particle removal that is accompanied by the least amount of heavy fluid. These centrifuges hence give three products against one inlet feed which are the solid particles, heavy fluid, and lighter fluid. The solids carry over some amount of the heavy liquid too (Dixon and Summers, 1985; Kim et al., 2013). Yet, another method to handle solids removal in a continuous feed is using the telescoping action within the bowl. In it, parts of the bowl are moved in overlapping motions and transfer the collected solids to the outlet as shown in Figure 5.6(a).

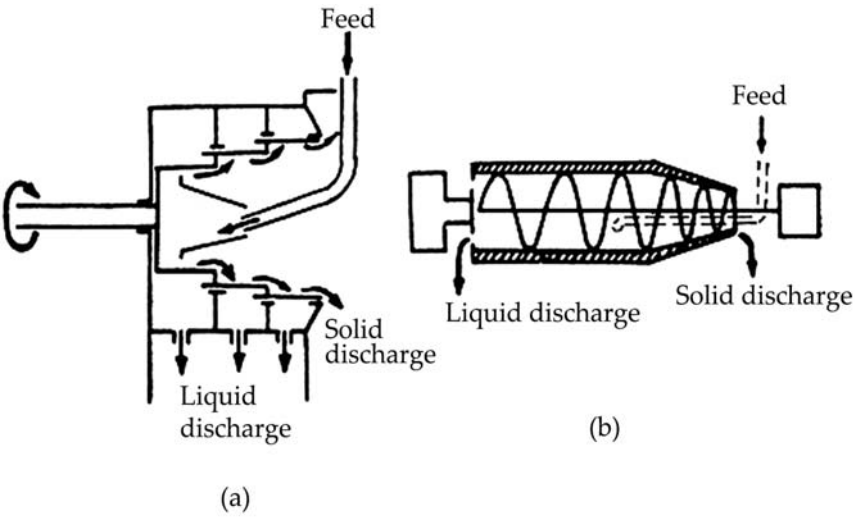


Figure 5.6. Solid /liquid centrifuges: (a) telescoping bowl; (b) horizontal bowl.

Source: https://slideplayer.com/slide/12578554/.

Figure 5.6(b) shows a horizontal bowl centrifuge fitted with a scroll discharge. This machine can discharge continuously. In it, the screw (or horizontal collection scroll) revolves within the conical-ended bowl and transfer along with the collected solid, while simultaneously discharging the liquid from the overflow at the machine's center and located opposite of the discharge outlet for solids. The important thing to consider is that relative to the bowl, the speed of the scroll must be kept less (Mulhem et al., 2006; Schütz et al., 2009). Take, for example, that the bowl's speed is 2000 rev/min, the optimum scroll speed would be between 2025 and 1975 rev/min, or 25 rev/min in relation to the bowl's speed. These differential speeds are kept by employing gearing between the driving shafts for the scroll and the bowl. The centrifuges fashioned in this manner can handle feeds with a solid content of a maximum of 30% (Henry Jr et al., 1977; Wallace et al., 2013).

5.5. FILTRATION

Filtration is yet another class in the domain of mechanical separations that involves the placement of a screen through which the feed liquid is forced through. The screen cuts off all particles greater than a certain size. The liquid collected is known as the filtrate, while the solids collected on the screen are called filter cake. In some cases, either the filtrate or the filter

cake is the product required (Sutherland, 1948; Rubio et al., 2002).

The fine pores in the filter screen are made available by the solid particles bed, the meshes and screens made metals or plastics, or the fabric filter clothes. In certain instances, a fine layer of filter cake or any other solid particle layer is placed onto the filter clothe to aid in creating adequately fine pores on the surface of the filter and this process is called to be a pre-coat (Berthod and Faure, 2015; Bouju et al., 2016).

To analyze the process of filtration, it is necessary to understand the system of flow. In it, the liquid is passed from the filter medium. The filter medium resists the flow basing off of the differential pressure acting across the filter. Hence, we can write the recognizable equation:

Rate of filtration = Driving force/resistance

In addition to the resistance offered by the filter medium (mesh, bed, filter cloth), the resistance offered by the accumulating filter cake is also to be considered. To calculate the filter-cake resistance, take the product of the thickness of the filter cake and its resistance per unit thickness, also known as the specific resistance of the filter cake. The filter medium's resistance and the precoat's resistance are jointly known to be the filter resistance. It is feasible to suppose the resistance of the filter for a hypothetical filter cake thickness. Using this thickness, we obtain the resistance for the filter cake by using the product of the assumed thickness and the filter cake's specific resistance. Hence, giving us the following equation to calculate the volumetric rate of flow (Langmuir, 1920; Kostanyan and Erastov, 2018):

$$dV/dt = (A\ \Delta P)/R$$

From the equation we can see that a directly proportional relationship exists between the fluid's viscosity and the total resistance, allowing us to write:

$$R = \mu r(L_c + L)$$

where; R denotes filter's resistance to the flow; μ is the fluid's viscosity; r represents the filter cake's specific resistance; L_c denotes the filter cake's thickness while; L is the assumed thickness of the filter medium and pre-coat; A represents the area of the filter; and lastly, ΔP indicates the drop in pressure over the filter.

Considering that the flow rate of the fluid along with the solid contents is known and assuming the solids are all completely retained by the filter, we can express the filter cake's thickness using:

$$L_c = wV/A$$

where; w represents fractional solid content over liquid volume; V denotes fluid volume passed through the filter; and A represents the filter area on which the filter cake is formed.

Resistance can be rewritten as:

$$R = \mu r\,[w(V/A) + L] \tag{11}$$

while the equation to denote flow in the filter, driven by the drop in the pressure is:

$$dV/dt = A\,\Delta P/\,\mu r[w(V/A) + L] \tag{12}$$

Then Eqn. (12) is taken to be the principal equation for the process of filtration. It shows the filtration rate in measurable quantities, sometimes estimated, often found from tables. Useful in predicting the performance of filters at a large scale based on their pilot or laboratory tests. Generally, the two applications using Eqn. (12) are filtration under constant pressure and at a constant flow rate.

5.5.1. Constant Rate Filtration

During the initial stages of the filtration cycle, the filter-cake layer accumulated is thin, so the total resistance is virtually offered by the pre-coat and the filter-medium, making the value of resistance constant nearly. Hence, the filtration process continues at a constant rate. Using Eqn. (12) we can find the amount of liquid passed through the filter over a given period. As the terms on the right-hand side of the equation are constant, integration becomes very easy (Anderson, 1966; Penrose et al., 2004):

$$\int dV/Adt = V/At = \Delta P/[\mu rw(V/A) + L]$$

or,

$$\Delta P = V/At \times \mu r[(wV/A) + L] \tag{13}$$

From Eqn. (13), the required drop in pressure to maintain the optimum flow rate can also be calculated. Moreover, by conducting multiple runs using different pressures, we can find the resistance offered by the filter cake.

5.5.2. Constant Pressure Filtration

Over time, as the filter cake has accumulated, the flow is now driven by the constant pressure differential. Influenced by these factors and keeping the term ΔP constant in Eqn. (12) we get,

$\mu r[w(V/A) + L]dV = A\,\Delta P dt$

Now integrating from the values $V = 0$ at $t = 0$, till $V = V$ at $t = t$

$\mu r[w(V^2/2A) + LV] = A\,\Delta Pt$

Now writing this as:

$tA/V = (\mu rw/2\Delta P) \times (V/A) + \mu rL/\Delta P$ (14)

$t/(V/A) = (\mu rw/2\Delta P) \times (V/A) + \mu rL/\Delta P$

Then Eqn. (14) depicts the practical situation most often occurred in industrial situations and is useful for those situations. The equation can be used in the performance prediction of a filtration plant-based off on the experimental runs (Yoshida et al., 2001; Griffith, 2010).

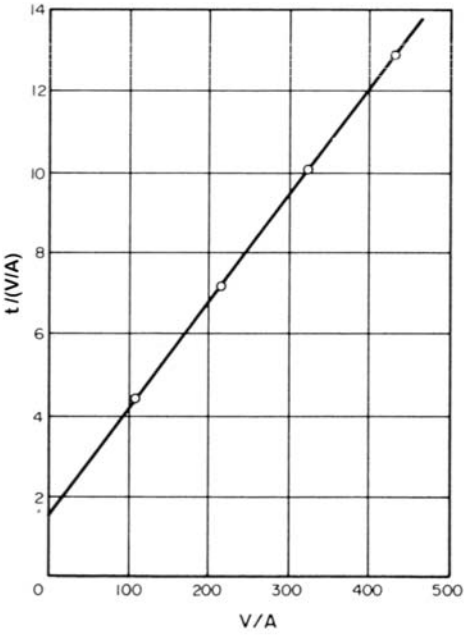


Figure 5.7. Filtration graph.

Source: https://nzifst.org.nz/resources/unitoperations/mechseparation5.htm.

In a test influenced by using uniform pressure and measuring and collecting the filtrate at regular intervals of time, the values can then be used to plot a **filtration graph** of $t/(V/A)$ against (V/A). From the relationship shown in Eqn. (14) we can see that the graph must be a straight line with the slope of this graph corresponding to $\mu rw/2\Delta P$. The intercept of the graph on *the t/*

(V/A) axis will provide the value $\mu rL/\Delta P$. As generally, μ, w, ΔP and A can easily be measured or are already known, we can use the value of intercept and slope of the graph to find L and r.

In the following graph, a few values for $t/(V/A)$ are plotted against their accompanying values of V/A in Figure 5.7. We can see in the graph, the slope is 0.0265, while the intercept 1.6.

5.5.3. Filter-Cake Compressibility

In the case of some filter cake, their specific resistance varies alongside the pressure drop acting across them. This is due to the cake becoming denser with higher pressure exerted and providing lesser and tinier flow passage. This phenomenon is known as filter-cake compressibility. Flocculent and soft materials tend to provide filter cakes with higher compressibility, while on the other hand, granular and harder materials, as in salt and sugar crystals are not affected much by the pressure. The empirical relationship below has been proposed to make room for cake compressibility (Edzwald, 1995; Qian and Wu, 2009):

$$r = r'\Delta(P)^s$$

where; r denotes the filter cake's specific resistance with pressure; P acting upon it; ΔP represents the drop in pressure over the filter; r' is the filter cake's specific resistance when the drop in pressure is 1 atm; and s denotes the material constant, known as its compressibility (Zhao et al., 2006).

This expression for r can be inserted into the filtration equations, such as Eqn. (14), and values for r' and s can be determined by carrying out experimental runs under various pressures.

5.5.4. Filtration Equipment

The following are the elementary components required by filtration equipment:

- Method to remove the excessive filter cake accumulated;
- Access of flow to and from filter medium;
- Providence of mechanical support to the filter medium.

In some cases, the washing of the filter cake may be necessary to remove any leftover solution. The pressure may be provided to the filter's upstream side, or maybe a vacuum is drawn downstream, and/or the combination of the two (Figure 5.8).

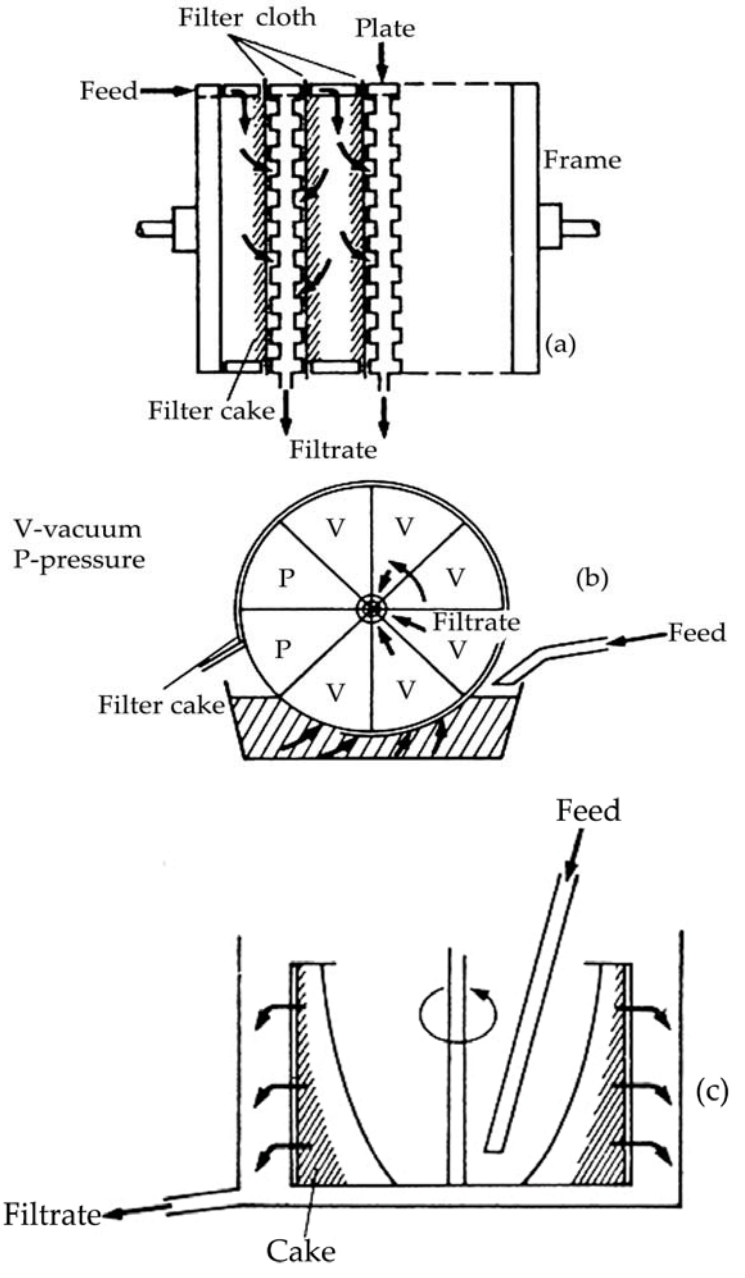


Figure 5.8. Filtration equipment: (a) frame and plate press; (b) rotary vacuum filter.

Source: https://slideplayer.com/slide/4537225/.

For filter equipment, the general parts are (Derksen, 2003):

- Rotary filters;
- Plate and frame filter press;
- Air filters;
- Centrifugal filters.

5.6. SIEVING

For the final class in the mechanical separation, we have sieving. In sieving, the restriction on the passage of the flow of certain particles is imposed using mechanical screens. The screens are placed consecutively, decreasing in size as they progress, resulting in a particle series made of different sizes. The fluid, generally air, is mostly ignored. In it, the material is agitated above a sloth or mesh screen with particles of a smaller size than the mesh openings passing through successfully under the force of gravity. The following factors affect the rate of throughput for the sieves (Richard et al., 2011; Cao et al., 2019):

- Methods employed to prevent the sticking or bridging of the particles within the sieve apertures;
- Sieve material's physical nature and tension;
- Particle's shape and nature;
- Shaking's amplitude and frequency.

Over time the standard sizes for sieves have evolved from 25 mm to down to 0.6 mm aperture size. The original mesh used to be the number of apertures present per inch. A logical combination for the series of sieve sizes to be used must be a fixed relation to the next smaller and to the next larger. The Tyler sieve series used as a standard in the United States follows the feasible ratio of 2:1 (Hoffmann et al., 1995). The numbers of the mesh are named to show the number of apertures per inch (= 2.54 cm). Using the best-suited choice sizes for the wire with which the sieves are woven the ratio is larger as the opening sizes are kept constant from one sieve to the next the ratio of 2:1 is large and the normal series is the ratio of $\sqrt{2}$:1 and the required intermediate series to make the adjacent ratio of series sieves set is $\sqrt[4]{2}$: 1. The British standard series has been made accessible over the standard size of wires and follows the same as the Tyler series, in which the aperture ratios are not consistent. Following the SI system, the aperture size is measure in mm (Kim et al., 2016).

To get reproducible results for accurate sieving, it is mandatory to make the process the standard procedure. The reports for the analysis are presented as the cumulative percentage of the larger material collected on the sieve or the material percentage retained on a sieve (Wu and Lee, 1998).

The sieve analysis results can be shown in various shapes, with the best being the cumulative analysis. This is given as the function of sieve aperture (*D*), the powder weight fraction *F*(*D*) represents the material passed through this and the larger sieves. This is irrespective of the situation occurring on the smaller sieves. This presents the cumulative fraction sums for all the particles smaller than that of the particles collected on the sieve concerned (Chen et al., 2017).

Hence,

F = F(D)

dF/dD = F'(D)

where; *F'(D)* is taken to be derivative of *F(D)* in respect to *D*.

So,

$$\int dF = \int F'(D)\, dD \qquad (15)$$

By integrating between the range D_1 and D_2 provide the cumulative fraction of particles between the two size ranges D_2 (larger) and D_1 this also represents provides the fraction of particles passing from sieve with aperture D_2 and is restrained on the of aperture D_1. The *F'*(*D*) graph provides particle size distribution analysis (Koyaguchi et al., 1990).

Particle size distribution is plotted in Figure 5.9 with graph *F*(*D*) smoothed. From this graph of *F'*(*D*) plotted, with the slope of *F*(*D*), is attained particle size distribution (Xu et al., 2017; Behera et al., 2019).

Eqn. (15) shows that the portion of particles of specified sizes can be found using the graph. The area under the *F'*(*D*) graph against the sizes of our interest over the total area under the graph gives us the required fraction for particles of our concerning sizes. From Figure 5.9 we can count the squares to obtain 13% in the range 300 μm (0.300 mm) and 350 μm (0.350 mm) and 9% in the range from 350 μm (0.350 mm) and 400 μm (0.400 mm).

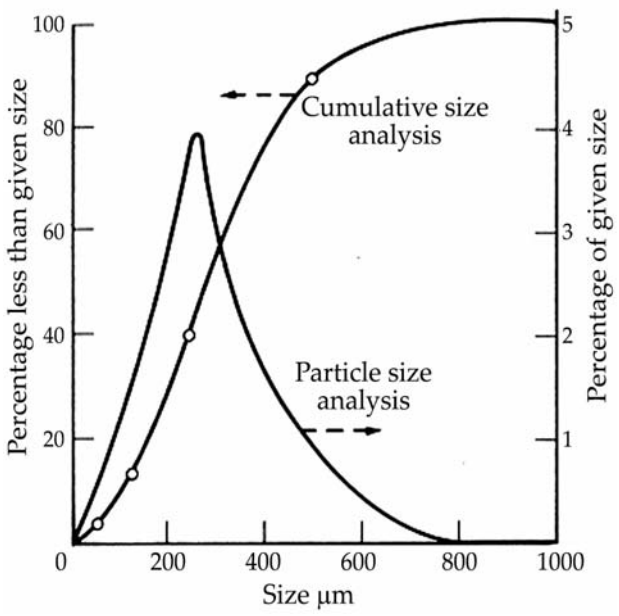


Figure 5.9. Particle-size analysis.

Source: https://nzifst.org.nz/resources/unitoperations/documents/UnitopsCh10.pdf.

In the case of sieving at an industrial level, it is not feasible to wait for the state of equilibrium to be reached. The term sieving efficiency was coined to cater to the fact that only the proportion of particles smaller than the intended size pass through the sieve. In equipment, often the sieves will be stacked along with the usage of a mechanical shaker (Behera et al., 2019).

The phenomenon of determining the particle size using sieve analysis must be approached with caution, especially in the case of non-spherical shaped particles, and must be accompanied by the powders microscopical examination. The distribution of sizes in the powders can provide a helpful approximation for parameters that are integral as in the performance features for a spray dryer, a separating cyclone, a milk powder's ease of dispersion when dissolved in water, or the surface area that is available for the reaction (Maxey and Patel, 2001; Bosse et al., 2005).

Sieves at the industrial level include rotary screens. Rotary screens are horizontal cylinders that are either covered or perforated by a screen, and the material is fed into it. As the cylinder rotates, particles of smaller size will pass through. Other examples of industrial level screens include multi-deck

screens in which the particles will fall through a series of sieves up until they have reached a sieve that is too small for the particles to fall through. Another example would be vibrating screens, usually vibrated using an eccentric weight. In the case of the vibrating screens, the amplitude and the frequency of the vibrations can considerably impact the results of the achieved separation. Screens are generally rated for their capacities and in terms of the fraction of particles passed per unit area in a specified amount of time. At an industrial range, particles of 50mm diameter and upwards can be easily screened (Smirnov et al., 2002; Winters et al., 2007).

In the flour-milling industry, continuous vibrating sieves are used. The sieves use a series of the mesh of apertures increasing in size as the particle moves along the screen. Hence the finer particles are being removed as the flour progresses. The shaking of the sieve is required to make the particles fall through as well as conveying the bigger particles onto the next segment. In certain situations, for the removal of bran air classification may be used below the sieves (Auzerais et al., 1988; Clennell et al., 1999).

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Chapter

6

Fundamentals of Adsorption Separation Technique

CONTENTS

6.1. INTRODUCTION

Due to urbanization and industrialization, the extreme release of heavy metals into the environment has led to a great challenge worldwide. As the heavy metal ions do not reduce into non-toxic end products so the presence of heavy metal ions is a major concern due to their toxicity to many life forms. One of the major problems that public and environmental health is facing is the contamination of water with toxic metal ions like Ni (II), Cr (III), Hg (II), As (III), Cd (II), Cu (II) (Anson et al., 2004). There are several important techniques like ion exchange, precipitation, adsorption, reverse osmosis (RO), electrochemical treatments, evaporation, membrane filtration, floatation, biosorption, and oxidation processes that are being applied to eradicate toxic metal ions from water and attain environmental purification are extensively used. Absorption is the one technique among these which is quite effective to eradicate toxic metal ions from water (Dąbrowski, 2001; Kast, 1981).

Up till now, several types of research have been conducted to pay attention to eliminate the heavy metals from polluted water through the adsorption process. In operation and design adsorption method can offer flexibility in several cases, will give high-quality treated wastes. Now, all over the world, adsorption is extensively acknowledged in environmental treatment applications. On the capacity of certain solid, solid-liquid adsorption systems are grounded to specially concentrate precise substances from solution to their surfaces. For the elimination of pollutants, this method can be applied like organics and metal ions from wastewater (Brunauer et al., 1938; Guendy, 2010).

In several fields' adsorption plays a significant role and facilitate different applications in industrial and chemical processes, for example, purification of air, separation of mixtures, wastewater, and water, heterogeneous catalysis, industrial catalytic process, purifying of vegetable oils, color elimination in sugar processing, drying, and cleansing of chemical substances, from the industrial waste recovery of valuable materials, adsorption of toxic gasses in the gas mask and separation of metal ores, etc. (Wilhelm et al., 2005; Qasim and Mane, 2013).

The surface phenomena that are characterized by the concentration of a chemical species from a solution onto or near the surfaces of pores of a solid or through its vapor phase is known as *adsorption*. When the substance's attractive energy with the solid surface is more than the consistent energy of the substance itself, this surface surplus usually occurs

(El-Geundi, 1991; Khulbe and Matsuura, 2018). If the solid material has a great surface area, the adsorptive uptake is improved. When the adsorption happens through London-van der Waals forces of the solid and adsorbable, it is known as *physical adsorption*. The adsorption is called *chemisorption* if the forces guiding adsorption are associated with chemical bonding forces. Nevertheless, the dissimilarity between chemisorption and physical adsorption is not always piercing. For that matter, we can take the example of the adsorption of polar vapors on polar solids that may drop under either arrangement, dependent on the adsorption energy (Gupta et al., 2012; Wang et al., 2012). If we take the thermodynamic opinion, the absorption of a substance from a solution or dilute vapor phase on a solid surface resembles a reduction in the liberty of motion of molecules and thus to a decrease in system entropy. The adsorption method as such must be exothermic to the degree that the –ve ΔH is more in magnitude than the related –ve $T\,\Delta S$ to keep a promising free-energy driving force (such as for ΔG to be –ve) (Figure 6.1).

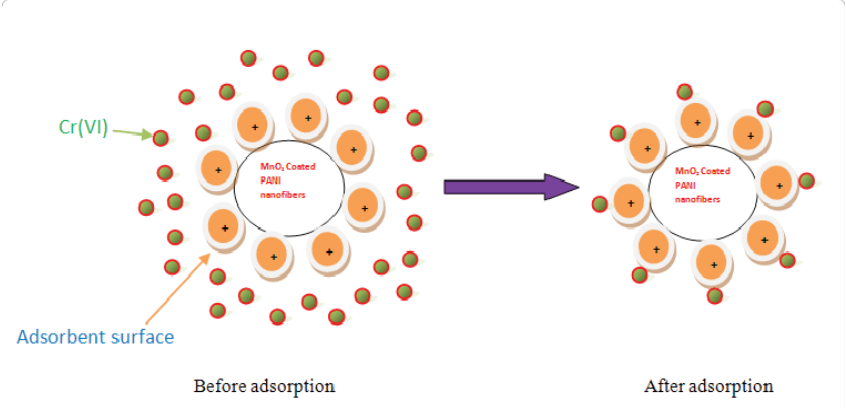

Figure 6.1. The phenomenon of adsorption of Cr (VI) on MnO_2 coated PANI polyaniline.

Source: https://www.researchgate.net/figure/Outline-of-proposed-mechanism-of-adsorption-of-CrVI-on-MPNF-adsorbent_fig2_316352217.

The amount of the vapor that is adsorbed is proportional to the mass of solid when a vapor is adsorbed on its pore space or an earlier unoccupied solid surface. The vapor uptake is depending on these factors 1) temperature (T), 2) the equilibrium fractional pressure of the vapor (P), and 3) the nature of the solid and vapor. At a fixed temperature for a vapor adsorbed

on a solid, the quantity that adsorbed per unit mass of the solid (Q) is then only a function of the equilibrium fractional pressure of the vapor (P). At a given temperature, the relation between P and Q is known as the *adsorption isotherm*. As a function of the relative pressure Q is taken, $P/P°$, where at temperature T to the saturation vapor pressure($P°$), P is controlled of the adsorbate. Usually, the controlled isotherm is more valuable, as it over a range of temperatures allows one to evaluate readily the remaining adsorption heats and other features of vapors. From solution for adsorption of solutes, by connecting C_e with Q one forms alike isotherm or with the comparative concentration, C_e/C_s, where C_s is taken as the solubility of the solute (Wang et al., 2012).

The surfaces of maximum solids are heterogeneous apart from rare cases where the microscopic structure of a solid surface is approximately unvarying, with the consequence that adsorption energies are adjustable. Successively the adsorption sites are occupied, from the sites of highest-energy to the sites of lowest-energy, with cumulative partial pressure or solute concentration. Therefore, with the raise of adsorption, the net molar heat of adsorption declines and disappears when solute concentration or vapor pressure approaches saturation. Typically, adsorption isotherms are nonlinear due to the limited active sites and the energetic heterogeneity of the solid. As a surface or a given site of the solid cannot be collected by two or more dissimilar kinds of adsorbates, the process of adsorption is essentially viable, which is contrary to a partition process. The porosity or surface area of the solid is typically the major factor affecting the sum of vapor adsorption; so, a powerful adsorbent must have a large surface area. Adsorption of a solute from solution is subject to competition by the solvent and other components in the solution. Therefore, a powerful adsorbent for single vapors is not necessarily a strong adsorbent for solutes from solution (Golimowski et al., 1985).

On an extensive variety of solids, numerous adsorption isotherms have been noted for vapors. As we can see, Brunauer (1945) assembled the isotherms into five primary classes, types I to V, as shown in Figure 6.1. Via Langmuir-type adsorption the Type I is categorized, which displays a monotonic method to a preventive value that resembles theoretically the completion of a surface monolayer (Brunauer, 1945).

For physical adsorption Type II is possibly most usual on comparatively open surfaces, in which from sub-monolayer to multilayer adsorption goes gradually; the isotherm shows a diverse concave-downward curving at some

low comparative pressure ($P/P°$) and high $P/P°$ an abruptly rising curve. The achievement of an adsorbed monolayer is being indicated by point B.

A type III isotherm shows a comparatively weak gas-solid interface, as shown by the adsorption of alkanes and water on nonporous low-polarity solids like Teflon (polytetrafluorethylene). In such a situation, on the solid surface, the adsorbate does not efficiently feast. We can see that the type IV and V isotherms are distinctive of vapor adsorption via capillary condensation on small adsorbent pores, through which the adsorption approaches an asymptotic rate as the saturation pressure is reached. The adsorption of organic vapors that are on activated carbon is naturally typed IV, while adsorption of water vapor on started carbon is type V (Figure 6.2) (Gupta et al., 2012).

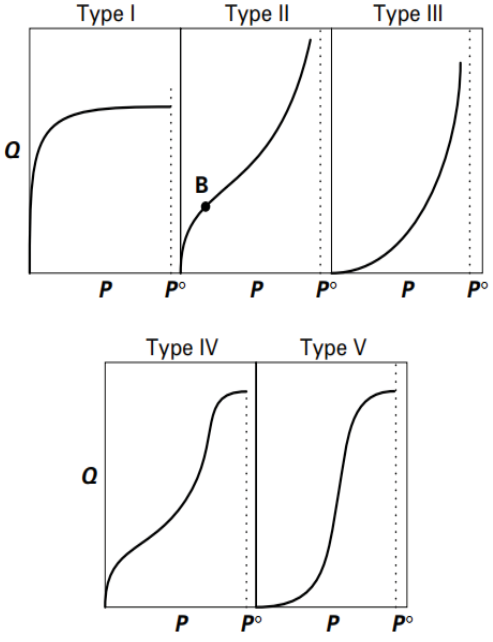

Figure 6.2. Different types of adsorption isotherms.

Source: https://onlinelibrary.wiley.com/doi/10.1002/0471264326.ch4.

An applied method to differentiate a type III vapor adsorption isotherm from a like shaped vapor divider isotherm is that the vapor divider should show a very huge uptake capacity, generally greater than 10% as per weight at $P/P° = 0.5$, whereas a type III vapor adsorption displays a very small

capacity, typically far <1% by weight, at $P/P° = 0.5$. At all boundaries, the adsorption phenomenon happens. The following kinds of interfaces can occur (Qasim and Mane, 2013):

- Solid-liquid;
- Liquid-gas;
- Solid-gas;
- Liquid-liquid;
- Solid-solid.

Though, the solid-liquid boundary has found several applications in many biological, chemical, and electrochemical processes.

6.2. TYPES OF ADSORPTION

Taking the nature of the forces in account there are two key types of adsorption method may be distinguished (Kühn, 1964; Golimowski et al., 1985):

- Chemisorption or chemical adsorption; and
- Physisorption or the physical adsorption.

The third type of adsorption, which is referred to as activated adsorption is also known (Figure 6.3).

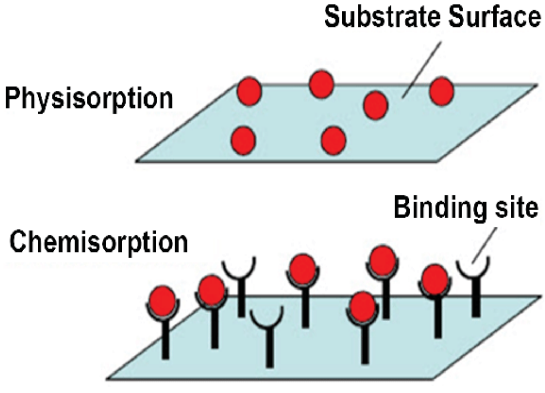

Figure 6.3. Chemisorption and physisorption phenomena.

Source: https://www.researchgate.net/figure/a-Depiction-of-physisorption-and-chemisorption-interaction-between-the-substrate_fig1_327315818.

6.2.1. Chemisorption

At a surface, the adsorption of a substance in several cases involves the creation of chemical bonds among the adsorbent and adsorbate because of sharing or transfer of electrons. Through the high degree of heat of adsorption, it is usually characterized such as, at high temperatures more than 20–150 Kcal/mole and can happen. In nonspecific and nature, it is regularly irreversible with both adsorbent and adsorbate. Because of high initial heat large amount of adsorption is a revealing of chemical adsorption to make somewhat like monolayer, subsequently by physical forces the formation of multilayer that is bound (Donohue and Aranovich, 1998; Wu et al., 2014).

6.2.2. Physisorption

Through the weak Van-der Waal's forces, the adsorbate and adsorbent are bound together. As such, there is no sharing or transfer of electron; however, new equilibrium modification takes place deprived of losing the unique association of electron along with their particular interacting species. Through the low heat of adsorption, the existence of these weak bonds is characterized generally <10 Kcal/mole [63–84 KJ/mol] (Al-Asheh et al., 2003). Just at a temperature under the boiling point of adsorbate, this adsorption is considerable. Concerning the adsorbent, it is reversible in non-specific and nature. Keeping in view the idea that physical adsorption may be to the development of multilayer, these guide to more dependency on the form of the adsorbate than that of solid adsorbent (Al-Asheh et al., 2003).

6.2.3. Activated Adsorption

The suitable amount of energy is that essential to conduct and activate the process of adsorption is called activated adsorption. For this type, the surface seemed to be catalytically active (Black and Waring, 1976).

6.3. METHODS FOR THE MEASUREMENTS OF ADSORPTION

Either by gravimetric or volumetric method, adsorption of gasses on solids is usually measured. But due to the existence of solvent in the research of adsorption from dissolved substance's solution is rather complicated (Thommes et al., 2015).

Usually, the solution of adsorbate and the amount of the adsorbent to give the equilibrium are kept in contact with each other at the specified

temperature and either directly by on the adsorbent surface the amount deposited or indirectly by calculating a decline in the amount of solution adsorbed, is measured. For the latter, general methods of analysis, for example, titrimetric, gravimetric, calorimetric, absorptiometric, and several electronic techniques are used (Li et al., 2014; Li and Zhang, 2017). But for the moderate and dilute solution or in case the adsorbents are not so good, more complex methods are employed such as the use of spectrophotometer and radioisotope. In the case of the indirect method, variation in the weight of adsorbent yields the amount of adsorption (Sing, 1985). For defining the amount of adsorbate adsorbed through substrate, the method which is used is the use of spectroscopy in the region of visible light. Due to their strong color, it is quite possible to track in the solution the variation in concentration of dyes by any typical absorptiometer. Thus, the outcome of change in the general variable of interest temperature, time, and concentration of the other reagent or dye, and the solution of pH can usually be studied. For the previous three to four decades, this technique has been found almost entirely accessible. In volatile solvents for solution comprising dissolved non-volatile solutes, by considering the residual solute and evaporating the solvent, the amount adsorbed can be calculated. On high polymer, through this method, the adsorption of fatty acid can be taken as. By calculating the solution's refractive index, the research of adsorption has also been carried out (Sing and Williams, 2004; Pokhrel and Viraraghavan, 2008).

The research of adsorption of metal ions and dyes from solution spectrophotometric technique is quite reliable, useful, and most of the time employed by several workers. The option of personnel mistakes is avoided with the aid of a spectrophotometer which assists in keeping the precision of results. It can be employed to very dilute solutions, which is the very significant feature of this instrument. We can determine the isotherm adsorption by employing this instrument. Several explanations can be given based on studies of isotherm, for instance, during the process involved interaction of nature, coverage factor, pore size and surface area of adsorbent and the accumulation of dye molecules. Through the calculation of adsorption of methylene blue, Wilson, and Kipling assessed the surface area of excellently divided solid. For estimation of the surface area of silica, alumina, graphite, carbon, zinc oxide same method was employed by several workers (Gowtham et al., 2007; Bonenfant et al., 2008).

In researches, it is concluded that the amount of heavy metal ions and dye adsorbed is reliant on the nature of adsorptive, for the adsorption of disperse dyes the method employed on textured polyester fibers came out to

be rise when the dye bath comprises a carrier when the previous treatment is specified to the adsorbent with few chemicals. Several workers have studied these types of outcomes (Hirasaki and Pope, 1974).

6.4. FACTORS INFLUENCING ADSORPTION

On a solid, the volume adsorbed from the solution be contingent upon various factors like nature of adsorbate and adsorbent, interfacial tension among adsorbent, concentration, and solution temperature of the system. The porosity of the adsorbent, pH of the solution, the existence of the extraneous material, duration of time offered and for the systems the procedure adopted. Some key factors are explained below (Farrah, 1982; Hammer et al., 1996).

6.4.1. Nature of Adsorbent

Primarily on the adsorbent's chemical composition, the nature of adsorbent relies on but for the adsorption the responsible surface is affected because of the occurrence of corners, edges, pores, pre-treatment, and cracks. On the solid adsorbent, several adsorption studies are achieved like cellulose, activated carbon, alumina, polymer, and starch, on these studies, adequate data are offered. From contact, pollution to the environment, distinction in conditions of manufacturing, and deficiency of appropriate attention to eradicating surface impurities might be adequate to source important variation in adsorption. Form aqueous solution wright has calculated the adsorption of the sequence of dibasic acid at low concentrations by Spheron and Graphon to determine the effect of elimination of oxygen complexes (Ho and McKay, 1998). Research of Shikalova et al. and Kiselev of phenanthrene and the adsorption of 6-methyl hept-1-one via dilute solution in n-heptane by adsorbent consuming an extensive range of polar character also determines the importance of surface (Aharoni and Ungarish, 1977). Pre-treatment like treating with chemicals, exposure to radiation, or heating is also significant. The nature of the surface is significantly changed by pre-treatment of the adsorbent with chemicals. Therefore, the adsorption of the acid orange II on alumina, it was shown that the adsorption volume of the adsorbent can be enhanced by the pre-treatment of the adsorbent with acid. On bentonite, the ITS (adsorption of thiram), treatment of acid with H_2SO_4 and at 110°C the heat treatment or at 200°C through the aqueous solution at 20°C has been calculated by Gonzalez et al. They came to know that by heating and also by acid wash adsorption rises. Ganichenko and his companies employed silica gels of three non-porous of the diverse degree

of hydration to calculate the adsorption of aliphatic alcohols through the solution of dilute carbon tetrachloride and improved adsorption with an increase in the amount of hydration of surfaces (Bailey and White, 1970; Martin and Al-Bahrani, 1978).

On titanium dioxide, the impact of an adsorbed water molecule on the adsorption performance of alcohols and acids has been studied. In the surface characteristic, the radiation with prevailing rays, in the same way, presents the changes of the mark and thus in real adsorption as stainless-steel displays great adsorption if phosphate and cerium ion are existing there. So, it is clear in the adsorption study that the chemistry of the surface plays a vital part (Lang and Williams, 1978).

6.4.2. Nature of Solute (Adsorbate)

From its solutions, the soluble matter's adsorption hinges on numerous factors like in the experimental solvents its solubility, physical state in solution, its chemical nature, etc. In the calculation of adsorption, solubility has a significant role. As per the study for a given solvent, a smaller amount or somewhat soluble adsorbate are more powerfully adsorbed than the considerable soluble ones. For a long time, the relation between the chain length and extent of adsorption of adsorbate has been known (Reyes et al., 1997; Luntz and Persson, 2005).

In the molecule, the impact of the growing number of aromatic rings was established by Shikalova and Kiselev. They worked on the adsorption of phenanthrene, naphthalene, and benzene in n-heptane solution and resulted in several aromatic rings that the adsorption improves. Solutes that make the slightest soluble adsorption products are those display propensity for micelles formation are generally adsorbed to a major extent. Fatty acids and several more compounds occur mostly in the form of dimeric in organic media, nevertheless in an adsorbed layer, their configuration relies on the comparative strength of their collaboration with the surface and of the connotation. So, on the non-polar solids, they are perhaps adsorbed like dimers with the main axis equivalent to the surface however in a vertical orientation on titanium and alumina dioxide. It has resulted that several solutes are usually toxic. The upsurge in the concentration of a heavy metal ion in receiving medium or water produces health risks to the human being (Berger et al., 1990).

Mining and industrial wastewaters are the root cause of the pollution of heavy metals Moreover, in developing countries, several industries are

functioned at a medium or small scale or even as a family business inside the housing buildings of the owner. A substantial pollution load can be generated through these smaller in several cases, and deprived of any facilities are cleared directly into the environment for wastewater treatment (Mohammad et al., 2009).

6.4.3. Nature of Solvent

In the adsorption, study solvent plays a vital part. Adsorption relies on the relation of solvent with the solute existing in the adsorbed layer.

It is founded that the solvent effects are significant in adsorption on polar surfaces, particularly when the solvent has an aromatic ring. On alumina is n-pentane the solvent order of adsorption of lauric acid, also back to the solvent effect (Tsai et al., 2011).

6.4.4. Influence of Temperature

In the study of adsorption, temperature plays a significant part. Comparable to gaseous adsorption, we can say that the adsorption from the liquid phase is also seen to rely on temperature. Generally, to larger adsorption, a lower temperature is advantageous such as a complete process is exothermic. In few situations, the adsorption is being enhanced by the high temperature. In fact, on the nature of adsorbate-adsorbent collaboration, the variation depends (Shumaker-Parry and Campbell, 2004).

The rise in adsorption is studied by Hockey and Mills with the temperature of the ester of n-fatty acids and lauric acid from benzene solution on silica. The hydrocarbon chains do not dislocate adsorbed benzene, they determined from calorimetric and infrared data. Everett has also reported the structuring at the boundary (Sing, 1998).

6.4.5. Influence of pH

On the pH of the solution, the study of adsorption depends. Usually, the oxide surface displays a surface charge, which relies on the pH of the solution. Whole oxides contain a well-defined pH at which this variation is zero. In determining the amount of adsorption, the pH of the solution has resulted as to be very operative particularly if the solid adsorbent is disposed to surface fluctuation because of changes in the concentrations of hydrogen, the huge distinction has been found with metal, metal oxides, and metal hydroxides as adsorbents (Belmabkhout et al., 2004).

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Chapter

7

Introduction to Electrophoretic Separation

CONTENTS

7.1. INTRODUCTION

For the analysis and separation of proteins in the foods, the electrophoretic techniques are among the best methods. In this chapter, we will review the general principles of analytical electrophoresis (EP) and the most significant detection and separation modes, such as the usage of clear reagents, and applications to food analysis/ verification (Andrews, 1986; Druml, 2001).

A common term that explains the separation and migration of charged particles (ions) underneath the influence of an electric field is called EP. There are two electrodes of opposite charge (cathode, anode) in an electrophoretic system that are connected by a conducting medium known as an electrolyte (Figure 7.1) (Halperin and Goldstein, 1999; Henger et al., 2000). From the differences in the velocity (v) the separation effect on the ionic particles occurs, which is the product of the field strength I and the particle's mobility (m):

$$v = mE \qquad (1)$$

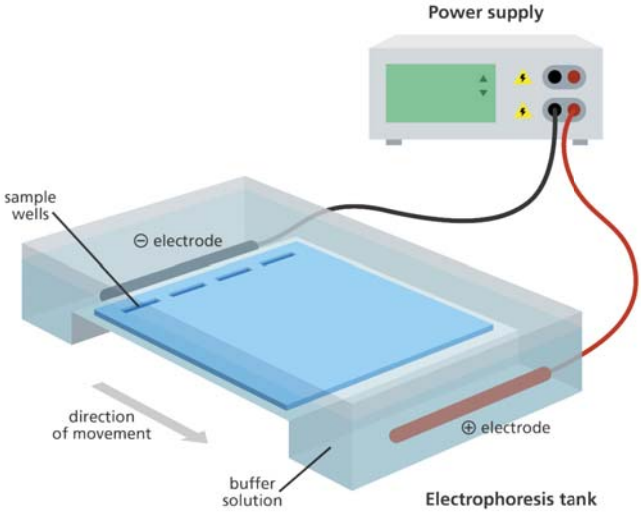

Figure 7.1. Schematic of electrophoresis mechanism.

Source: https://www.yourgenome.org/facts/what-is-gel-electrophoresis.

If we want to determine the mobility (m) of an ionic particle, it is done by the charge, shape, and particle size, and during the separation, the temperature is constant under specific electrophoretic conditions. By the electrical

parameters (power, voltage, current) the electrophoretic conditions are characterized, and factors like viscosity, pH value, ionic strength, pore size, etc., explains the medium in which the particles are moving (Chrambach, 1985; Westermeier, 1990).

In EP, the major challenge is the removal of heat generated by the passage of electric current. In the rates of migration, any temperature difference causes variations via the medium, which in the bands of separated molecules results in the distortion. Undoubtedly, at a constant temperature, it would be perfect if electrophoretic analyzes could be carried out. The basic characteristics and their different separation mode are given in Table 7.1 (Jorgenson, 1986; Jorgenson and Philips, 1987).

Table 7.1. Modes of Electrophoresis and Basic Characteristics of Systems

Mode	Characteristics
Isoelectric focusing	No molecular sieving effect, stable, and linear pH gradient, continuous electrolyte system
Isotacho-phoresis	Passage at the same velocity, concentrating effect, discontinu-ous electrolyte system
Zone electro-phoresis	Sieving effect probable dependent on support medium, con-tinuous ionic strength and pH, continuous electrolyte systems

7.2. FORMATS OF ELECTROPHORESIS (EP)

Generally, all methods of EP can be taken as either 'support medium,' where anti-convective support matrices overpower the thermally determined convection-diffusion and currents in the electrophoretic medium, or in a 'free solution,' via which no anti-convective stabilizers are employed (Kellum, 2000).

Through the further factors in supporting media, the sharpness and mobility of separations can be influenced. These contain IE effects and adsorption with the matrix, electro-endosmosis inside the support matrix, and inhomogeneity. Moreover, support media give imagining of separated zones in a common format where slabs, foils, and strips can be effortlessly detained, stained, and manipulated in ways not probable for free solutions (Oh, 2000; Khanna and Kurtzman, 2001).

The most commonly practiced formats and techniques of EP in food analysis are listed in Table 7.2. Gels made from agarose and polyacrylamide are the supportive media of choice nowadays. For the work of routine screening, the usage of cellulose acetate strips is common where the viable accessibility of ready-to-use material, the comfort of handling, and speed are appropriate attributes. Thin-layer and paper EP (for example, on silica gel) is effectively employed for the study of high-molecular-weight lipopolysaccharides and polysaccharides (Kraut, 2000; Koeppen and Stanton, 2001).

Table 7.2. Important Electrophoretic Techniques and Types of Detection in Food Analysis*

Format	Electrophoretic Technique	Type of Detection
In free solution	Capillary isotachophoresis	On-line optical
	Capillary zone electrophoresis	On-line electrical
In non-sieving medium	Zone electrophoresis in agarose	On-line thermal Staining for proteins
	Isoelectric focusing on agarose	Amido black silver, Coomassie blue
In sieving medium	Zone electrophoresis in polyacrylamide: • with pore size gradients • in multiphasic gel and buffer system • inhomogeneous gel and buffer system • in the existence of SDS cleaner Isoelectric concentrating in polyacrylamide	phosphoproteins, lipo-, ikipe-
		assays for enzyme activity
		Colloidal gold Localization of specific constituents
		Immunoprinting, immunofixation
		Spoiling on the immobilized matrix: normal staining approaches immunoblotting

*These sorts of detection are appropriate to both the non-sieving and sieving media.

7.2.1. Agarose Gel

It is derived from agar and it is an extremely decontaminated polysaccharide, a normal product of red seaweed. The agarose materials which are

commercially accessible display different, characterized stages of electro-endosmosis, because of the presence of carboxyl and sulfate groups in agar. On agarose gel even though EP has been obscured by the usage of polyacrylamide in the study of several glycoproteins and proteins, in applications, it remains vital where nonrestrictive gel and a very large pore size hence essential, for example, in immune-electrophoretic methods, especially those depending on an immunodiffusion stage the separation of very large molecules with an average hydrodynamic radius greater than 5–10 nm, like lipoproteins, antibodies, few membrane proteins, viruses, and nucleic acids (Remer, 2000; Rose and Post, 2001).

Though agarose gels are quite rigid, they are less elastic than polyacrylamide and so are more easily dithering and need cautious handling (Figure 7.2) (Silver et al., 2000; Sugiura et al., 2000).

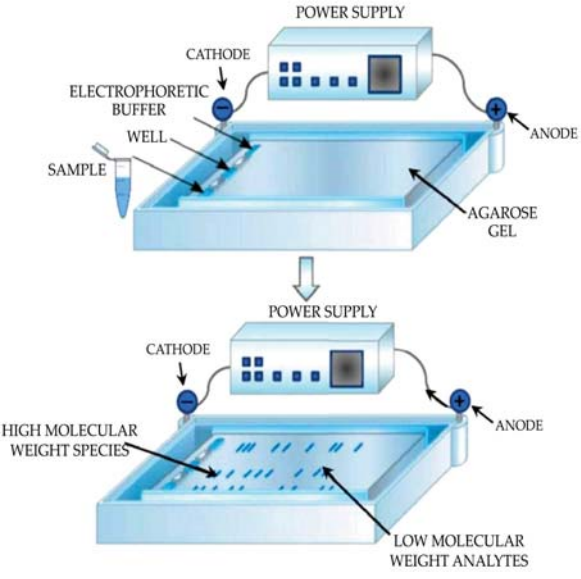

Figure 7.2. Setup of agarose gel electrophoresis.

Source: https://www.sciencedirect.com/topics/chemistry/agarose-gel-electrophoresis.

7.2.2. Polyacrylamide Gel (PAG)

Quite ideal for making well-defined, chemically inert matrices of variable, and mechanically stable pore size, PAG (polyacrylamide gel) is perhaps

the most commonly employed gel medium in electrophoretic food analysis nowadays. The gels are mechanically completely transparent, easy to handle, and strong. Because of the comparatively small pore size, diffusion is restricted, so PAG generally offers a better resolution for maximum proteins rather than other gel media (Bishop et al., 1967).

Via the polymerization of acrylamide monomers, PAG is produced with the cross-linking co-monomer *N*,*N*^{I}-methylenebisacrylamide, in the existence of free radicals generally given by the photochemical initiator riboflavin or the chemical initiator ammonium persulfate. Via the accumulation of *N*^{I}, *N, N,* and *N*^{I}-tetramethylethylenediamine, the polymerization reaction is controlled, which offers a source of tertiary amines (Konarska and Sharp, 1986).

By monitoring both the acrylamide's concentration and the relative proportion of the cross-linking agent BIS to acrylamide, the pore size of the gel can be simply adjusted. This outcome is well-distinct molecular separating properties, giving an extra separation effect reliant on molecular size (Talmadge and Roy, 1993).

7.2.3. Sample Pretreatments

If sample proteins are not soluble or responsible for precipitation or accumulation during an electrophoretic separation, unfolding, and dissociating agents like nonionic detergents or urea for example, octyl glucoside, are mixed with the gel or to the sample. Uncharged thiol reducing agents like the unscented compounds dithioerythritol or dithiothreitol or β-mercaptoethanol are employed to keep free thiol groups in proteins against disulfide bond formation and oxidation (Heuer et al., 1997). Certainly, these mixtures will also slash disulfide bonds,

they can be mixed through a separating agent like urea or, rather, SDS (sodium dodecyl sulfate) to source the thoughtful dissociation of aggregates and proteins into their essential subunits.

In SDS-PAG EP this method is successfully employed in which samples are run without and with the usage of these thiol dropping agents to form an approximation of the degree of protein cross-linking by intramolecular- and/or inter disulfide bonds. Through dialysis or ultrafiltration (UF), the isoelectric focusing must be desalinized (McCormick, 1988; Schmalzing et al., 1993).

7.3. GEL ELECTROPHORESIS (EP)

In a chemically inert gel matrix, the gel EP is zone EP, like agarose or polyacrylamide. In a small volume, the sample is employed like a narrow zone, for example, in gel slots. When the electric field is employed, each sample component travels as per its mobility in a gel medium of ionic strength and constant pH. Through exploiting the differential rate of migration, the separation into 'pure zones' is attained, although minimizing zone dispersion because of diffusion and heat convection (Figure 7.3) (Lesse et al., 1990; Lareu et al., 1994).

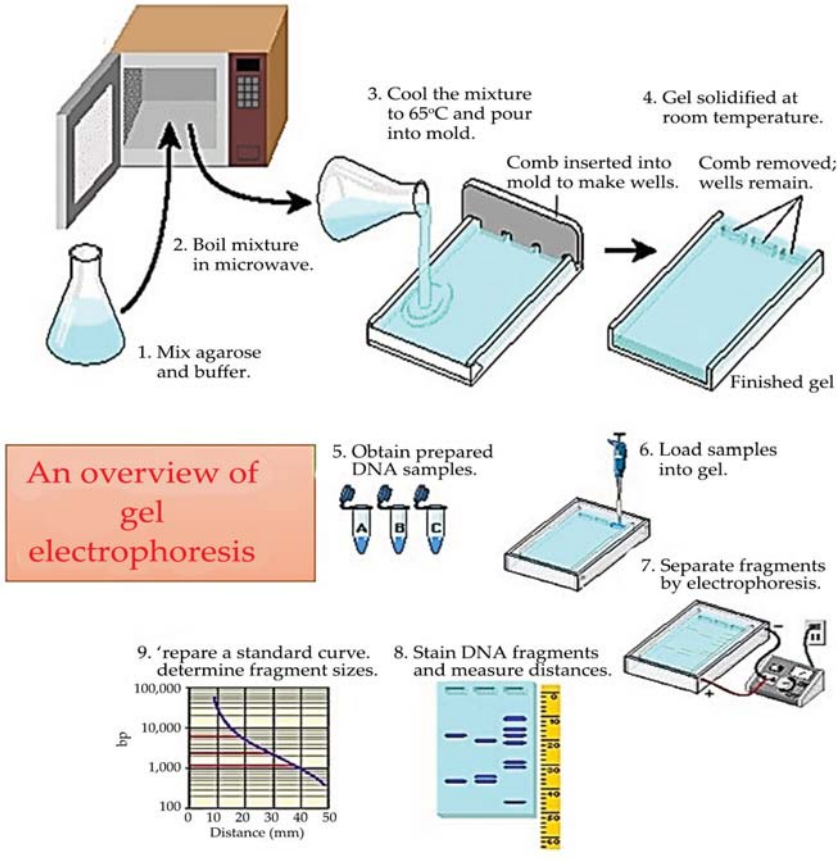

Figure 7.3. Flowchart of gel electrophoresis.

Source: http://universe84a.com/collection/gel-electrophoresis/.

There are three dissimilar geometrical forms in which gel EP can be taken: vertical cylinders (rods), vertical slabs, or horizontal slabs of gel, often also taken as tube gels. In thinner layers, slab gels are favored to tube gels or thicker slabs, as they give sharper zones, faster separation, and more effective and fast cooling and subsequent staining. In PAG EP, for the horizontal system, there is a pure preference to employ ultrathin gel layers polymerized onto transporter foils. The benefits of horizontal versus vertical systems are compactable to hand, more effective cooling, decreased material prices, availability of entirely automatic systems, and flexibility to other forms of EP like isoelectric focusing (Schultz and Kennedy, 1993; Shimura and Kasai, 1997).

7.4. PAG ELECTROPHORESIS (EP)

In the study of complex mixtures of proteins, PAGE (polyacrylamide gel electrophoresis) is the most extensively employed method. PAGE of proteins can generally be classified as follows (Laurell, 1966; Meyers et al., 1976):

- Multiphasic (discontinuous) systems, where the nonrestrictive large pore gel, a stacking gel, is on the top layer of the small-pore gel, unraveling gel. With different barriers, each gel layer is formed, which may vary in ionic strength, and ion mobility, and pH.
- Homogeneous systems by using continuous buffer media and by using a single separating gel.

In the assembling gel of a multiphasic system, in the isotachophoresis (ITP) mode, sample components are detached having no molecular sieving effect. After clearing the boundary between separating gel and stacking, the components of the sample are then detached by charge and size in the normal way. The high resolving power of this technique, which is frequently taken as disc PAGE, is because of the creation of very sharp zones generated by the buffer discontinuities and gel. The generation of thin starting zones makes disc PAGE quite appropriate for the usage with dilute sample solutions (Figure 7.4) (Arnott et al., 1974; Balgude et al., 2001).

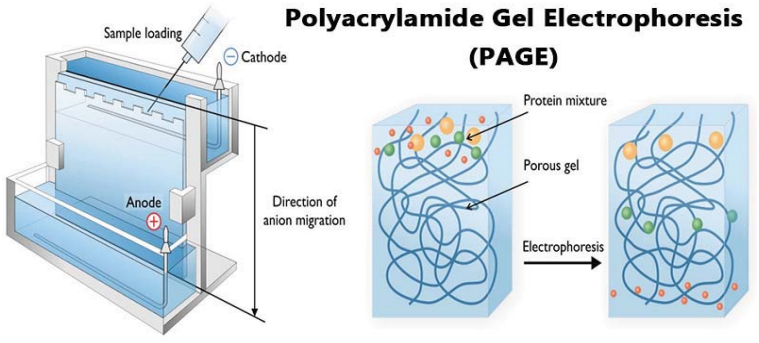

Figure 7.4. Flowchart of polyacrylamide gel electrophoresis process.

Source: https://microbenotes.com/polyacrylamide-gel-electrophoresis-page/.

Gradient PAGE offers a resolution that is greater than that of a gel of a single concentration. In tubes or slabs, polyacrylamide can be cast in which continuously the concentration of acrylamide rises over the length of the gel, thus producing an increasing sieving effect because of reducing pore size (Swank and Munkres, 1971).

In the existence of the anionic cleaner SDS PAGE of food, proteins are most effective. Around the polypeptide backbone SDS wraps, collapsing noncovalent protein aggregates (tetramers, dimers, etc.), and in basic charge of the proteins avoids the differences. Like this, SDS alters the proteins to shafts of –ve charges with equivalent charge densities, such as it discusses a –ve charge to the polypeptide chains in an amount to their length and conveys the similar free solution movement to all proteins, irrespective of their identity. By the sieving effects separation of SDS-denatured proteins mainly happens, with lower-molecular-weight proteins traveling more quickly over the gel. In blend with pore size gradients and irregular barrier systems, SDS-PAGE can be an even more influential and effective technique for protein separation (Loening, 1967; Fried and Crothers, 1981).

7.5. ISOTACHOPHORESIS (ITP)

Separation in ITP or displacement electrophoresis is because of mobility difference of charged ions in the different solutions of discontinuous electrolyte system, including a leading as well as terminating electrolyte. Sample placement between the two electrolytes is done. Cations and anions can be checked separately, but both cannot be checked simultaneously.

Leading electrolytes with higher mobility anion are required for anionic calculation like chlorides and vice versa. Negative ions travel to the anode in order of their mobility (Trudel and Asselin, 1989; Raymond et al., 1994).

Sample separation during the migration happens between the terminating and leading electrolytes. Every individual sample moves as a "pure" band separately upon the establishment of equilibrium. Stacking of each pure band is done between the next highest and the lowest mobility sample component. Sample analyte zones are formed following the leading ion zone acting as a locomotive in an anion train, and preceding the terminating ion zone (glucose) in the order of decreasing mobility at a velocity similar to the leading electrolyte zones. Electric field strength varies in each analyte zone in a step-wise manner, causing the concentrating effect. No diffusion can take place in the preceding or following bands due to the concentration effect introducing sharp boundaries between the bands, and diffusional broadening is prevented (Figure 7.5) (Chang et al., 2016).

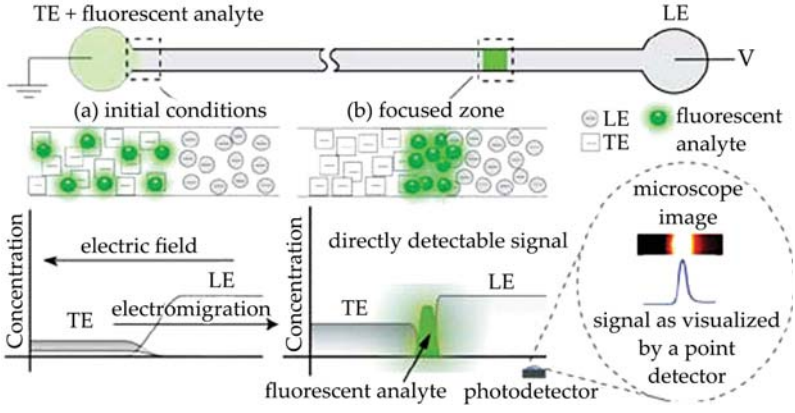

Figure 7.5. Representation of "peak-mode" isotachophoresis using single-interface injection.

Source: https://www.researchgate.net/figure/Schematic-of-peak-mode-isotachophoresis-with-single-interface-injection-A_fig3_44696028.

Small-bore capillary tubes using a free solution id usually done for analytical scale ITP, with a well-defined pH, constant current, and on-line detection. Rectangular peaks, rather than Gaussian-shaped curves are seen in the resolved band emergence. The measure of electric field strength can identify the bands, by using a capillary channel containing pair of microelectrodes. The band length gives the quantitative information.

Usually, electrical detection is employed, but UV and thermal detection is also applied for ITP measurements (Ünlü et al., 1997; Muyzer and Smalla, 1998).

7.6. IMMUNO-ELECTROPHORETIC TECHNIQUES

It is the combination of an electrophoretic step with followed antigen-antibody complexes precipitation. The usual method in food analysis is the migration of the antigenic protein into or through an antibody-containing gel. pH and buffer values are adjusted to let only the antigens migrate, very slow or no migration occurs in the antibodies and is present evenly in the gel during electrophoresis. The most used techniques are (Altland et al., 1980):

1. **Counter Immune-Electrophoresis:** Also termed as crossed-over immune-electrophoresis: Antigens travel towards uncharged antibodies electrophoretically, moved by electroendosnotic flow in the counter-current, and as a result, precipitating arcs are formed.
2. **Grabar/Williams Method:** Immuno-diffusion stage preceded by normal zone electrophoresis. Precipitating arcs are formed through the electrophoretically separated components assisted diffusion of antigen from gel troughs that are cut parallelly.
3. **Laurell Rocket Techniques:** Electrophoretic transportation by antibodies containing gel slab in which the antibodies are immobilized by selecting sufficient pH. In this way, rocket-shaped precipitate peaks are formed which have the concentration of antigen and height and area related linearly. Not for complex mixtures, but it allows a quantitative analysis of antigens.
4. **Crossed Immune-Electrophoresis:** Isoelectric focusing or one-dimensional agarose zone electrophoresis follows the antibody-containing gel electrophoresis in the second dimension, mountain-like precipitation peaks are formed. In this method, quantitative as well as qualitative analysis of peak area is possible.

The opaque precipitate formation will be perfectly adequate and clear relatively in a transparent gel. After the non-precipitated protein removal, the detection sensitivity can be increased by protein staining techniques, as well as other methods like enzyme tagged, fluorescent, or radiolabeled reactants (Florvaag et al., 1986). The limitation in this process is the availability of specific antibodies used to avoid cross-reaction with non-targeted proteins (Jung et al., 2006).

7.7. CAPILLARY ZONE ELECTROPHORESIS (CZE)

Along with versatility and power, conventional methods lack automation and need extensive labor as well as time. Instrumental methods for free solution electrophoresis have been developed in the last 10 years, which provide online detection. Currently used techniques are capillary electrophoresis (CE) and capillary zone electrophoresis (CZE). Capillaries with 20–200 mm inside diameter are used in free solution electrophoresis for CZE. Least zone broadening and heat dissipation in the electrophoretic medium by the molecular convection and diffusion are aided by these small dimensions. High-efficiency separations due to the usability of high voltages are achieved. A separate reservoir containing a high-voltage electrode and the same electrolyte is used for every capillary (Hirschfeld et al., 1960). The inertness of capillary influences the CZE strongly, especially in buffer ions or analytes polar interactions. Inert capillary separations have the basis on the charged molecules' mobility in an electric field like in conventional zone electrophoresis. And the separating forces of electro-endosmotic flow and electrophoretic migration are used in non-inert capillaries CZE (Figure 7.6) (Wallingford and Ewing, 1987).

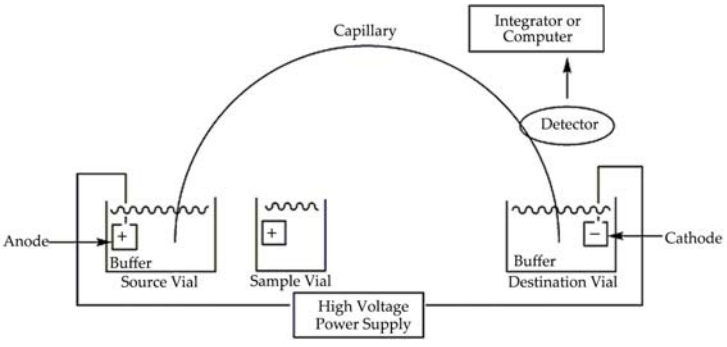

Figure 7.6. Schematic representation of CZE process.

Source: https://en.wikipedia.org/wiki/Capillary_electrophoresis.

7.8. APPLICATION TO FOOD ANALYSIS

The primary targets of Electrophoresis (EP) in food analysis are peptides and proteins. Using the EP technique, the quantification of proteins, assessing homogeneity, and characterization of molecular architecture can be done. Protein yield increase, protein-protein interaction, and enzymatic

protein degradation have been characterized by using electrophoresis. The studies of processing treatments as well as compositional parameters have helped highlight the usefulness of mentioned features on the texture as well as the flavor of protein-based foods. It holds special value in dairy science and technology where new developments have been made due to the quantification and electrophoretic separation of the milk proteins as well as peptides (Rodríguez-Ramírez et al., 2011). The major milk proteins' simultaneous determination by IEF has been demonstrated in Figure 7.7. The protein distribution changes have been followed by this efficient procedure in accelerated ripened cheeses and similar methods.

In complex food systems, isoelectric focusing and SDS have been used on larger peptides in ultrathin and horizontal PAG slabs. Subtle differences in molecular properties that can occur due to genetic variations or posttranslational modifications have also been determined from these methods. THE two-dimensional EP approach gives the ultimate protein separation. One dimension isoelectric focusing, when combines with the 2D-SDS gradient page EP, gives the best resolution. Proteolytic breakdown and verification of the intact primary structure of proteins after processing can also be done. These high-resolution techniques are used to follow proteolytic breakdown and to verify the intact primary structure of food proteins after certain processing treatments.

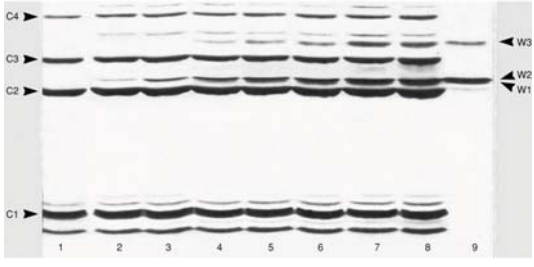

Figure 7.7. Simultaneous determination of major milk proteins using isoelectric focusing in ultrathin-layer PAG.

Source: https://www.sciencedirect.com/science/article/pii/B012227055X014097.

Note: C1, aS1-casein B; C2, b-casein A2; C3, b-casein A1; C4, aS2-casein; W1, a-lactalbumine; W2, b-lactoglobuline A; W3, b-lactoglobuline B. Samples from left: recombined milk powders with casein: whey protein ratio of 100:0 (1), 98:2 (2), 95:5 (3), 90:10 (4), 80:20 (5), 70:30 (6), 60:40 (7), 50:50 (8), and 0:100 (9)

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Chapter

8

Membrane Separation Technology

CONTENTS

8.1. INTRODUCTION

In the food and pharmaceutical industries, there is a need for effective separation techniques for achieving high-grade products to support the elimination or recovery of toxic or substantial constituents from industrial effluents and the population with high-quality water (Padaki et al., 2015; Shenvi et al., 2015). Still, one of the leading hurdles in the chemical industries is the separation, concentration, and purification of molecular mixtures. Therefore, several separation methods are being applied recently, e.g., distillation, anion exchange, precipitation, extraction, crystallization, and adsorption. Recently, a group of processes that utilize semi-permeable membranes being the separation barriers is used to add these traditional separation techniques (Lonsdale, 1982; Ho and Sirkar, 2012).

A technology that judiciously separates (or fractionates) materials through minute gaps or/and pores in the molecular composition of a continuous structure are known as membrane separation. Membrane separations are categorized by the separation driving force and by pore size. These categorizations are: (i) microfiltration (MF); (ii) ultrafiltration (UF); (iii) ion-exchange (IE); and (iv) reverse osmosis (RO).

Figure 8.1 demonstrates the examples of variant substances with near correspondence to the pore size of the membrane separation process that might be employed.

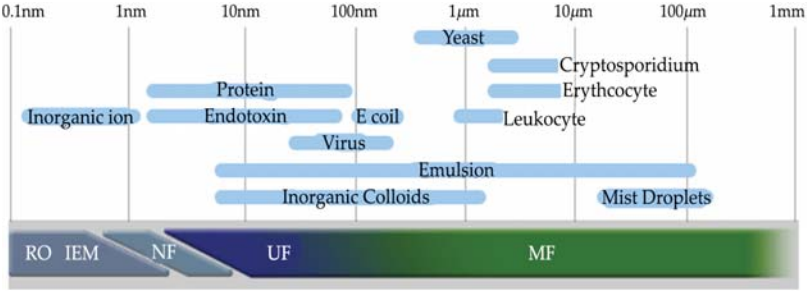

Figure 8.1. A chart demonstrating ranges of membrane separation processes.

Source: https://www.asahi-kasei.co.jp/membrane/microza/en/kiso/kiso_1.html.

At first introduced as an analytical tool in biomedical and chemical laboratories, membranes, and membrane processes rapidly grown into industrial techniques and products with considerable commercial and technical significance (Bhattacharyya and Butterfield, 2003; Strathmann,

2004). Currently, membranes are being operated on a big scale to produce potable water from the sea and brackish water, to clean industrial effluents and restore beneficial components, to separate gasses and vapors in petrochemical procedures, and to concentrate, fractionate, or purify macromolecular mixtures in the drug and food industries. They also play an important role in storage systems and energy conversion, drug delivery devices, chemical reactors, and artificial organs (Osada and Nakagawa, 1992; Drioli and Romano, 2001).

8.2. MEMBRANE SEPARATION PROCESSES

Membranes permit certain ions or molecules to infiltrate using diffusion and sometimes using specially designed 'facilitated diffusion.' The rate of passage is based on the concentration, pressure, and temperature of the solutes or molecules on any side of the membrane, also the permeability of the membrane to every solute. This relies upon solubility characteristics, chemistry, or solute size (Figure 8.2).

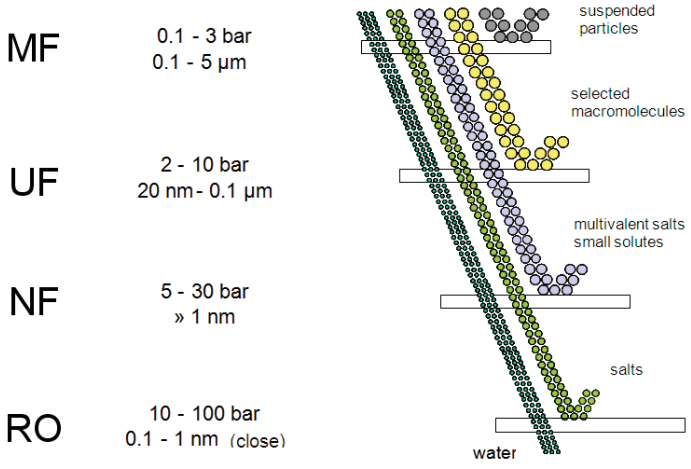

Figure 8.2. Conventional ranges and properties of various membrane separation processes.

The tubular, spiral-wrap, capillary, and flat sheets are included in membrane configurations and are used to isolate particles approximately ranging from 10 µm and less. The following are various different membrane separation processes.

8.2.1. Microfiltration

Microfiltration (MF) gives a physical obstacle to a broad range of pathogens, microorganisms, and suspended particles greater than 0.1 μm. It is a significant step in a vast range of treatment applications, involving municipal and industrial wastewater treatment and potable water treatment, where it serves as a disinfectant and filter both. Typically, below 2 bar pressure is required for microfiltration for the membrane separation process (Figure 8.3).

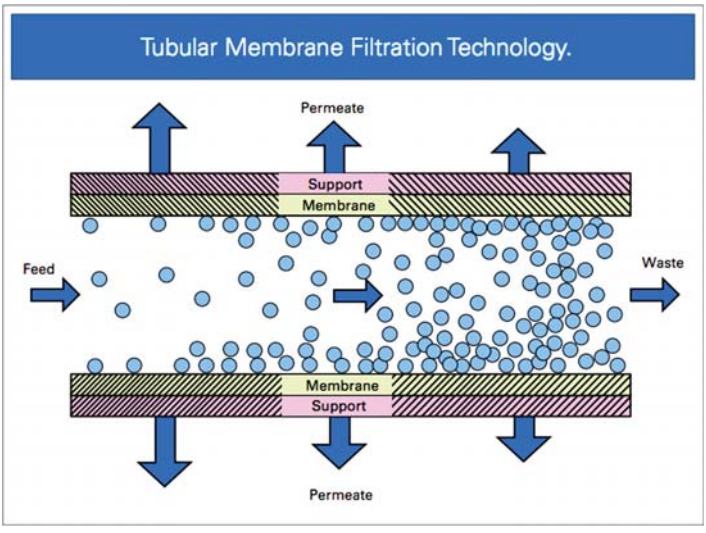

Figure 8.3. Microfiltration membrane filtration process.

Source: http://www.porexfiltration.com/learning-center/technology/what-is-microfiltration/.

8.2.2. Ultrafiltration (UF)

For the removal of macromolecules and particulates from water in wastewater treatment and potable water, ultrafiltration (UF) is used. It is used either along with these processes being an integrated treatment process in water with high suspended solids or as a substitute for present tertiary and secondary filtration. The pore size of UF membranes is between 100 nm and 2 nm and need 1 to 10 bar pressure to function (Figure 8.4).

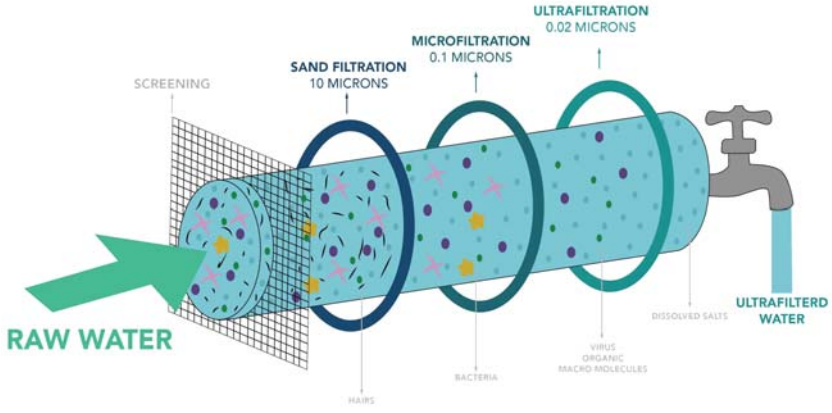

Figure 8.4. The ultrafiltration membrane process.

Source: https://crystalquest.com/pages/what-is-ultrafiltration.

8.2.3. Nanofiltration (NF)

Nanofiltration (NF) is applied in water treatment applications having fewer total dissolved solids. It is used for the elimination of organic by-products and water softening that may consequent from prior disinfection treatment. NF is a productive water softening solution when keeping scale-forming hydrated divalent ions at the time of passing smaller hydrated monovalent ions. NF is obtained with membranes having pore sizes between 2 and 1 nm needing 3 to 20 bar pressure (Figure 8.5).

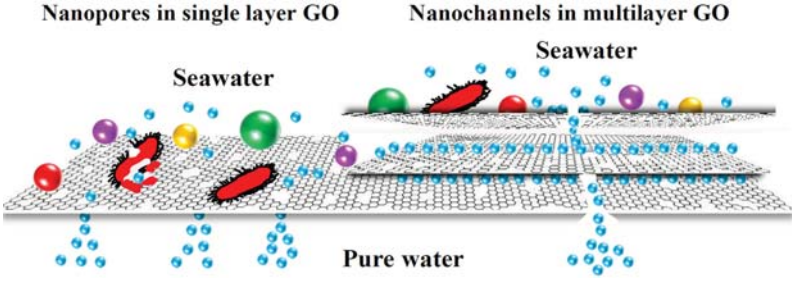

Figure 8.5. Graphene-based nanofiltration membrane.

Source: https://www.sciencedirect.com/science/article/abs/pii/S0011916417308767.

8.2.4. Reverse Osmosis (RO)

For the separation of ions, salts, and small organic molecules from feed water, reverse osmosis (RO) is used which utilizes membranes having pores less than 1 nm. RO is utilized in ultrapure, treating potable, and process water, and is the main process in seawater and brackish desalination. The high pressures (usually up to 80 bar) are required by RO to operate due to the low porosity of these membranes (Figure 8.6).

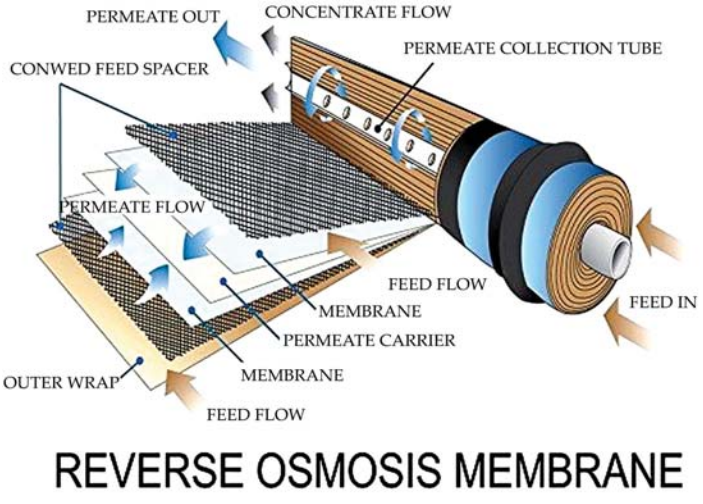

Figure 8.6. Reverse osmosis membrane structure.

Source: https://www.pinterest.com/pin/644155552935826017/.

8.3. MEMBRANE TECHNOLOGY

The presence of membranes is from the 18th century when separating two stages from each other via restricting the movement of constituents from it in a choosy style. They serve as a barrier. A lot of effort has been made to make membranes convenient for different multiple applications.

Basically, membranes can be classified as anisotropic or isotropic. In composition and physical structure, the isotropic membranes are consistent that is broadly used in microfiltration membranes. These membranes can be microporous; therefore, their permeation fluxes are rationally high compared to when they are nonporous (dense) and their administration is very much limited due to low permeation fluxes (de Morais Coutinho et al., 2009;

San Roman et al., 2010). At the same time, anisotropic membranes that are especially engaged in the procedures of RO, appear to be non-uniform at the membrane area and are formed of different layers with distinct configurations and structures. The membrane's thin selective layer is supported by a thicker and greatly permeable layer.

Regarding membrane material makeup, they are classified as either inorganic or organic membranes. For the formation of origin membranes, synthetic organic polymers are used. In many situations, synthetic organic polymers are utilized to form membranes for pressure-driven separation procedures (microfiltration, RO, UF, and NF). These consist of polyethylene (PE), cellulose acetate, polytetrafluoroethylene (PTFE), and polypropylene, among others. On the other side, materials like ceramics, silica, metals, or zeolites are used for forming inorganic membranes.

Different driving forces are in charge of the passage of media over the membranes. They are (i) equilibrium-based membrane procedures, (ii) non-equilibrium-based membrane procedures, (iii) non-pressure driven procedures, and (iv) pressure-driven procedures. Figure 8.7 illustrates an overview of some of these methods depending on their driving forces, and the following passages describe separately detailed membrane processes (Kumar et al., 2013; Alzahrani and Mohammad, 2014).

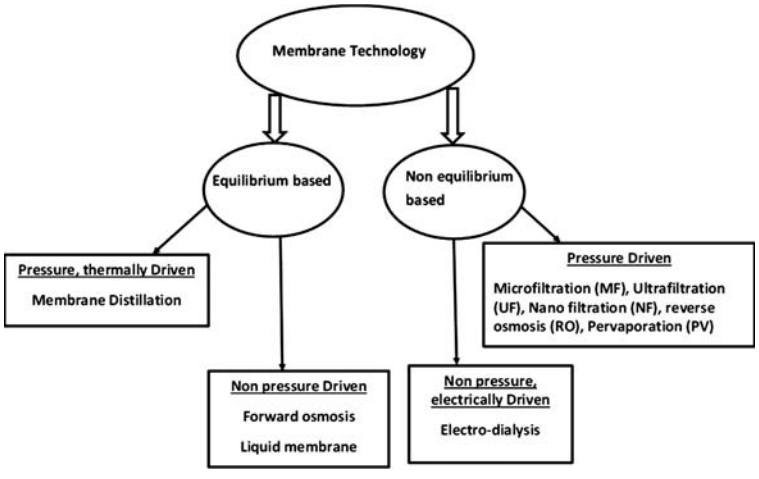

Figure 8.7. Diagrammatic illustration of certain membrane procedures.

To improve the water quality while working in the water industry for discharge, re-use, or use in the environments, membrane technology incorporates membranes able to remove contaminants, e.g., bacteria, and

protozoa down to ions. The membranes range between non-porous and finely porous. Some of the advantages of membrane technology are its modular character, the standard of the product water, the fairly small footprint, allowing application at a very large or small scale, and, sometimes, the less use of energy (Bilad et al., 2014; Wei et al., 2014). Continuous growth in membranes will be experienced in the water industry in the near future due to increasing water shortage together with stable development in energy requirement, cost, and membrane performance (Guo et al., 2012; Wei et al., 2014).

It has been cleared that for the removal of some of the adulterants, traditional water treatments are failed, moreover, new adverse compounds can be created in the disinfection phase (for example, trichloromethane is produced by the reaction between chlorine and natural organic matter). Membrane procedures can be a response for a contemporary physical treatment of drinking water aside from wastewater recovery.

Different membrane procedures having different separation ranges have existed (3, 4). Table 8.1 has exhibited the control of membrane operations in water treatments. Nano-filtration (NF) and RO operate at a higher pressure (10e-0 bar for RO, and 8–20 bar for NF) comparable to microfiltration (MF) and ultra-filtration (UF), which are low-pressure procedures (0.1–2 bar for MF, and 2–10 bar for UF). MF and UF efficiently remove microorganisms, suspended solids (MF), and colloids (UF). Moreover, being popular for the desalting of seawater and brackish water, RO is operating to eliminate low molecular weight organic compounds as well, either they are of the synthetic or natural source from water (Bhattacharjee et al., 2017). Active in water softening by the removal of magnesium and calcium ions and for disposing of some simple organic compounds, the nano-filtration is a pretty immature membrane process. To replace individual purification stages in traditional water treatment plants or to design an integrated membrane procedure, different membrane processes can be combined successfully. Nevertheless, technological and economical determinants mainly limit a broader diffusion of membrane techniques. Economically, there is still a high cost of certain types of membrane (especially those initiated from ceramic materials), and the price increases as the expected water quality increases. Even though the price of a RO plant has decreased due to raised diffusion of this technology in the consideration of water desalination, MF, and UF are quite cheap in comparison to that (Barelli et al., 2008; Munirasu et al., 2016).

Table 8.1. The Membrane Used in the Membrane Process for Water Treatments

Membrane Process	Membrane Type	Material	Application
Microfiltration	Porous-symmetrical or asymmetrical	Polymeric Ceramic	Sterilization Removal of colloids and suspended solids
Ultrafiltration	Porous-symmetrical or asymmetrical	Polymeric	Removal of macromolecules and viruses
Nanofiltration	Dense or nano-porous asymmetrical	Polymeric	Removal of organic compounds
Reverse osmosis	Dense composite or skinned	Polymeric	Removal of salts

At the same time, a separate study of the functional environments (i.e., management of scaling and fouling phenomena), and the suitable membrane material (i.e., chemical stability in challenging circumstances such as antifouling properties, chlorine-containing water) are required by each newly possible application of membrane processes. The presence of scaling may be because of high levels of magnesium hydroxide or silica, calcium sulfate, or fluoride or carbonate, whereas fouling might happen due to organic or inorganic colloids, metal oxides, or bacteria, or other microorganisms. On membrane composition, the research effort is now focused on these problem's solutions. The academics and industry are concentrating on their research on the development of membranes having higher chemical stability for the recovery of wastewater, the improvement of modules and membranes with antifouling properties, the formation of large membrane surfaces with homogeneous characteristics, and minimizing energy consumption amid applications by an appropriate study of the equipment and membrane module. For the improvement of the operation of the membrane process in the production of drinking water a suitable option of the module design, pretreatments, membrane structure, and material, and the operational modes to obtain the primary membrane performance are essential (Shuit et al., 2012; Yeo et al., 2012).

The membranes used in the various applications differ highly in their method of operation, structure, and function. However, because all

membranes have various identical aspects, they are assumed to be attractive tools for the isolation of molecular mixtures. The separation must occur in such a way that it does not be a reason for any chemical change in the elements of a mixture in a physical method at a circulating temperature. It is mandatory for applications in downstream processing of bio-products, multiple drug delivery systems, artificial organs, and the drug and food industry, where temperature-sensitive items should be handled repeatedly. Moreover, membrane properties may be changed and personalized for particular separation tasks. While membrane processes are especially well-regulated and non-complex as compared to traditional separation methods. They are suitable for substantial continuous functions same as for batch-wise treatment of minute quantities (Suárez et al., 2012; Humpert et al., 2016).

Vague in structure and function, synthetic membranes are used broadly as powerful scientific and technical tools in modern industrialized society. People normally associate the thought of membrane to same as a filter, i.e., a device that can separate various components from a mixture depending upon their size. Apart from that, a membrane can be too complicated in both function and form. It could be a part of a micrometer or a millimeter wide. It may be heterogeneous or homogeneous, anisotropic or isotropic, liquid or solid in its structure. Its electrical resistance can be from a ratio of an Ohm to millions of Ohm (Atadashi et al., 2011; Samaei et al., 2018).

Still another characteristic of membranes is perm selectivity that relies on the unlikeness in the transport rates of various constituents that exist in the membrane matrix. A measure of the rate at which a given element is moved via the membrane at various circumstances of concentration, electric field, temperature, or/and pressure is called the permeability of a membrane. The transportation of a constituent is established through the chemical nature of the permeating material and the electric charge, the size of the permeating constituents, the structure of a membrane, together with the driving force such as the electric potential gradient, concentration, or pressure across the membrane. The phenomenon of coupled, facilitated, or active transport is observed on the transportation of certain constituents from the membrane may be excited by a chemical reaction occurring in the membrane, supported by specific chemical compounds, or restrict with the transport of other constituents (Suárez et al., 2012; Humpert et al., 2016).

Usually, a membrane is an obstacle that interacts with and/or separates two different regions while controlling the movement of matter and energy

among these regions. The flexibility of the membrane's arrangement and functions provide a comprehensive and complete definition of it. A membrane may serve as a contacting barrier that most of the time holds contacting two regions within which the exchange takes place. It may act as a selective barrier as well where it manages the movement of components within the two regions strictly (Wenten, 2002; Krishnamoorthy et al., 2014). Artificial synthetic membranes and biological membranes (that are a portion of living organisms) can be varied. Biological membranes are able to perform complex and certain transport movements in living organisms. This membrane can finish the job speedily and effectively with less energy expenditure using active transport continuously (Muhamad et al., 2016; Pulido, 2016).

Generally, there is minimal selectivity and energy efficiency of synthetic membranes while having only passive transport characteristics because they do not have a function or structure as complicated as biological membranes. However, they have symbolic chemical and mechanical stability, especially at a higher temperature (Joscelyne and Trägårdh, 2000; Lau et al., 2012). The selectivity of synthetic membranes is governed by a porous framework based on the uniform structure or their size depending on the solubility and solute diffusivity. For distinct permeating components, the driving force is definitely as vital as the permeability of the membrane, that is only one of the determinants of the flux from the membrane. An electric potential driving force functions only in the existence of a charged particle as compared to concentration, pressure, or temperature gradients are a few driving forces that act evenly on all the constituents (Koros and Fleming, 1993; Pinelo et al., 2009). Various membrane processes modeled after consolidating different membranes and driving forces are RO, membrane reactors, electrodialysis, pervaporation, ultra-, micro-, and NF, gas separation, Donnan dialysis, membrane distillation (MD), membrane-based solvent extraction, membrane contactors, etc. Whereas, the practicable applications of the membrane structures and processes are more divergent indeed (Matsuura, 2001; Gugliuzza et al., 2017). Around the 1970s, there is a beginning of the large-scale industrial usage of membranes, to grow high-quality and potable industrial water by desalination and purification subsequently membranes became a largely used means in process engineering with a tremendous technical and commercial effect. Currently, three major fields are using membranes. Firstly, they are used in therapeutic systems and artificial organs where there is still no other valid alternative for membrane operations. Secondly, the drug and food industry encompasses applications for the

formation of ultra-pure water and the segregation of the molecular mixture. Even though the technical and commercial advantages are offered by the membranes, substitutes also exist (Lee et al., 2011; Gugliuzza and Drioli, 2013). In the end, applications for seawater desalination and wastewater purification uses membranes because they are technically beneficial. Still, there are alternate techniques such as distillation and biological treatment that compete with it on the basis of the whole economy. Due to more advancement, the separation process is carried out by newel membranes more effectively resulting in the common unit means in process engineering procedures using methods like membrane contactors and membrane reactors (Ho et al., 1992; Drioli et al., 1999; Marcano et al., 2002; Klaassen et al., 2005). The large-scale function of membranes is rapidly reaching outside the limits of its present level.

8.4. HISTORICAL IMPROVEMENTS OF MEMBRANES AND MEMBRANE PROCEDURES

Four decades ago, the technical use of membrane procedures is started, and synthetic membranes are one of the recent progress. In the mid of the 18th century, there was a discovery of the osmosis process, and Nollet also made the first-ever documented study of the membrane phenomenon. He noticed that when the pure water is brought in contact with a water-ethanol mixture, then a pig's bladder preferentially meets with ethanol (Nollet, 1752). By chance, Nollet was the first one who ever recognizes the relationship between the semi-permeable membrane and osmotic pressure. The diffusion of gasses was analyzed by Graham using numerous media and found out that unique permeabilities are exhibited by rubber to various gasses. He performed more studies on transportation of mass in semi-permeable membranes (Graham, 1866; Van de Witte et al., 1996).

Traube was the one who initially introduces the artificially made semi-permeable membrane by the precipitation of cupric ferrocyanide on a smooth layer of porous porcelain. Natural materials, e.g., animal bladder and gum elastics, were used in about all the works based on membrane permeation (Traube, 1867). Pfeffer employed artificial membranes in his fundamental studies on osmosis (Pfeffer, 1877). Most of the theoretical treatment and explanation corresponding to mass transport from the membrane and osmotic phenomenon has a source from the studies of Fick. The diffusion in liquids was described by him as a result of concentration gradients, whereas the thermodynamic interpretation was presented by van't

Hoff for the osmotic pressure of dilute solutions (Fick, 1855; van't Hoff, 1887). Later, Planck and Nernst suggested the flux equation for electrolytes under the effect of an electrical potential gradient or a driving force of a concentration (Nernst, 1888; Planck, 1890). With Donnan elaborating the theory of membrane potentials and membrane equilibria in the existence of electrolytes in his typical publications, the initial past of membrane science stops at most of the basic phenomena theoretically proposed and adequately described (Donnan, 1911; Parhi, 2013).

The gateway to membrane technology and science in a new phase was marked at the beginning of the 20th century. A method for forming the initial synthetic membranes was formulated by Bechhold such that by drenching a filter paper in a mixture of nitrocellulose in glacial acetic acid (Bechhold, 1908). The membranes having varied permeabilities can be recreated and prepared by changing the fraction of acetic acid to nitrocellulose. The nitrocellulose membranes were also employed by Zsigmondy in his works such as ultrafilters to separate thin particles and macromolecules from an aqueous solution (Zsigmondy et al., 1918). Many other researchers continued their conducted researches (Elford, 1931; Mc Bain et al., 1931). The corporation among electro-osmosis, streaming potential, and electrodialysis was dealt with in a monograph (Prausnitz et al., 1931). In 1937, in accordance with a patent (Zsigmondy, 1922), Sartorius GmbH initiated the advancement of a chain of nitrocellulose membranes with unique pore sizes. These membranes are used by microbiological laboratories in analytical applications. The formation of the first successfully operating hemodialyzer is the ground for the broad-scale application of membranes in the area of biomedical sciences (Kolff et al., 1944; Sazali et al., 2019).

With a bit of practical application, most of the time, membranes had been a topic of scientific interest in the initial days of membrane technology and science. However, in the 1950s, it changed, turning to the rapid improvement in the membrane-based industry when the practical usage of membranes in technically suitable applications became the focus of interest. The advancement of the formation for new membranes with brilliant thermal and specific transport property and mechanical stability commences after the creation of a huge amount of synthetic polymers because of the developments in polymer chemistry. The membrane transport qualities are explained by the healthy theory deduced from the thermodynamic of irreversible processes (Staverman, 1952; Kedem et al., 1961; Schlögl, 1964). Another way for explaining membrane processes is provided by hypothesizing a few membrane transport models, e.g., the model of a

solution-diffusion membrane (Merten, 1966). The features of IE membranes with their practical usage were also uncovered in the comprehensive study (Spiegler, 1958). In membrane technology and science, the development of cellulose acetate-based RO membrane demonstrated to be a breakthrough because it gives high fluxes and increased salt rejection at moderate hydrostatic pressure (Reid et al., 1959; Loeb et al., 1964). This winded up being one of the leading steps towards the management of RO membranes being an effective tool for the growth of potable water from the sea (Belfort, 1988; Mohshim et al., 2013). Sourirajan and Loeb fabricate a membrane that consists of an asymmetric composition with a dense outer skin, it regulated the membrane flux and selectivity whereas its tremendously porous framework gives it mechanical strength (Aptel et al., 1968; Liu et al., 1977). It was exposed that the structure of asymmetric cellulose acetate membranes was formed on a phase inversion process which incorporates a uniform polymer solution to be changed into a two-phase system such as a polymer lean phase forming the liquid-filled membrane pores and a solid polymer-rich phase contributing to the solid polymer structure (Kesting, 1971; Strathmann et al., 1975, 2011). Not so long after that for the development of synthetic membranes, polymers, e.g., polyamides, polyethylene, polyacrylonitrile, polysulfone, were used as elementary materials. In contrast with cellulose esters, mechanical strength and greater chemical and thermal stability were exhibited by these polymers. Besides that, cellulose acetate was used as the primary material for the formation of RO membranes before the fabrication of interfacial-polymerized composite membranes (Cadotte et al., 1981; Riley et al., 1967). As compared to the membranes developed from cellulose acetate, withal, these membranes represented elevated rejection, largely higher chemical and mechanical stability, and fluxes. The first membranes prepared for RO desalination and alternate applications were created as flat sheets later they were implemented in a formal spiral wound module (Bray, 1968; Westmoreland, 1968). A unique approach was provided to the membrane geometry by an independent hollow fiber membrane having a wall thickness ranging from just 6 to 7 microns (Mahon, 1966). The asymmetric hollow fiber membranes were designed by the Du Pont Corporation with its prevailing application in seawater and brackish desalination (Schultz and Keating, 1971; Laine et al., 2003).

After the development of effective membranes, modules, appropriate membrane housing assemblies, were proposed. The mandates for preparing such modules involve considerable membrane packing density, low cost, module replacement or readiness for membrane, governance of concentration

polarization, and reliability. Three different designs were hollow fiber or capillaries, tubes, and flat sheets, in which membranes were constructed. Nowadays, spiral wound modules are used, whereas hollow fiber membrane modules were used in pervaporation and gas separation. Today, in medical applications, blood, and kidney oxygenator capillary membranes play a significant part. Mostly, ultra-, and micro-filtration utilize tubular membranes (Wijmans and Baker, 1995; Ghernaout et al., 2018).

There was a practice of electrodialysis on an industrial scale even before when the RO was utilized for brackish water or sea desalination on a big scale. The logs of electrodialysis register in the earlier times to the preparation of the first multi-cell stack (Meyer et al., 1940). The initial reliable IE membranes maintaining both admirable electrolyte conductivity and exceptional ion-permselectivity result in contemporary electrodialysis into practical use (Juda et al., 1953). For the desalination of brackish water, Ionics Inc did the initial commercial usage of electrodialysis. The reason at the back of the success of Ionics was their jam-packed stacking, their membranes, and their procedure of operation called electrodialysis reversal (Gekas and Hallström, 1987; Zhang et al., 2012). This approach ensured the membrane stack with a recurrent self-cleaning technique by that allowing long-term constant function at increased concentrations of scaling materials without the requirement for the mechanical cleaning of the stack (Nagasubramanian et al., 1977; Katz, 1979).

At the beginning of the 1960s, bio-polar membranes came into being, which was a thoroughly new field for practicing electrodialysis, To obtain bases and acids from the matching salt. The large-scale separation of vapors and gasses is another field in the industry for the exploitation of membranes. Monsanto Inc. founded the gas separation (Henis et al., 1980).

8.5. ADVANTAGES AND LIMITATIONS OF MEMBRANE PROCEDURES

There is a direct competition between the membrane procedures and the more conventional water treatment process in most of the applications, e.g., water desalination and purification. Membrane processes have proven to be obvious to operate, and are more energy-efficient, leading to higher product quality, rather than that of traditional techniques. The same has been noticed in the processes of concentration, purification, and separation of medical or pharmaceutical applications, or drug and food products. Moreover, these

processes have the benefit to operate at a circulating temperature without any degradation or change of products (Bechhold, 1908; Lokhandwala et al., 2010).

RO or electrodialysis can be used for the desalination of water is an efficient procedure relying upon conditions, e.g., the needed capacity of the desalination plant, water quality, and energy cost. As compared to desalination, distillation is normally considered to be an inexpensive method, and for units with large capacity, and in power plant case might be linked with the desalination unit. Membrane processes, micro-, and ultra-filtration are battling with carbon adsorption, biological treatment, IE, flocculation, and bed filtration for wastewater treatment, and surface water purification (Bray, 1968; Bhattacharyya et al., 2003). Membrane procedures might be more costly in these applications, however, they maintained better water quality. The alliance of customary water treatments and membrane processes might bring a high product water quality combining with an economical and reliable treatment. Still, the membrane processes long-term consistency has not been confirmed, which is a disadvantage of membrane processes in many applications, especially in the chemical and petrochemical industry. (Kurihara and Sasaki, 2017). Moreover, sometimes intense pretreatment is required by the membrane processes based on membrane fouling due to chemical interaction with water constituents and their sensitivity towards polarization, concentration. As the membranes are not very robust mechanically, hence a breakdown in the operating process may decay the membrane. Luckily, great developments have been made recently, particularly in RO seawater desalination, in advancing membranes, they have all-around enhanced performance and also show minimized sensitive operational errors, and enhanced chemical and thermal stability (Hoek et al., 2002; Mohammad et al., 2012).

8.6. ECONOMIC CONSIDERATIONS AND ENVIRONMENTAL AFFECTS

Membrane processes are considered to be much energy-efficient as compared to numerous other separation techniques. However, the energy needs of a process are just one element in the eventual cost with the investment-related expenses mostly participating largely in the complete process and maintenance. The pre-and post-treatment are the other determinants that should be kept in mind such as required product quality, particularly the content of the feed mixture that has to be treated (Donnan, 1911; Cadotte

and Petersen, 1981). For example, process expenditure is firmly based on the structure of the water feed which may require various membrane processes in water treatment. For the purification of surface water and some wastewater, micro-and ultra-filtration can be used. In these processes, there are fewer energy demands but micro- and ultra-filtration are competing with biological treatments which need even low power or sand bed filtration. RO, the alone economic seawater desalination process is battling with several distillation methods. RO has been verified to be a strongly efficient process in terms of energy expenditure. However, the essential point to contemplate is that in RO, the pressure-generating pumps employ combustion or electric engines. In contrast with the basic energy attained from fossil fuels, these engines retain efficiency of below 40%, whereas such energy may be operated directly for heating logic in the distillation processes. For the transportation of ions from the feed to the concentrated solution, electrodialysis is practiced (Drioli and Romano, 2001; Drioli and Giorno, 2020). As the current desired for the desalination process is directly proportional to the number of ions that require to be removed from the feed solution, the energy expenditure increases with a hike in feed solution concentration. Besides that, several alternate aspects find out the entire economics of a process, e.g., various operating expenses and investment, or the feed solution and pre-and post-treatment processes of the product water. The plant's capacity in the whole cost plays an important role too. Whereas, in the case of the distillation process, an immense decrease in expenses can be found with a raise in the capacity of the plant. In RO, there is a little effect of the scale-up factor to some extent (Fick, 1855; Elford, 1931). Normally, in seawater desalination, RO has a tremendous cost-benefit on the other competing processes. Distillation processes have a definite technical and economic limitation over electrodialysis and RO in the desalination of brackish water. The same can be spoken for the desalting and purification of surface water for domestic or industrial use. However, here RO and UF produce higher quality product water. The membrane holds back not only salt but also other dissolved and dispersed water elements in these processes as long as the permeate, i.e., the product is chiefly free of all contaminant. The product water of electrodialysis may constitute bacteria, viruses, particles, and different other pollutants because only ionic components are obtained from the feed stream (Mahon, 1966; Marcano and Tsotsis, 2002). However, the study of the above-mentioned water has been stated in a generalized tone and quite simply. A combination of procedures may be appropriate based on the feed water composition and the required product water quality. For example,

when ultra-pure water is required for some industrial applications, a series of procedures may be performed, such as RO with IE methods to remove all ions and micro-filtration as a "point-of-use-filter" to remove indications of particles. Microfiltration is generally used along with RO as a pre-treatment procedure (Henis and Tripodi, 1980; Graham, 1995).

There is relatively less environmental influence on all the membrane processes. There is neither production of heart nor is any utilization of dangerous chemicals that desire to be discharged. RO produces the only emission in the desalination that is a concentrated brine solution. In seawater desalination, the brine results in minute problems as this solution can be released directly into the sea, but it can be the reason for problems in the situation of brackish water desalination; hence, brine post-treatment techniques might be important. Moreover, additional processing of the concentrated discharge may be needed in surface water treatment (Ho and Sirkar, 2012).

Generally, there is a better quality of the product obtained from pressure-driven membrane processes and are not connected to any health risks. Therefore, post-treatment processes are required somewhat. Sometimes, chlorination may be used to guarantees the required sterility of potable water, especially when long-term storage is expected in a hot temperature (Merten, 1966; McBain and Kistler, 2002).

8.7. THE MEMBRANE-BASED INDUSTRY

A membrane-based industry brought into existence together with the preparation of membrane procedures and products. With a growth rate of over 10% annually, this industry has a business of several billion US$ per year and is increasing. The framework of the industry is really heterogeneous in terms of their principal perspective towards the market and the size of the companies. Many companies have attention to the formation of membranes only. A range of membrane products are offered, e.g., hollow fibers, flat sheets, or capillaries with unique properties and for several applications approaching from fuel and waste cell separators and seawater desalination to medical devices and surface water treatment to an equipment producer or an end-user. Other companies make membrane devices or complete systems. These companies bought the modules or membranes as basic component from one or different membrane, membranes to show and build the actual plant and generally also use it, confirming the buyers a specific amount of the product of a given quality (Meyer and Strauss, 1940; Verbrugge and

Hill, 1990). Usually, these companies grant clients a solution to separation desires which may be a combination of separation techniques, e.g., different biological and chemical treatment processes, precipitation, IE, flocculation, and carbon adsorption along with membrane procedures. Based on their specific application understanding in different markets, they are substantial in the membrane industry despite the sale of membrane modules and membranes to any of these firms normally is not really high (Nollet, 1752; Osada and Nakagawa, 1992).

Finally, some companies supply the system layout, the plant activity, and the membranes. Large-scale applications, e.g., the formation or hemodialysis of potable water from seawater or brackish is the focal point for them. Moreover, they distribute the water and manage the plant, and additionally, supplying tools for the formation of potable water for seawater desalination. Notwithstanding, companies making artificial kidneys also administer dialysis stations. The industry is facing noticeable variation marked by acquisitions and mergers due to the call for water supply systems and membranes are increasing very speedily while changing constantly (Pfeffer, 1877; Reid and Breton, 1959).

Concerning the membrane market, there are various small market segments in the pharmaceutical, chemical, and food industry, analytical laboratories, and specifically in the treatment and recycling of industrial effluents and also some other large market segments, e.g., hemodialysis, seawater desalination, and the production of brackish or ultra-pure water. Many small companies are active in market places such as treating particular wastewater streams or providing their services to the drug and food or chemical industry. The larger markets for water desalination and hemodialysis are supervised by a somewhat small number of large organizations (Ettisch, 1932; Cohen and Cooley, 1965).

8.7.1. Membrane and Membrane Module Manufacturers

Segments of notable chemical companies are usually producers of membranes. Usually, a series of membrane products to be used in few applications are indicated by these bases such as water desalination and purification, hemodialysis, gas separation, or bio-production. The companies that prepare the membranes, most of them also made suitable modules. Normally employed in water desalination and purification, flat sheet membranes are generally organized in spiral wound membranes, whereas hollow fiber membranes modules are used by gas separation. Allocating a

relatively extensive membrane area per unit volume, a large amount of pre-treatment might be required by both of these applications. The capillary type membrane modules predominate the market corresponding to hemodialysis that is already occupied in the preparation of ultra-filtration and ultra-pure water. Usually used in refining some wastewaters and in chemical and food processing industries, tubular modules or plate-and-frame are conducted (Spiegler, 1958; Riley et al., 1967).

However, there may be varied constitutional material together with the manufacturing process utilized by the firms for their membranes. Asymmetric membrane structures are constructed by some companies for micro-and ultra-filtration and RO from polyamides, cellulose esters, and polysulfone. The composite membranes having a porous polysulfone may be constructed by other companies that support structure together with a polyamide type barrier layer formed by interfacial polymerization (Woermann and Spei, 1964).

8.7.2. System Manufacturers

Various extensive and heterogeneous organizations propose and prepare membrane water treatment systems. Nearly all of these organizations specialize in particular applications, for example, the recycling and processing of wastewater from the food, metal, chemical, or pharmaceutical treatment industry or the improvement of potable and industrial wastewater. These types of firms are quite small,

whereas the rest are smaller parts of a larger corporation, where membrane processes only assist slightly to the whole enterprise activity (Traube, 1867). This activity may be infatuated, e.g., general water treatment, and consist of a complete chain of different processes, i.e., IE, different chemical or biological treatment processes, flocculation, and carbon adsorption or precipitation. Membrane comes up to be like a commercially existing product for these organizations.

Even though the business of membrane modules and membranes to any of these firms is often not extremely high, they are influential in the membrane industry because of their specific application understanding in various markets. Leading utility organizations that deliver modules, membranes, and systems as a complete package are exempted from this rule. These enterprises have secured their membrane supply by the collection of medium or small size membrane producers (Staverman, 1952; Strathmann, 2004).

8.8. THE FUTURE OF MEMBRANE SCIENCE AND TECHNOLOGY

Despite the traditional membrane procedures and applications, the recent membrane procedures such as membrane contractors and membrane reactors are improving at an industrial level. In process engineering, they are becoming accepted unit operations, participating in the entire influence of membrane engineering on either industrial manufacturing (Ho et al., 1992; Drioli et al., 1999). It has a particular significance that all membrane processes are organized with the requirements of the sustainable industrial development and process intensification technique. The fundamental concepts of chemical process rationalization, miniaturization, and process intensification are now aimed in all of the production methods. Immense operational flexibility, increased operational simplicity and energy efficiency, maintaining intrinsic characteristics of environmental compatibility, elevated transport selectivity, and membrane procedures are essential tools for contemporary molecular separations and chemical modifications overwhelming the current limitations of the traditional industrial processes. The membrane market includes a great number of small market segments in the analytical laboratories, chemical, pharmaceutical, or food industry, and chiefly in the recycling and processing of industrial wastewater. Some other large market segments are also included, e.g., hemodialysis, and seawater or brackish desalination, or the preparation of ultrapure water. It is rather hard to predict the future membrane market accurately (Westmoreland, 1968; Zeman and Zydney, 2017).

Nowadays, the demands of potable and industrial water of satisfactory quality are increasing enormously, while globally, the origins of freshwater with the demanded quality are decreasing gradually. The waste surface and polluted waters are being incorporated by the energy-efficient and economical techniques for the preparation of superior quality water from seawater and brackish sources. Presumably, the membrane industry will persist to advance in the predictable future in this field. The same can be envisioned for medical life support systems, in several applications in the gas separation and chemical process industry. However, the improvement also relies upon more developments orbiting around membranes with increased fluxes and improved selectivity together with improved thermal, chemical, and mechanical stability. When an increase in the valuable life of the membranes is provided by long-term experience in large plants, then the processes are more reliable and affordable (Zsigmondy and Bachmann, 1918; Aryal, 2013).

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Separation Technologies

Separation processes hold a crucial place in most of the contemporary chemical, physicochemical, and pharmaceutical processes. The conventional separation processes include distillation, adsorption, absorption, filtration, chromatography, etc. These separation technologies have been in practice for decades, and their pertinent methodologies are being upgraded regularly. On the contrary, novel separation technologies such as membrane processes, supercritical fluid extraction, and membrane bioreactors processes are getting a reputation in modern plants. This book is predominantly designed to highlight these innovative separation technologies. The imminent water crisis faced by humans by poor sanitation and water stress signifies one of the utmost challenges for this century. Therefore, it is essential to explore the alternatives for recycling water and preserving water. Various separation technologies have emerged as promising candidates to fight the water crisis. Although there are many books that discuss different separation technologies, but this book contains the latest and comprehensive overview of the contemporary separation techniques. Different chapters of the book provide a unique information that focuses on the rising importance of separation techniques. The book contains eight chapters which are written in a reader-friendly manner. Each chapter starts with a general introduction of the topic followed by specific details about the topic. Chapter 1 focuses on providing the fundamentals of various separation techniques. Separation configurations and principles are also discussed in the chapter. Distillation is one of the essential separation processes with a wide variety of applications. Chapter 2 offers a detailed discussion about various types of distillation processes. Chapter 3 discusses the comprehensive overview of chromatographic processes and their principles. A detailed overview of different types of chromatography is presented in the chapter. Solvent extraction processes have gained significant attention in the past few years due to their robust separation methodologies. Chapter 4 discusses the principles and applications of solvent extraction processes for separating different material components. Most of the separation processes were carried out using mechanical techniques in the past. Presently, those mechanical techniques have been upgraded to meet the current standards of separation technologies. Chapter 5 briefly discusses the mechanical separation processes, which include sedimentation, centrifugation, filtration, and sieving. The separation techniques based on adsorption phenomenon have gained immense attention during the last few decades due to their excellent performance. Chapter 6 focuses on providing a detailed overview of different principles of adsorption separation techniques. Chapter 7 presents a comprehensive information about electrophoretic separation processes. Different types of electrophoretic processes are discussed in the chapter. Today, the world is shifting towards efficient and green separation technologies for treating wastewater and chemicals. Membrane processes are suitable for providing the required results with reasonable economic costs. Chapter 8 introduces the readers with the fundamentals of membrane processes and their principles.

This book is intended for students (both graduate and undergraduate-level) of chemical engineering, membrane engineering, and environmental engineering. Moreover, the professionals associated with concerned with the pharmaceutical industry, chemical industry, and water industry can also benefit from this book.

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