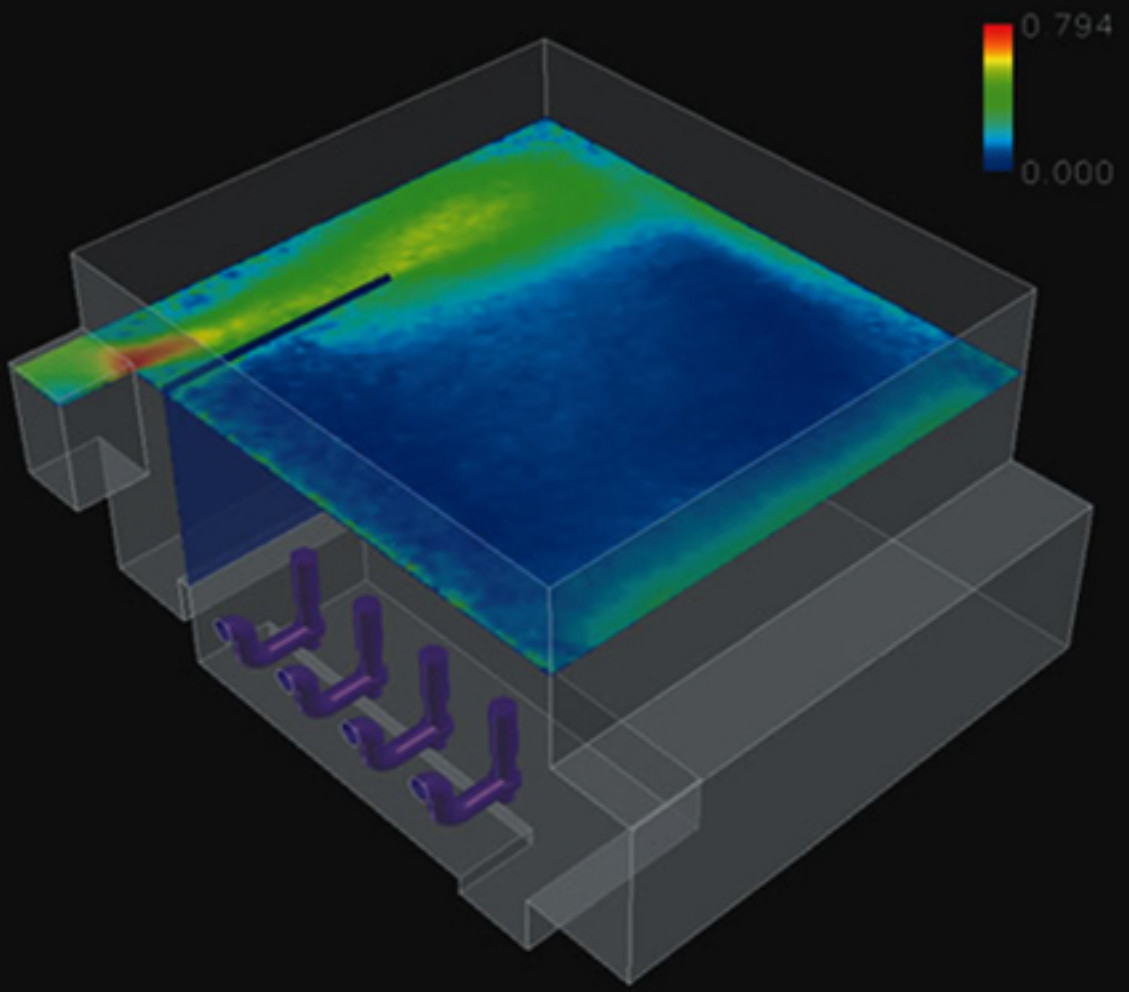
Basic Technology and Tools in Chemical Engineering Field



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Chapter- 1

Centrifugation

Centrifugation is a process that involves the use of the centrifugal force for the separation of mixtures with a centrifuge, used in industry and in laboratory settings. More-dense components of the mixture migrate away from the axis of the centrifuge, while less-dense components of the mixture migrate towards the axis. Chemists and biologists may increase the effective gravitational force on a test tube so as to more rapidly and completely cause the precipitate ("pellet") to gather on the bottom of the tube. The remaining solution is properly called the "supernate" or "supernatant liquid". The supernatant liquid is then either quickly decanted from the tube without disturbing the precipitate, or withdrawn with a Pasteur pipette.

The rate of centrifugation is specified by the acceleration applied to the sample, typically measured in revolutions per minute (RPM) or *g*. The particles' settling velocity in centrifugation is a function of their size and shape, centrifugal acceleration, the volume fraction of solids present, the density difference between the particle and the liquid, and the viscosity.

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

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

Centrifugation in biotechnology

Microcentrifuges and superspeed centrifuges

In microcentrifugation, centrifuges are run in batch to isolate small volumes of biological molecules or cells (prokaryotic and eukaryotic). Nuclei is also often purified via microcentrifugation. Microcentrifuge tubes generally hold 1.5-2 mL of liquid, and are spun at maximum angular speeds of 12000-13000 rpm. Microcentrifuges are small and have rotors that can quickly change speeds. Superspeed centrifuges work similarly to microcentrifuges, but are conducted via larger scale processes. Superspeed centrifuges

are also used for purifying cells and nuclei, but in larger quantities. These centrifuges are used to purify 25-30 mL of solution within a tube. Additionally, larger centrifuges also reach higher angular velocities (around 30000 rpm) and also use a larger rotor.

Ultracentrifugation

Ultracentrifugation makes use of high centrifugal force for studying properties of biological particles. While microcentrifugation and superspeed centrifugation are used strictly to purify cells and nuclei, ultracentrifugation can isolate much smaller particles, including ribosomes, proteins, and viruses. Ultracentrifuges can also be used in the study of membrane fractionation. This occurs because ultracentrifuges can reach maximum angular velocities in excess of 70000 rpm. Additionally, while microcentrifuges and supercentrifuges separate particles in batch, ultracentrifuges can separate molecules in batch and continuous flow systems.

In addition to purification, analytical ultracentrifugation (AUC) can be used for determination of macromolecular properties, including the amino acid composition of a protein, the protein's current conformation, or properties of that conformation. In analytical ultracentrifuges, concentration of solute is measured using optical calibrations. For low concentrations, the Beer-Lambert law can be used to measure the concentration. Analytical ultracentrifuges can be used to simulate physiological conditions (correct pH and temperature).

In analytical ultracentrifuges, molecular properties can be modeled through sedimentation velocity analysis or sedimentation equilibrium analysis. In sedimentation velocity analysis, concentrations and solute properties are modeled continuously over time. Sedimentation velocity analysis can be used to determine the macromolecule's shape, mass, composition, and conformational properties. During sedimentation equilibrium analysis, centrifugation has stopped and particle movement is based on diffusion. This allows for modeling of the mass of the particle as well as the chemical equilibrium properties of interacting solutes.

Centrifugation analysis

Lamm equation

Particle dispersion and sedimentation can be analyzed using the Lamm equation. The calculation of the sedimentation coefficient and diffusion coefficient is useful for determining the physical properties of the molecule, including shape and conformational changes. However, the Lamm Equation is most ideal for modeling concentrations of ideal, non-interacting solutes. Chemical reactions are unaccounted for by this equation. Additionally, for large molecular weight particles, sedimentation is not always smooth. This may lead to the overestimation of the diffusion coefficient, or oscillation effects at the bottom of a solution cell.

Sigma analysis

Sigma analysis is a useful tool determining centrifuge properties. It is similar to the continuity equation that relates volumetric flow rate Q, fluid velocity u, and flow path cross-sectional Area A:

$Q = uA$

In the case of sigma analysis, u is replaced by v_g,the settling velocity at centripetal acceleration of g (9.81 m/s^2), Σ replaces area, and is a property of the type of centrifuge, and Q is the input fluid flow rate. Σ has the same units as area.

$Q = 2v_g\Sigma$

Other applications

- Separating textile.
- Removing water from lettuce after washing it in a salad spinner
- Separating particles from an air-flow using cyclonic separation.
- The clarification and stabilization of wine

Clarification and stabilization of wine

The clarification process can bring out the clarity and brightness of a wine.

The **clarification and stabilization of wine** in winemaking involves removing insoluble and suspended materials that may cause a wine to become cloudy, gassy, form unwanted sediment deposit or tartaric crystals, deteriorate quicker or develop assorted wine faults due to physical, chemical or microbiological instability. These processes may include fining, filtration, centrifugation, flotation, refrigeration, barrel maturation, pasteurization and racking. Most of these processes will occur after the primary fermentation and before the wine is bottled. The exception is for white wine production which will usually have the must separated from some of the grape skins and particles prior to fermentation so as

to avoid any unwanted maceration. The timing and exact methods used will vary by producer, depending on the desired finish product -- such as a completely bright and clear wine or a wine that still retains some of the flavor and color phenols. Some of the materials that are removed from the must during this stage of winemaking include dead yeast cells (lees), bacteria, tartrates, proteins, pectins, various tannins and other phenolic compounds, and pieces of grape skins, pulp, stems and gums.

Clarifying wine

A method of natural clarification takes place as wine ages in the barrel with suspended particles gradually precipitating and collecting at the bottom.

In wine tasting, a wine is considered "clear" when there are no visible particles suspended in the liquid and (mostly in regards to white wines) there is some degree of transparency. This is demonstrated by holding the glass up to a piece of paper with writing and seeing if one could read through the wine. Some red wine grape varieties have a naturally high concentration of coloring phenols that make the wine more translucent or opaque. For these wines, the brightness or "vividness" of the wines color is considered. A wine with a lot of suspended particles will appear less clear and more dull in brightness. While lack of clarity and brightness may not negatively affect the taste of wine, it may make the wine less visually appealing to the consumer. In the modern wine industry there has been a premium placed on wines being both clear and bright. To achieve this, wines are usually clarified through some means in order to remove suspended particles.

These suspended particles are normally insoluble solids such as lees, fragments of grape skins, pulp and seeds as well as colloids that are not visible to the unaided eye like gums, pectins, proteins, tartrates, active yeast and bacteria. The forces of gravity can achieve natural clarification through the process of settling (French *débourbage*) where the larger suspended particles gradually settle to the bottom of the storage vessel. The wine is then siphoned or "racked" off the compact solids into a new container. This natural process can be very lengthy, sometimes requiring many months or even a couple years as well as

several rackings in order to produce a perfectly clear wine. Producers can accelerate the process by using fining agents, filtration or flotation.

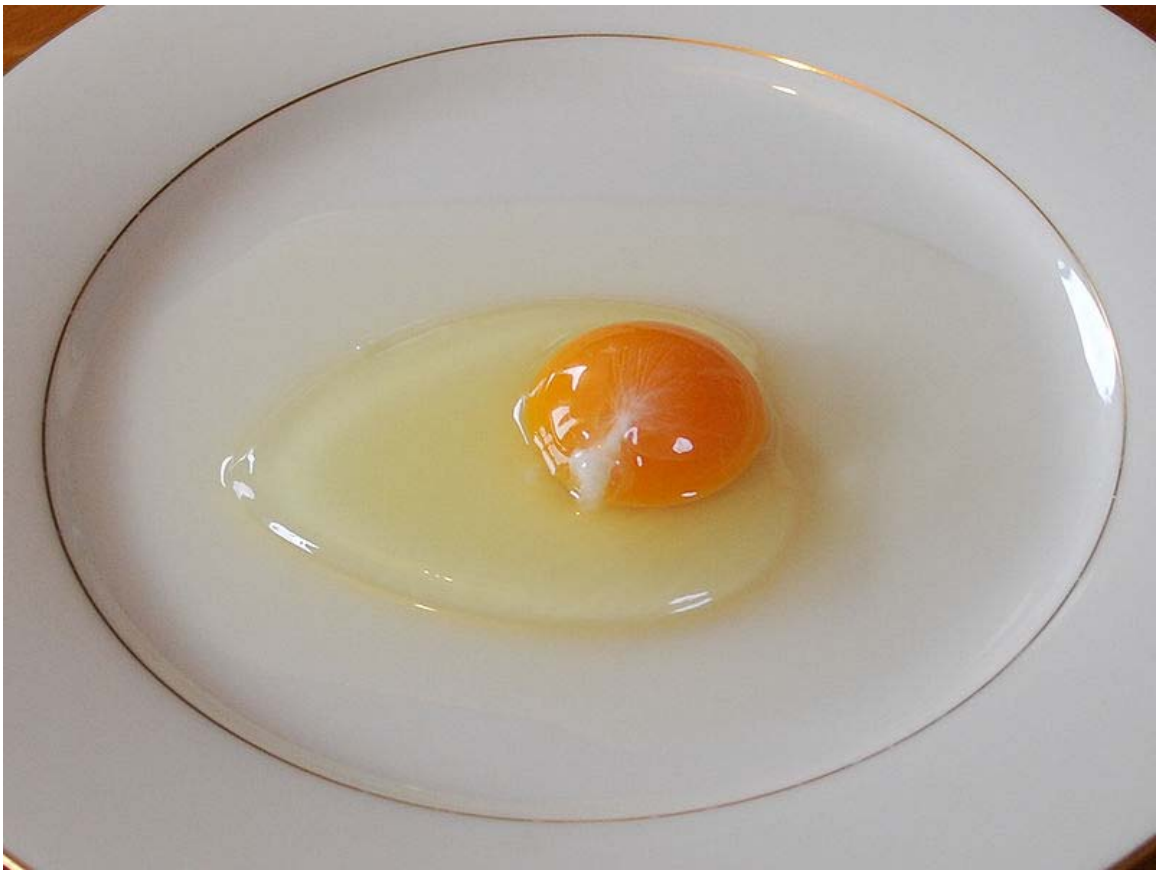
The timing of these methods vary depending on producer and type of wine being made. White wine, particularly aromatic varieties such as Riesling and Sauvignon blanc, is often settled and racked soon after the grapes are crushed and pressed. This is to minimize maceration and exposure to the phenolic compounds present in the grapes' skin, seeds and stems that can leach out into the must and cause the wine to prematurely brown in color as well as diminish the fruit flavors of the wine. Other varieties, such as Chardonnay, may spend some time in contact with the skins and particulate matter in order to gain complexity before being settled and racked. To aid in this clarification of white wine, pectin-splitting enzymes, sulfur dioxide and fining agents such as bentonite are added to the must to encourage the agglomeration and settling of the colloids while the holding tank is brought to low temperatures and held for 24 hours prior to rack. While most red wines are clarified after fermentation, the pectin-splitting enzymes may be added prior to fermentation to make post-fermentation clarification easier.

Fining

Bentonite

In winemaking, fining is the process where a substance (fining agent) is added to the wine to create an adsorbent, enzymatic or ionic bond with the suspended particles, making them a larger molecule that can precipitate out of the wine easier and quicker. Unlike filtration, which can only remove particulates (such as dead yeast cells and grape fragments), fining is effective in removing soluble substances such as polymerized tannins, coloring phenols and proteins. Given enough time in a stable environment, many of these suspended particles would gradually precipitate out on their own. The use of fining agents speeds up the process at lower cost. White wines are fined to remove particles that may cause the wine to brown or lose color as well as removing heat-unstable proteins that could cause the wine to appear hazy or cloudy should it be exposed to high temperatures after bottling. Red wines are fined for the same reason but also for the added benefit of reducing the amount of bitter, astringent tannins which makes these

wines smoother and more approachable sooner after bottling and release. Throughout history a wide range of substances have been used as fining agents, such as dried blood powder, but today there are two general types of fining agents — organic compounds and solid/mineral materials.



Winemakers can use the whites of eggs (discarding the yolk) as a fining agent

Organic compounds used as fining agents are generally animal based, which may bring concerns for a vegan diet. The most common organic compounds used include egg whites, casein derived from milk, gelatin and isinglass obtained from the bladders of fish. Pulverized minerals and solid materials can also be used as fining agents with bentonite clay being one of the most common fining agent used due to its effectiveness in absorbing proteins and some bacteria. Activated carbon derived from charcoal is used to remove some phenols that contribute to browning colors as well as some particles that produce "off-odors" in the wine. In a process known as **blue fining**, potassium ferrocyanide is used to remove copper and iron particles that may have entered the wine through the use of metal winery and vineyard equipment, vineyard sprays such as the bordeaux mixture, and the use of bentonite as a fining agent . Due to the potential of potassium ferrocyanide forming hydrogen cyanide, its use is highly regulated and is illegal in many wine producing countries. Other inorganic materials use include silica and kaolin.

Some countries, such as Australia and New Zealand, have wine labeling laws that require the use of fining agents that may be an allergenic substance to appear on the wine label, as there may be trace amounts of the substance still in the wine. However a study conducted by the University of California, Davis Department of Viticulture and Enology found that no detectable amount of inorganic fining agents such as bentonite are present in wine that has been fined and only negligible trace amount of proteinaceous agents such as egg whites can be detected.

As with filtration, there is the risk of some loss of flavor with fining due to desirable flavor molecules being precipitated out along with the more undesirable particles. Some producers of premium wine will do less fining or do it much later in the production process in order to leach as much flavor and aromatics from the phenols before they are removed. Still, fining is considered a less harsh process than filtration, with its advocates believing that it better mimics the natural clarification and stabilization process.

Filtration

Depth filters are often made from diatomaceous earth *(sample pictured)*

While fining clarifies wine by binding to suspended particles and precipitating out as larger particles, filtration works by passing the wine through a filter medium that captures particles that are larger than the hole size of the medium. Complete filtration may require a series of filtering through progressively finer filters which can be expensive but will be considerably quicker than letting gravity naturally settle the wine and using racking to siphon the clear wine out. Most filtration in a winery can be classified as either depth filtration or surface filtration.

Depth filtration is often the first type of filtration a wine sees after fermentation when the wine is pushed through a thick layer of pads made from cellulose fibers, diatomaceous earth or perlite which traps the particles and can be removed from the wine. If the producers wish to further filter the wine, they may go to surface filtration. Surface filtration involves running the wine along a thin film of polymer material filled with holes tinier than the particles that are being filtered out. Running the wine parallel to filter surface (known as "Cross-flow" surface filtration) will minimizing the amount of potential clogging of the filter. Most membranes are made from plastic or ceramic.

Another step in surface filtration, usually taking place right before bottling, is microfiltration where the fine is passed through a membrane with holes small enough to trap yeast and bacteria cells. An alternative to filtration is centrifugation where wine is put through a centrifuge decanter and gravity separates the particles from the wine.

The use of filtration is a controversial subject in winemaking with some producers feeling that the technique strips the wine of too much of its natural flavors and characteristics. Some producers will add the phrase "unfiltered" to their wine label as a marketing tool. Wine can go through a natural clarification and stabilization process by aging in a wood barrel where the subtle oxidative effects can aid in the precipitation of larger particles (particularly proteins, tartrates and malates). This process takes time, however, and producers who bottle their wine too early, and without the assurance of sterile bottling equipment, can risk microbial contamination and instability. Wines that have not been filtered are much more likely to develop sediment as the wine ages.

Flotation

The winemaking technique of flotation was adapted from the froth flotation process used in the mining industry for ore refining. In this process, small bubbles of air (or compressed nitrogen) are injected into the bottom of a tank. As the bubbles rise through the must, grape solids have a natural tendency to cling to the bubbles creating a froth that can be removed from the wine. This technique has to take place prior to fermentation since the biological activity of yeast cells serves as an inhibitor to the flocculation needed for the froth to form. Since phenolic compounds that are prone to oxidation and browning are highly reactive to the air bubbles, wines that have gone through the flotation process are often more resistant to oxidative browning if exposed to air later.

Stabilization

The process of cold stabilization causes tartrates to crystallize and precipitate out of the wine. These crystals, while harmless, can look like shards of broken glass if they develop in the wine bottle.

As a chemical substance dependent on the activity of microorganisms and complex chemical reaction, wine can be very unstable and reactive to changes in its environment. After bottling, a wine can be exposed to extremes in temperatures and humidity as well as violent movement during transportation and storage that can encourage the wine to go through additional chemical changes that may produce faults or undesirable traits to emerge in the wine. These can include carbonic gas, formation of sediment deposits or tartaric crystals, hazy or cloudy appearance, rapid deterioration of flavor and spoilage. Eliminating suspended particles in a wine can increase the stability of a wine and prevent some of these undesirable characteristic to emerge. The process of clarification does, in itself, increase the stability of the wine by removing some of these particles. Conversely, the process of stabilization can also increase the clarity and brightness of a wine.

Temperature instability

Tartaric acid is the most prominent acid in wine with the majority of the concentration present as potassium acid salt. During fermentation, these tartrates bind with the lees, pulp debris and precipitated tannins and pigments. While there is some variance among grape varieties and wine regions, generally about half of the deposits are soluble in the alcoholic mixture of wine. The crystallization of these tartrates can happen at unpredictable times if the wine is exposed to low temperature. These crystals can appear in a wine bottle looking like broken glass (though they are in fact harmless) and their presence may be undesirable to consumers. To prevent this from happening after the wine has been bottled, winemakers stabilize the wine by putting it through a **cold stabilization** process where it is exposed to temperatures below freezing to encourage the tartrates to crystallize and precipitate out of the wine. Some white grape varieties (such as Muscat) have significant quantities of proteins that are "heat-unstable" and will coagulate if the wine is exposed to excessive amount of heat fluctuations, making the wine appear hazy and cloudy. Winemakers will use fining agents, such as bentonite, to remove these proteins and increase the heat stability of the wine.

Microbiological instability

Dead yeast cells (lees-*pictured*), still present in a wine can make wine look hazy and cloudy. Active yeast cells can trigger a secondary fermentation.

Both active yeast cells and bacteria may be present in a wine after it has gone through the fermentation process and is bottled. If the wine still contains some residual sugar, the active yeast cells will initiate a secondary fermentation process inside the bottle that will create dissolved carbonic gas as a by-product. When the wine is opened, it will be gassy or "sparkling". While this may be a desirable trait for some wines (such as Champagne where a deliberate initiation of a secondary fermentation is part of production), it is not desirable for wines intended to be still or non-sparkling--such as Burgundy Pinot noir or a Washington Merlot. The easiest way to stabilize the wine is to ensure that there is no fermentable sugars left in the wine but in cases where some residual sugar in desirable (such as to balance the acidity of the wine) other methods can be taken to stabilize the wine. One method is sterile filtration and bottling which ensures that no active yeast are present in the wine. Another method is the addition of sulfur dioxide and sorbic acid which can inhibit the growth of yeast cells.

Modern advances in hygiene has eliminated many of the bacterial concerns that contribute to wine stability. Historically the presence of bacteria played a larger role in the developing of various wine faults. The primary concern in modern wineries is the presence of acetobacter which can turn wine into vinegar and lactic acid bacteria that can initiate malolactic fermentation which may not be desirable for certain wines. Acetobacter is active in the presence of oxygen so taking preventative measures, such as the use of sulfur dioxide, can suppress the growth of acetobacter. Eliminating the presence of fermentable sugars and malic acid can inhibit the growth of lactic acid bacteria and stabilize the wine.

Other methods of stabilization

The clarification methods of fining and filtration also act to stabilize wine by removing some of the same particles that can promote instability. The subtle oxidation that occurs with oak barrel aging has a naturally stabilizing effect on the wine.

Pasteurization

A wine can be stabilized by a method of heat sterilization, commonly known as pasteurization. The purpose of this technique is to bring the wine up to high enough temperatures that all micro-organisms in the wine (namely yeast and bacteria) are killed. For kosher wines, the production of *mevushal* wines (literally "cooked" or "boiled") heat sterilizes the wine to where non-Jews and non-observant Jews can handle the wine and still maintain its kosher status. In the process of pasteurization, wines are brought up to temperatures of 185°F (85°C) for a minute and then quickly cooled to a temperature of 122°F (50°C) where it is kept for up to 3 days. The wine may then be allowed to cool down to room temperature or be bottled "hot" and cooled by water sprays. This process can be rough on a wine and could diminish flavors and aging potential. A more gentle procedure known as flash pasteurization has been developed which heats the wine to 205°F (95°C) for a few seconds, followed by rapid cooling.

Premium wine production

Not all producers decide to thoroughly clarify and stabilize their wines, believing that some of a wine's flavor, aging potential and complexity come from some of the suspended particles. Wine experts, such as Tom Stevenson, notes that techniques like filtration can improve wine quality in moderation but can also diminish quality if used excessively. The consumers of some premium wines (such as Bordeaux and Port) may expect to see tartrates and sediment from a wine that has been aging and not thoroughly filtered.

Chapter- 2

Crystallization

Crystallization is the (natural or artificial) process of formation of solid crystals precipitating from a solution, melt or more rarely deposited directly from a gas. Crystallization is also a chemical solid-liquid separation technique, in which mass transfer of a solute from the liquid solution to a pure solid crystalline phase occurs.

Frost crystallization on a shrub.

Process

Time-lapse of growth of a citric acid crystal. The video covers an area of 2.0 by 1.5 mm and was captured over 7.2 min.

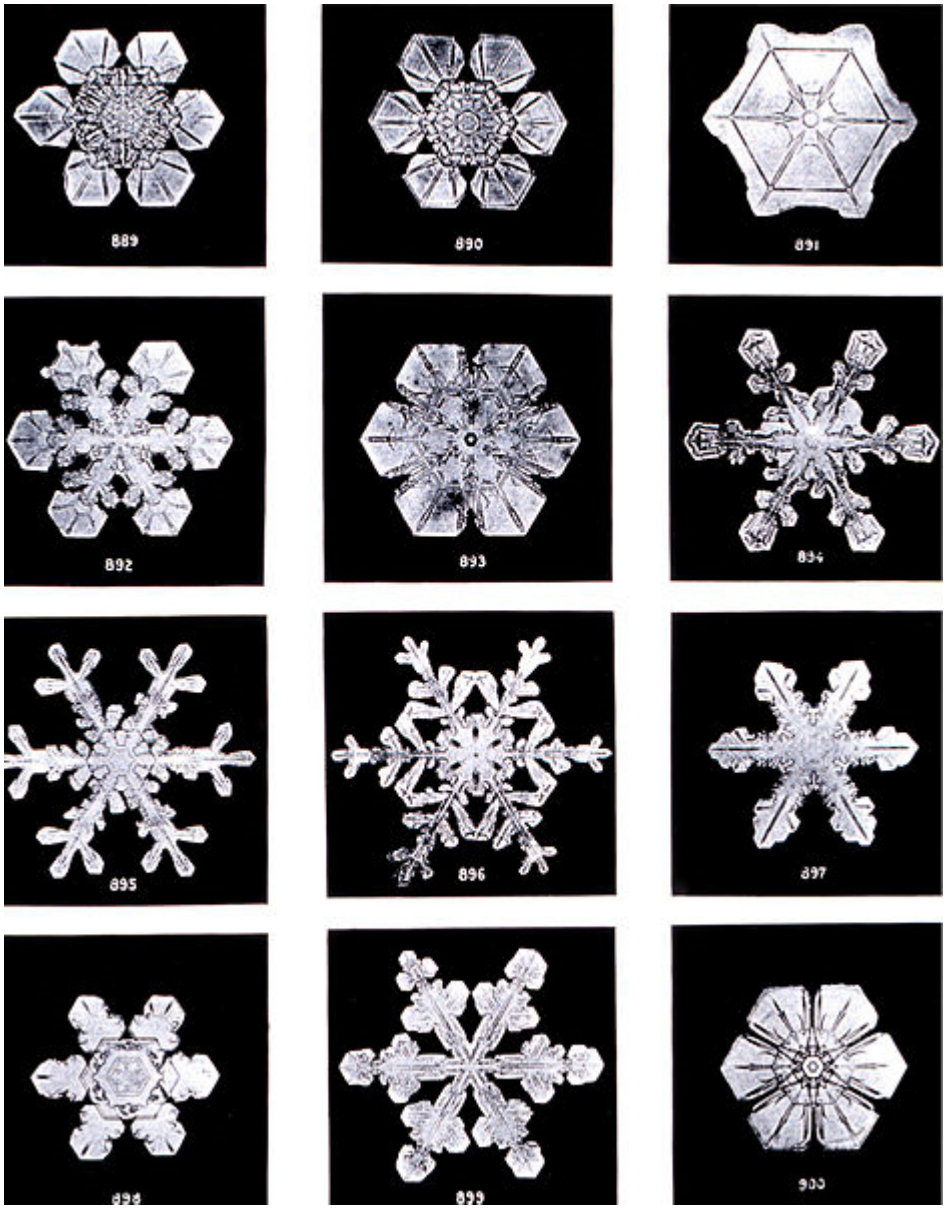
The crystallization process consists of two major events, *nucleation* and *crystal growth*. *Nucleation* is the step where the solute molecules dispersed in the solvent start to gather into clusters, on the nanometer scale (elevating solute concentration in a small region), that becomes stable under the current operating conditions. These stable clusters constitute the nuclei. However when the clusters are not stable, they redissolve. Therefore, the clusters need to reach a critical size in order to become stable nuclei. Such critical size is dictated by the operating conditions (temperature, supersaturation, etc.). It is at the stage of nucleation that the atoms arrange in a defined and periodic manner that defines the crystal structure — note that "crystal structure" is a special term that refers to the relative arrangement of the atoms, not the macroscopic properties of the crystal (size and shape), although those are a result of the internal crystal structure.

The *crystal growth* is the subsequent growth of the nuclei that succeed in achieving the critical cluster size. Nucleation and growth continue to occur simultaneously while the supersaturation exists. Supersaturation is the driving force of the crystallization, hence the rate of nucleation and growth is driven by the existing supersaturation in the solution. Depending upon the conditions, either nucleation or growth may be predominant over the other, and as a result, crystals with different sizes and shapes are obtained (control of crystal size and shape constitutes one of the main challenges in industrial manufacturing,

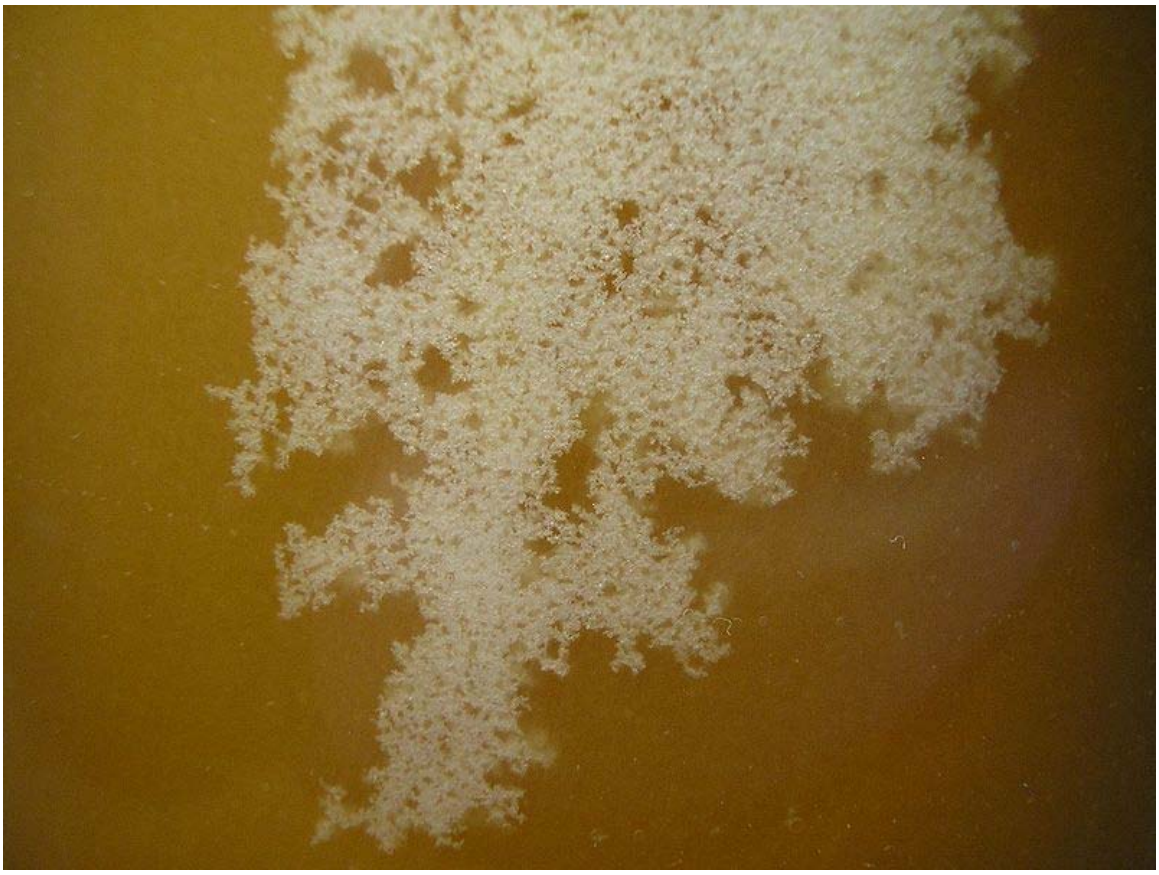
such as for pharmaceuticals). Once the supersaturation is exhausted, the solid-liquid system reaches equilibrium and the crystallization is complete, unless the operating conditions are modified from equilibrium so as to supersaturate the solution again.

Many compounds have the ability to crystallize with different crystal structures, a phenomenon called polymorphism. Each polymorph is in fact a different thermodynamic solid state and crystal polymorphs of the same compound exhibit different physical properties, such as dissolution rate, shape (angles between facets and facet growth rates), melting point, etc. For this reason, polymorphism is of major importance in industrial manufacture of crystalline products.

Crystallization in nature



Snowflakes are a very well known example, where subtle differences in *crystal growth* conditions result in different geometries.



Crystallized honey

There are many examples of natural process that involve crystallization.

Geological time scale process examples include:

- Natural (mineral) crystal formation;
- Stalactite/stalagmite, rings formation.

Usual time scale process examples include:

- Snow flakes formation;
- Honey crystallization (nearly all types of honey crystallize).

Artificial methods

For crystallization to occur from a solution it must be supersaturated. This means that the solution has to contain more solute entities (molecules or ions) dissolved than it would

contain under the equilibrium (saturated solution). This can be achieved by various methods, with (1) solution cooling, (2) addition of a second solvent to reduce the solubility of the solute (technique known as antisolvent or drown-out), (3) chemical reaction and (4) change in pH being the most common methods used in industrial practice. Other methods, such as solvent evaporation, can also be used. The spherical crystallization has some advantages (flowability and bioavailability) for the formulation of pharmaceutical drugs

Applications

There are two major groups of applications for the *artificial crystallization* process: *crystal production* and purification.

Crystal production

From a material industry perspective:

- *Macroscopic crystal* production: for supply the demand of natural-like crystals with methods that "accelerate time-scale" for massive production and/or perfection.
    - *Ionic crystal* production;
    - *Covalent crystal* production.
- *Tiny size crystal*s:
    - *Powder, sand and smaller sizes*: using methods for powder and controlled (nanotechnology fruits) forms.
        - *Mass-production*: on chemical industry, like salt-powder production.
        - *Sample production*: small production of tiny crystals for material characterization. Controlled recrystallization is an important method to supply unusual crystals, that are needed to reveal the molecular structure and nuclear forces inside a typical molecule of a crystal. Many techniques, like X-ray crystallography and NMR spectroscopy, are widely used in chemistry and biochemistry to determine the structures of an immense variety of molecules, including inorganic compounds and bio-macromolecules.
    - Thin film production.

Massive production examples:

- "Powder salt for food" industry;
- Silicon crystal wafer production.
- Production of sucrose from sugar beet, where the sucrose is crystallized out from an aqueous solution.

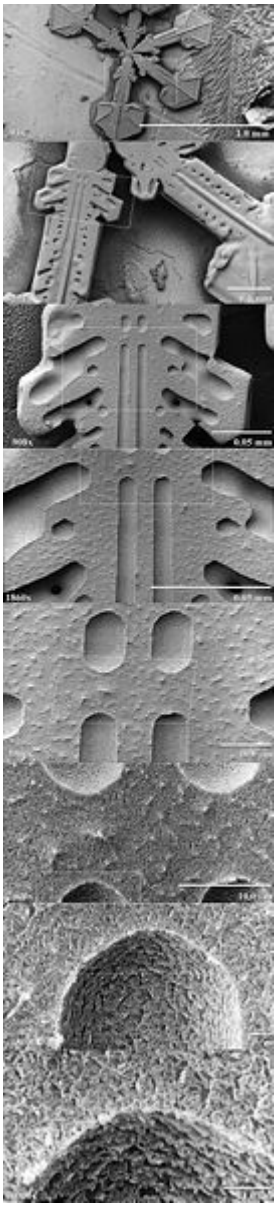
Purification

Used to improve (obtaining very pure substance) and/or verify their purity.

Crystallization separates a product from a liquid feedstream, often in extremely pure form, by cooling the feedstream or adding precipitants which lower the solubility of the desired product so that it forms crystals.

Well formed crystals are expected to be pure because each molecule or ion must fit perfectly into the lattice as it leaves the solution. Impurities would normally not fit as well in the lattice, and thus remain in solution preferentially. Hence, molecular recognition is the principle of purification in crystallization. However, there are instances when impurities incorporate into the lattice, hence, decreasing the level of purity of the final crystal product. Also, in some cases, the solvent may incorporate into the lattice forming a *solvate*. In addition, the solvent may be 'trapped' (in liquid state) within the crystal formed, and this phenomenon is known as *inclusion*.

Thermodynamic view



Low-temperature SEM magnification series for a snow crystal. The crystals are captured, stored, and sputter coated with platinum at cryo-temperatures for imaging.

The nature of a crystallization process is governed by both thermodynamic and kinetic factors, which can make it highly variable and difficult to control. Factors such as impurity level, mixing regime, vessel design, and cooling profile can have a major impact on the size, number, and shape of crystals produced.

Now put yourself in the place of a molecule within a pure and *perfect crystal*, being heated by an external source. At some sharply defined temperature, a bell rings, you must leave your neighbours, and the complicated architecture of the crystal collapses to that of a liquid. Textbook thermodynamics says that melting occurs because the entropy, S, gain

in your system by spatial randomization of the molecules has overcome the enthalpy, H, loss due to breaking the crystal packing forces:

$$T(S_{liquid} - S_{solid}) > H_{liquid} - H_{solid}$$

$$G_{liquid} < G_{solid}$$

This rule suffers no exceptions when the temperature is rising. By the same token, on cooling the melt, at the very same temperature the bell should ring again, and molecules should click back into the very same crystalline form. The entropy decrease due to the ordering of molecules within the system is overcompensated by the thermal randomization of the surroundings, due to the release of the heat of fusion; the entropy of the universe increases.

But liquids that behave in this way on cooling are the exception rather than the rule; in spite of the second principle of thermodynamics, crystallization usually occurs at lower temperatures (supercooling). This can only mean that a crystal is more easily destroyed than it is formed. Similarly, it is usually much easier to dissolve a perfect crystal in a solvent than to grow again a good crystal from the resulting solution. The nucleation and growth of a crystal are under kinetic, rather than thermodynamic, control.

Equipment for crystallization

1. *Tank crystallizers*. Tank crystallization is an old method still used in some specialized cases. Saturated solutions, in tank crystallization, are allowed to cool in open tanks. After a period of time the mother liquid is drained and the crystals removed. Nucleation and size of crystals are difficult to control. Typically, labor costs are very high.
2. *Scraped surface crystallizers*. One type of scraped surface crystallizer is the Swenson-Walker crystallizer, which consists of an open trough 0.6 m wide with a semicircular bottom having a cooling jacket outside. A slow-speed spiral agitator rotates and suspends the growing crystals on turning. The blades pass close to the wall and break off any deposits of crystals on the cooled wall. The product generally has a somewhat wide crystal-size distribution.
3. *Double-pipe scraped surface crystallizer*. Also called a *votator*, this type of crystallizer is used in crystallizing ice cream and plasticizing margarine. Cooling water passes in the annular space. An internal agitator is fitted with spring-loaded scrapers that wipe the wall and provide good heat-transfer coefficients.
4. *Circulating-liquid evaporator-crystallizer*. Also called *Oslo crystallizer*. Here supersaturation is reached by evaporation. The circulating liquid is drawn by the screw pump down inside the tube side of the condensing stream heater. The heated liquid then flows into the vapor space, where flash evaporation occurs, giving some supersaturation.The vapor leaving is condensed. The supersaturated liquid flows down the downflow tube and then up through the bed of fluidized and agitated crystals, which are growing in size. The leaving saturated liquid then goes back as a recycle stream to the heater, where it is joined by the entering

fluid. The larger crystals settle out and slurry of crystals and mother liquid is withdrawn as a product.

5. *Circulating-magma vacuum crystallizer*. The magma or suspension of crystals is circulated out of the main body through a circulating pipe by a screw pump. The magma flows though a heater, where its temperature is raised 2-6 K. The heated liquor then mixes with body slurry and boiling occurs at the liquid surface. This causes supersaturation in the swirling liquid near the surface, which deposits in the swirling suspended crystals until they leave again via the circulating pipe. The vapors leave through the top. A steam-jet ejector provides vacuum.
6. *Continuous oscillatory baffled crystallizer* (*COBC*). The COBC is a tubular baffled crystallizer that offers plug flow under laminar flow conditions (low flow rates) with superior heat transfer coefficient, allowing controlled cooling profiles, e.g. linear, parabolic, discontinued, step-wise or any type, to be achieved. This gives much better control over crystal size, morphology and consistent crystal products.

Chapter- 3

Crystallization (engineering aspects)

In chemical engineering **crystallization** occurs in a **crystallizer**. Crystallization is a unit operation through which a chemical compound, dissolved in a given solvent, precipitates under certain conditions to allow successive separation between the phases.

Crystallization is therefore an aspect of precipitation, obtained through a variation of the solubility conditions of the solute in the solvent, as compared to precipitation due to chemical reaction.

History

Crystallization is one of the pristine unit processes. It may be assumed that our ancestors used sodium chloride found in crevices of the surface rocks after drying caused by the sun: this process is still in use in modern solar ponds.

Other crystallization processes, for example sucrose production (this is the crystalline product with the largest world production, followed by sodium chloride), or in pigment manufacturing, were used in ancient times. These substances were sometimes produced by crystallizing the solutes of some more or less natural brine.

In more recent times, the fast expansion of the chemical industry has required a thorough study of the dynamics of crystallization, and this unit operation is now used in many industrial manufacturing areas: table salt, sugar, sodium sulfate, urea, just to name a few, are produced by crystallization from solutions.

Crystallizer technology has progressed alongside with the new processes. Once simple tanks in which, through cooling, evaporation or maybe through pH variation a crystal was obtained, nowadays continuous machines ensure a remarkable consistency in the product characteristics. Among the first models of modern crystallizers were probably the *calandria* type, being today the standard crystallizer for sucrose, and the *Oslo*, named after the Norwegian capital, since it was developed to produce salt in a climate not particularly fit for solar ponds, salt being widely used in Norway in stockfish production. The Oslo type was probably the first crystallizer designed specifically for the control of crystal growth.

Crystallization dynamics

As mentioned above, a crystal is formed following a well-defined pattern, or structure, dictated by forces acting at the molecular level. As a consequence, during its formation process the crystal is in an environment where the solute concentration reaches a certain critical value, before changing status. Solid formation, impossible below the solubility threshold at the given temperature and pressure conditions, may then take place at a concentration higher than the theoretical solubility level. The difference between the actual value of the solute concentration at the crystallization limit and the theoretical (static) solubility threshold is called supersaturation and is a fundamental factor in crystallization dynamics. Supersaturation is the driving force for both the initial nucleation step and the following crystal growth, both of which could not occur in saturated or undersaturated conditions.

Nucleation

Nucleation is the initiation of a phase change in a small region, such as the formation of a solid crystal from a liquid solution. It is a consequence of rapid local fluctuations on a molecular scale in a homogeneous phase that is in a state of metastable equilibrium. Total nucleation is the sum effect of two categories of nucleation - primary and secondary.

Primary nucleation

Primary nucleation is the initial formation of a crystal where there are no other crystals present or where, if there are crystals present in the system, they do not have any influence on the process. This can occur in two conditions. The first is homogeneous nucleation, which is nucleation that is not influenced in any way by solids. These solids include the walls of the crystallizer vessel and particles of any foreign substance. The second category, then, is heterogeneous nucleation. This occurs when solid particles of foreign substances cause an increase in the rate of nucleation that would otherwise not be seen without the existence of these foreign particles. Homogeneous nucleation rarely occurs in practice due to the high energy necessary to begin nucleation without a solid surface to catalyse the nucleation.

Primary nucleation (both homogeneous and heterogeneous) has been modelled with the following:

$$B = \frac{dN}{dt} = k_n (c - c^*)^n$$

- B is the number of nuclei formed per unit volume per unit time.
- N is the number of nuclei per unit volume.
- k_{np} is a rate constant.
- c is the instantaneous solute concentration.
- c^* is the solute concentration at saturation.
- $(c-c^*)$ is also known as supersaturation.

- n is an empirical exponent that can be as large as 10, but generally ranges between 3 and 4.

Secondary nucleation

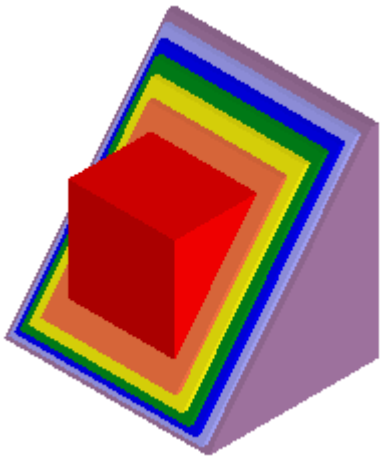
Secondary nucleation is the formation of nuclei attributable to the influence of the existing microscopic crystals in the magma. The first type of known secondary crystallization is attributable to fluid shear, the other due to collisions between already existing crystals with either a solid surface of the crystallizer or with other crystals themselves. Fluid shear nucleation occurs when liquid travels across a Crystal at a high speed, sweeping away nuclei that would otherwise be incorporated into a Crystal, causing the swept-away nuclei to become new crystals. Contact nucleation has been found to be the most effective and common method for nucleation. The benefits include the following

- Low kinetic order and rate-proportional to supersaturation, allowing easy control without unstable operation.
- Occurs at low supersaturation, where growth rate is optimum for good quality.
- Low necessary energy at which crystals strike avoids the breaking of existing crystals into new crystals.
- The quantitative fundamentals have already been isolated and are being incorporated into practice.

The following model, although somewhat simplified, is often used to model secondary nucleation:

$$B = \frac{dN}{dt} = k_1 M_T^j (c - c^*)^b$$

- k_1 is a rate constant.
- M_T is the suspension density.
- j is an empirical exponent that can range up to 1.5, but is generally 1.
- b is an empirical exponent that can range up to 5, but is generally 2.



Crystal growth

Crystal growth

Once the first small crystal, the nucleus, forms it acts as a convergence point (if unstable due to supersaturation) for molecules of solute touching - or adjacent to - the crystal so that it increases its own dimension in successive layers. The pattern of growth resembles the rings of an onion, as shown in the picture, where each colour indicates the same mass of solute; this mass creates increasingly thin layers due to the increasing surface area of the growing crystal. The supersaturated solute mass the original nucleus may *capture* in a time unit is called the *growth rate* expressed in kg/(m^2*h), and is a constant specific to the process. Growth rate is influenced by several physical factors, such as surface tension of solution, pressure, temperature, relative crystal velocity in the solution, Reynolds number, and so forth.

The main values to control are therefore:

- Supersaturation value, as an index of the quantity of solute available for the growth of the crystal;
- Total crystal surface in unit fluid mass, as an index of the capability of the solute to fix onto the crystal;
- Retention time, as an index of the probability of a molecule of solute to come into contact with an existing crystal;
- Flow pattern, again as an index of the probability of a molecule of solute to come into contact with an existing crystal (higher in laminar flow, lower in turbulent flow, but the reverse applies to the probability of contact).

The first value is a consequence of the physical characteristics of the solution, while the others define a difference between a well- and poorly designed crystallizer.

Crystal size distribution

The appearance and size range of a crystalline product is extremely important in crystallization. If further processing of the crystals is desired, large crystals with uniform size are important for washing, filtering, transportation, and storage. The importance lies in the fact that large crystals are easier to filter out of a solution than small crystals. Also, larger crystals have a smaller surface area to volume ratio, leading to a higher purity. This higher purity is due to less retention of mother liquor which contains impurities, and a smaller loss of yield when the crystals are washed to remove the mother liquor. The theoretical crystal size distribution can be estimated as a function of operating conditions with a fairly complicated mathematical process called population balance theory (using population balance equations).

Main crystallization processes

The main factors influencing solubility are, as we saw above:

- Concentration
- Temperature

So we may identify two main families of crystallization processes:

- Cooling crystallization
- Evaporative crystallization

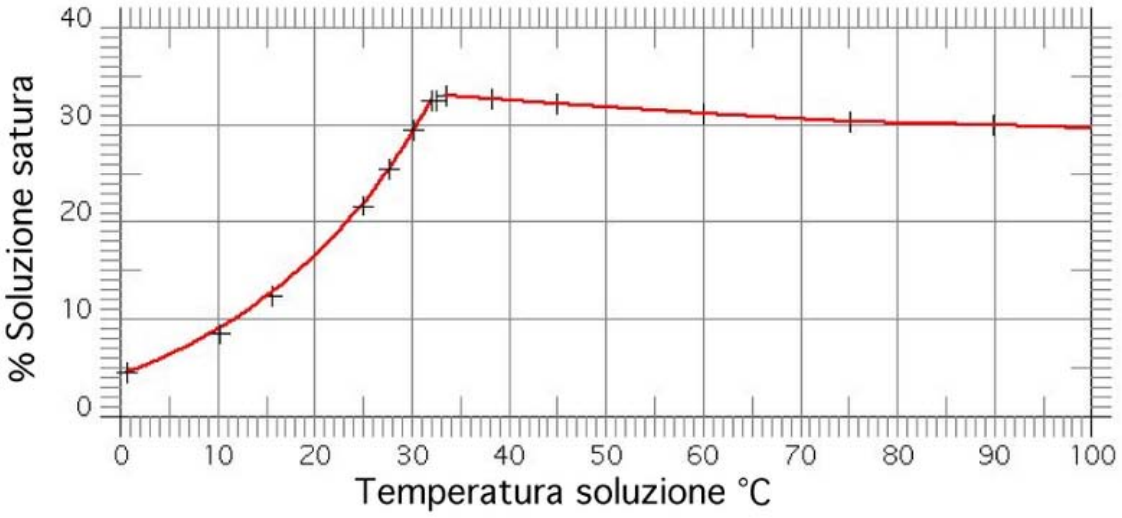
This division is not really clear-cut, since hybrid systems exist, where cooling is performed through evaporation, thus obtaining at the same time a concentration of the solution.

A crystallization process often referred to in chemical engineering is the Fractional crystallization. This is not a different process, rather a special application of one (or both) of the above.

Cooling crystallization

Application

Most chemical compounds, dissolved in most solvents, show the so-called *direct* solubility that is, the solubility threshold increases with temperature.



Solubility of the system Na_2SO_4 - H_2O

So, whenever the conditions are favourable, crystal formation results from simply cooling the solution. Here *cooling* is a relative term: austenite crystals in a steel form well above 1000 °C. An example of this crystallization process is the production of Glauber's salt, a crystalline form of sodium sulphate. In the picture, where equilibrium temperature is on the x-axis and equilibrium concentration (as mass percent of solute in saturated solution) in y-axis, it is clear that sulphate solubility quickly decreases below 32.5 °C. Assuming a saturated solution at 30 °C, by cooling it to 0 °C (note that this is possible thanks to the freezing-point depression), the precipitation of a mass of sulphate occurs corresponding to the change in solubility from 29% (equilibrium value at 30°C) to approximately 4.5% (at 0°C) - actually a larger crystal mass is precipitated, since sulphate entrains hydration water, and this has the side effect of increasing the final concentration.

There are of course limitation in the use of cooling crystallization:

- Many solutes precipitate in hydrate form at low temperatures: in the previous example this is acceptable, and even useful, but it may be detrimental when, for example, the mass of water of hydration to reach a stable hydrate crystallization form is more than the available water: a single block of hydrate solute will be formed - this occurs in the case of calcium chloride);
- Maximum supersaturation will take place in the coldest points. These may be the heat exchanger tubes which are sensitive to scaling, and heat exchange may be greatly reduced or discontinued;
- A decrease in temperature usually implies an increase of the viscosity of a solution. Too high a viscosity may give hydraulic problems, and the laminar flow thus created may affect the crystallization dynamics.
- It is of course not applicable to compounds having *reverse* solubility, a term to indicate that solubility increases with temperature decrease (an example occurs with sodium sulphate where solubility is reversed above 32.5 °C).

Cooling crystallizers

The simplest cooling crystallizers are tanks provided with a mixer for internal circulation, where temperature decrease is obtained by heat exchange with an intermediate fluid circulating in a jacket. These simple machines are used in batch processes, as in processing of pharmaceuticals and are prone to scaling. Batch processes normally provide a relatively variable quality of product along the batch.

The *Swenson-Walker* crystallizer is a model, specifically conceived by Swenson Co. around 1920, having a semicylindric horizontal hollow trough in which a hollow screw conveyor or some hollow discs, in which a refrigerating fluid is circulated, plunge during rotation on a longitudinal axis. The refrigerating fluid is sometimes also circulated in a jacket around the trough. Crystals precipitate on the cold surfaces of the screw/discs, from which they are removed by scrapers and settle on the bottom of the trough. The screw, if provided, pushes the slurry towards a discharge port.

A common practice is to cool the solutions by flash evaporation: when a liquid at a given T_0 temperature is transferred in a chamber at a pressure P_1 such that the liquid saturation temperature T_1 at P_1 is lower than T_0, the liquid will release heat according to the temperature difference and a quantity of solvent, whose total latent heat of vaporization equals the difference in enthalpy. In simple words, the liquid is cooled by evaporating a part of it.

Evaporative crystallization

Another option is to obtain, at an approximately constant temperature, the precipitation of the crystals by increasing the solute concentration above the solubility threshold. To obtain this, the solute/solvent mass ratio is increased using the technique of evaporation. This process is of course insensitive to change in temperature (as long as hydration state remains unchanged).

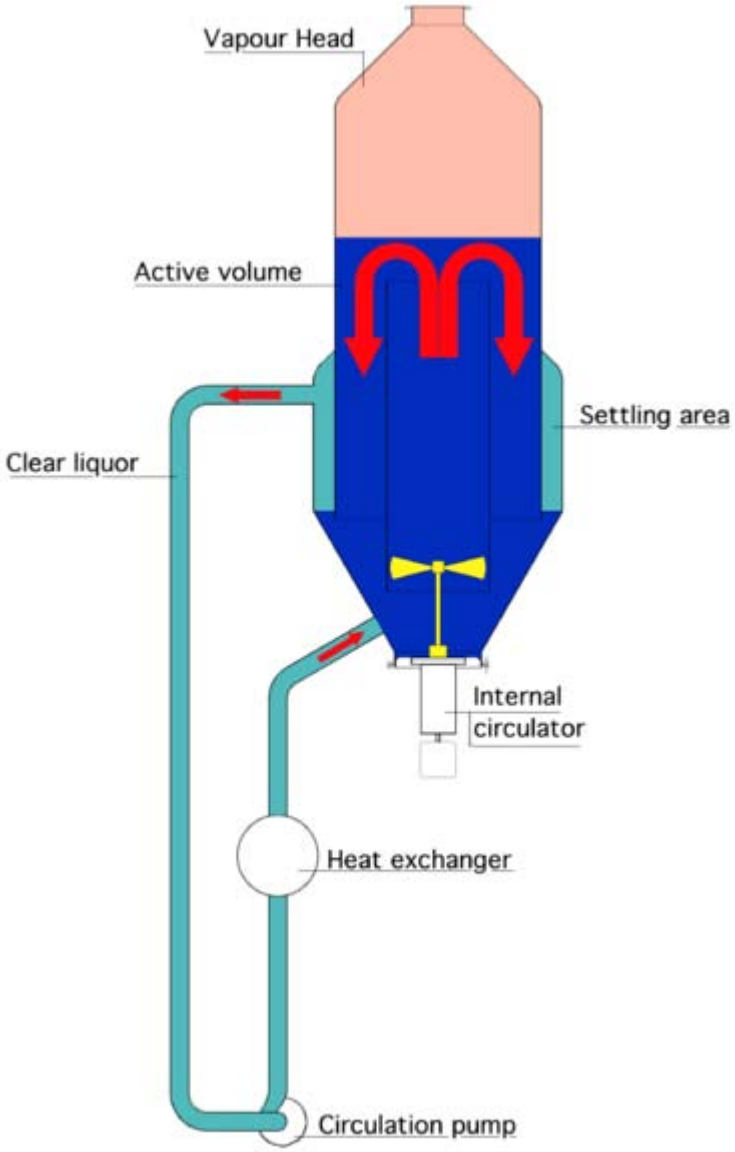
All considerations on control of crystallization parameters are the same as for the cooling models.

Evaporative crystallizers

Most industrial crystallizers are of the evaporative type, such as the very large sodium chloride and sucrose units, whose production accounts for more than 50% of the total world production of crystals. The most common type is the *forced circulation* (FC) model. A pumping device (a pump or an axial flow mixer) keeps the crystal slurry in homogeneous suspension throughout the tank, including the exchange surfaces; by controlling pump flow, control of the contact time of the crystal mass with the supersaturated solution is achieved, together with reasonable velocities at the exchange surfaces. The Oslo, mentioned above, is a refining of the evaporative forced circulation crystallizer, now equipped with a large crystals settling zone to increase the retention time (usually low in the FC) and to roughly separate heavy slurry zones from clear liquid.

The DTB crystallizer

DTB Crystallizer



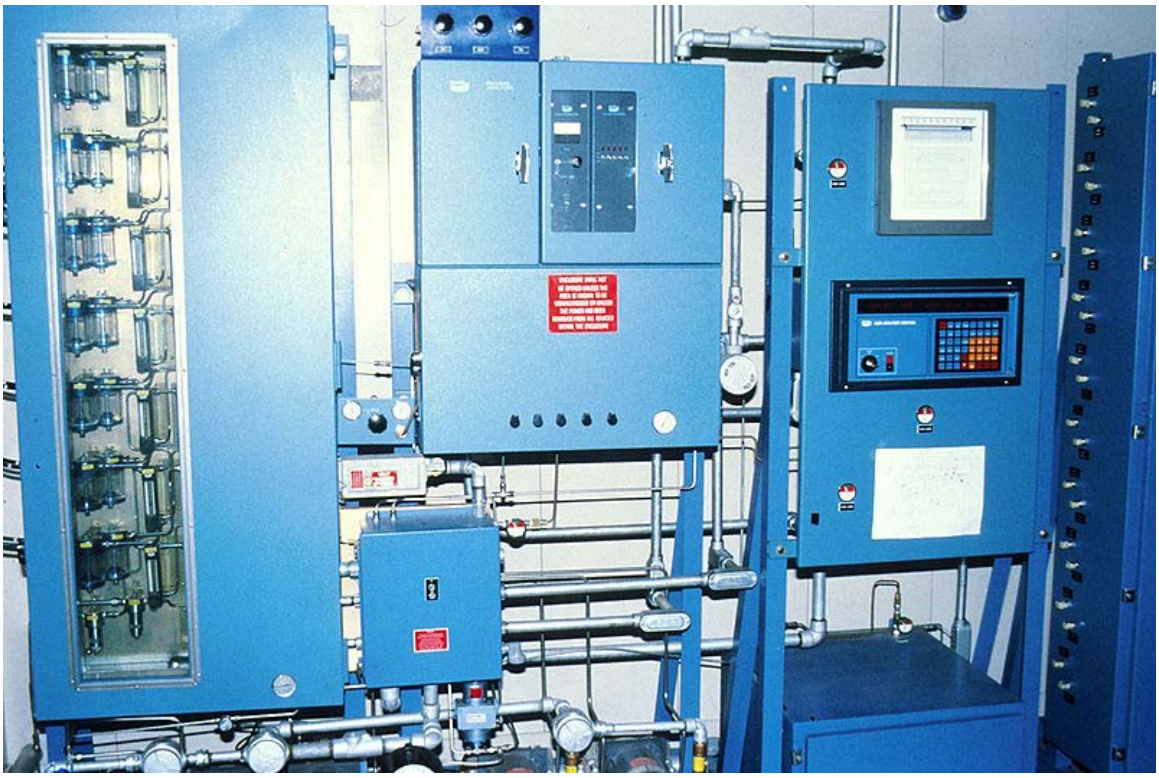
Schematic of DTB

Whichever the form of the crystallizer, to achieve an effective process control it is important to control the retention time and the crystal mass, to obtain the optimum conditions in terms of crystal specific surface and the fastest possible growth. This is achieved by a separation - to put it simply - of the crystals from the liquid mass, in order to manage the two flows in a different way. The practical way is to perform a gravity settling to be able to extract (and possibly recycle separately) the (almost) clear liquid, while managing the mass flow around the crystallizer to obtain a precise slurry density elsewhere. A typical example is the DTB (*Draft Tube and Baffle*) crystallizer, an idea of Richard Chisum Bennett (a Swenson engineer and later President of Swenson) at the end of the 1950s. The DTB crystallizer has an internal circulator, typically an axial flow mixer - yellow - pushing upwards in a draft tube while outside the crystallizer there is a

settling area in an annulus; in it the exhaust solution moves upwards at a very low velocity, so that large crystals settle - and return to the main circulation - while only the fines, below a given grain size are extracted and eventually destroyed by increasing or decreasing temperature, thus creating additional supersaturation. A quasi-perfect control of all parameters is achieved. This crystallizer, and the derivative models (Krystal, CSC, etc.) could be the ultimate solution if not for a major limitation in the evaporative capacity, due to the limited diameter of the vapour head and the relatively low external circulation not allowing large amounts of energy to be supplied to the system.

Chapter- 4

Chromatography



Pictured is a sophisticated gas chromatography system. This instrument records concentrations of acrylonitrile in the air at various points throughout the chemical laboratory.

Chromatography (from Greek χρῶμα *chroma* "color" and γράφειν *graphein* "to write") is the collective term for a set of laboratory techniques for the separation of mixtures. It involves passing a mixture dissolved in a "mobile phase" through a *stationary phase*, which separates the analyte to be measured from other molecules in the mixture based on differential partitioning between the mobile and stationary phases. Subtle differences in a

compound's partition coefficient result in differential retention on the stationary phase and thus **changing** the separation.

Chromatography may be preparative or analytical. The purpose of preparative chromatography is to separate the components of a mixture for further use (and is thus a form of purification). Analytical chromatography is done normally with smaller amounts of material and is for measuring the relative proportions of analytes in a mixture. The two are not mutually exclusive.

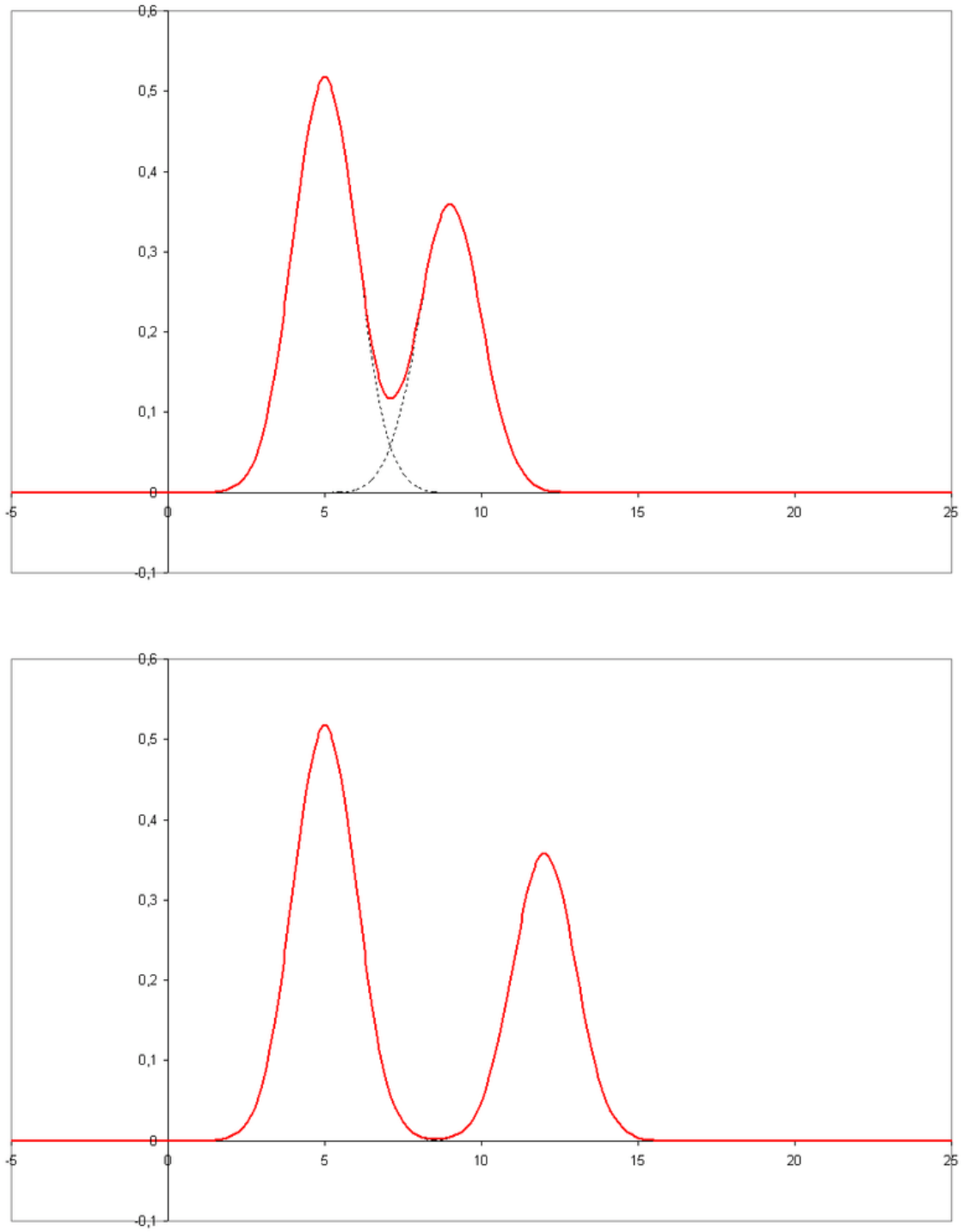
History

The **history of chromatography** begins during the mid-19th century. Chromatography, literally "color writing", was used—and named— in the first decade of the 20th century, primarily for the separation of plant pigments such as chlorophyll. New types of chromatography developed during the 1930s and 1940s made the technique useful for many types of separation process.

Chromatography became developed substantially as a result of the work of Archer John Porter Martin and Richard Laurence Millington Synge during the 1940s and 1950s. They established the principles and basic techniques of partition chromatography, and their work encouraged the rapid development of several types of chromatography method: paper chromatography, gas chromatography, and what would become known as high performance liquid chromatography. Since then, the technology has advanced rapidly. Researchers found that the main principles of Tsvet's chromatography could be applied in many different ways, resulting in the different varieties of chromatography described below. Simultaneously, advances continually improved the technical performance of chromatography, allowing the separation of increasingly similar molecules.

Chromatography terms

- The **analyte** is the substance to be separated during chromatography.
- **Analytical chromatography** is used to determine the existence and possibly also the concentration of analyte(s) in a sample.
- A **bonded phase** is a stationary phase that is covalently bonded to the support particles or to the inside wall of the column tubing.
- A **chromatogram** is the visual output of the chromatograph. In the case of an optimal separation, different peaks or patterns on the chromatogram correspond to different components of the separated mixture.



Plotted on the x-axis is the retention time and plotted on the y-axis a signal (for example obtained by a spectrophotometer, mass spectrometer or a variety of other detectors) corresponding to the response created by the analytes exiting the system. In the case of an optimal system the signal is proportional to the concentration of the specific analyte separated.

- A **chromatograph** is equipment that enables a sophisticated separation e.g. gas chromatographic or liquid chromatographic separation.
- **Chromatography** is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (the mobile phase) moves in a definite direction.
- The **eluate** is the mobile phase leaving the column.
- The **eluent** is the solvent that will carry the analyte.
- An **eluotropic series** is a list of solvents ranked according to their eluting power.
- An **immobilized phase** is a stationary phase which is immobilized on the support particles, or on the inner wall of the column tubing.
- The **mobile phase** is the phase which moves in a definite direction. It may be a liquid (LC and CEC), a gas (GC), or a supercritical fluid (supercritical-fluid chromatography, SFC). The mobile phase consists of the sample being separated/analyzed and the solvent that moves the sample through the column. In the case of HPLC the mobile phase consists of a non-polar solvent(s) such as hexane in normal phase or polar solvents in reverse phase chromotagraphy and the sample being separated. The mobile phase moves through the chromatography column (the stationary phase) where the sample interacts with the stationary phase and is separated.
- **Preparative chromatography** is used to purify sufficient quantities of a substance for further use, rather than analysis.
- The **retention time** is the characteristic time it takes for a particular analyte to pass through the system (from the column inlet to the detector) under set conditions.
- The **sample** is the matter analyzed in chromatography. It may consist of a single component or it may be a mixture of components. When the sample is treated in the course of an analysis, the phase or the phases containing the analytes of interest is/are referred to as the sample whereas everything out of interest separated from the sample before or in the course of the analysis is referred to as waste.
- The **solute** refers to the sample components in partition chromatography.
- The **solvent** refers to any substance capable of solubilizing other substance, and especially the liquid mobile phase in LC.
- The **stationary phase** is the substance which is fixed in place for the chromatography procedure. Examples include the silica layer in thin layer chromatography

Chromatography is based on the concept of partition coffecient. Any solute will partition between two immissible solvents. When we make one solvent immobile (by adsorption on a solid support matrix) and another mobile it results in most common applications of chromatography. If matrix support is polar (e.g. paper, sillica etc.) it is forward phase chromatography, and if it is non polar (C-18) it is reverse phase.

Techniques by chromatographic bed shape

Column chromatography

Column chromatography is a separation technique in which the stationary bed is within a tube. The particles of the solid stationary phase or the support coated with a liquid stationary phase may fill the whole inside volume of the tube (packed column) or be concentrated on or along the inside tube wall leaving an open, unrestricted path for the mobile phase in the middle part of the tube (open tubular column). Differences in rates of movement through the medium are calculated to different retention times of the sample.

In 1978, W. C. Still introduced a modified version of column chromatography called **flash column chromatography** (flash). The technique is very similar to the traditional column chromatography, except for that the solvent is driven through the column by applying positive pressure. This allowed most separations to be performed in less than 20 minutes, with improved separations compared to the old method. Modern flash chromatography systems are sold as pre-packed plastic cartridges, and the solvent is pumped through the cartridge. Systems may also be linked with detectors and fraction collectors providing automation. The introduction of gradient pumps resulted in quicker separations and less solvent usage.

In expanded bed adsorption, a fluidized bed is used, rather than a solid phase made by a packed bed. This allows omission of initial clearing steps such as centrifugation and filtration, for culture broths or slurries of broken cells.

Planar chromatography

Thin layer chromatography is used to separate components of chlorophyll

Planar chromatography is a separation technique in which the stationary phase is present as or on a plane. The plane can be a paper, serving as such or impregnated by a substance as the stationary bed (paper chromatography) or a layer of solid particles spread on a support such as a glass plate (thin layer chromatography). Different compounds in the sample mixture travel different distances according to how strongly they interact with the stationary phase as compared to the mobile phase. The specific Retention factor (R_f) of each chemical can be used to aid in the identification of an unknown substance.

Paper chromatography

Paper chromatography is a technique that involves placing a small dot or line of sample solution onto a strip of *chromatography paper*. The paper is placed in a jar containing a shallow layer of solvent and sealed. As the solvent rises through the paper, it meets the sample mixture which starts to travel up the paper with the solvent. This paper is made of cellulose, a polar substance, and the compounds within the mixture travel farther if they are non-polar. More polar substances bond with the cellulose paper more quickly, and therefore do not travel as far.

Thin layer chromatography

Thin layer chromatography (TLC) is a widely employed laboratory technique and is similar to paper chromatography. However, instead of using a stationary phase of paper, it involves a stationary phase of a thin layer of adsorbent like silica gel, alumina, or cellulose on a flat, inert substrate. Compared to paper, it has the advantage of faster runs, better separations, and the choice between different adsorbents. For even better resolution and to allow for quantification, high-performance TLC can be used.

Displacement chromatography

The basic principle of displacement chromatography is: A molecule with a high affinity for the chromatography matrix (the displacer) will compete effectively for binding sites, and thus displace all molecules with lesser affinities. There are distinct differences between displacement and elution chromatography. In elution mode, substances typically emerge from a column in narrow, Gaussian peaks. Wide separation of peaks, preferably to baseline, is desired in order to achieve maximum purification. The speed at which any component of a mixture travels down the column in elution mode depends on many factors. But for two substances to travel at different speeds, and thereby be resolved, there must be substantial differences in some interaction between the biomolecules and the chromatography matrix. Operating parameters are adjusted to maximize the effect of this difference. In many cases, baseline separation of the peaks can be achieved only with gradient elution and low column loadings. Thus, two drawbacks to elution mode chromatography, especially at the preparative scale, are operational complexity, due to gradient solvent pumping, and low throughput, due to low column loadings. Displacement chromatography has advantages over elution chromatography in that components are resolved into consecutive zones of pure substances rather than “peaks”. Because the process takes advantage of the nonlinearity of the isotherms, a larger column feed can be separated on a given column with the purified components recovered at significantly higher concentrations.

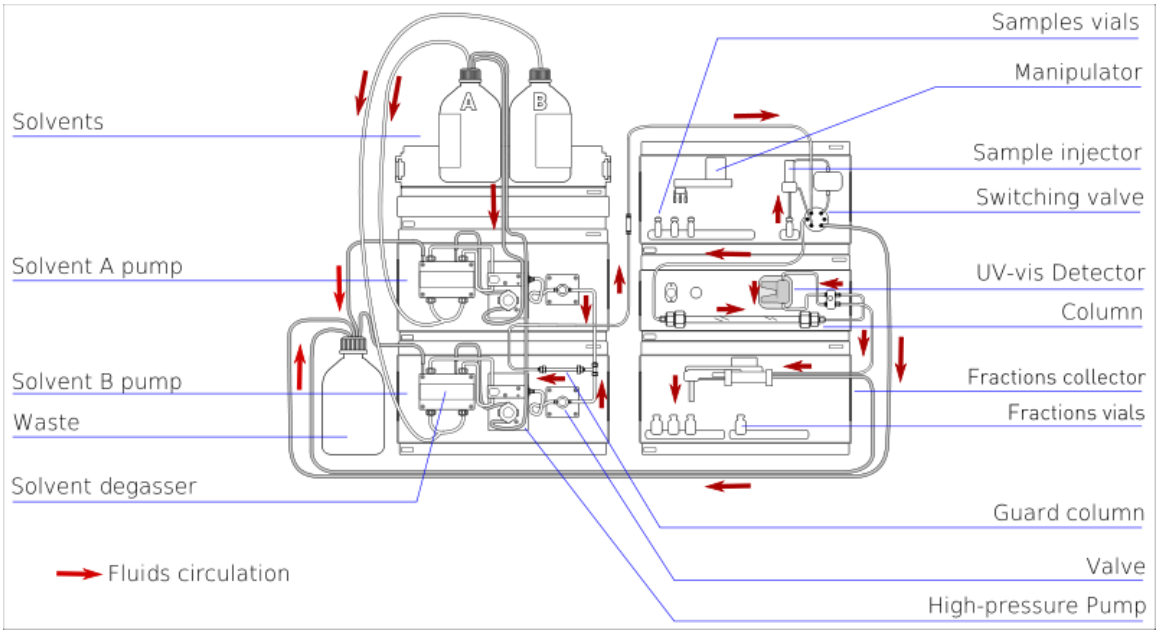
Techniques by physical state of mobile phase

Gas chromatography

Gas chromatography (GC), also sometimes known as Gas-Liquid chromatography, (GLC), is a separation technique in which the mobile phase is a gas. Gas chromatography is always carried out in a column, which is typically "packed" or "capillary" (see below) .

Gas chromatography (GC) is based on a partition equilibrium of analyte between a solid stationary phase (often a liquid silicone-based material) and a mobile gas (most often Helium). The stationary phase is adhered to the inside of a small-diameter glass tube (a capillary column) or a solid matrix inside a larger metal tube (a packed column). It is widely used in analytical chemistry; though the high temperatures used in GC make it unsuitable for high molecular weight biopolymers or proteins (heat will denature them), frequently encountered in biochemistry, it is well suited for use in the petrochemical, environmental monitoring and remediation, and industrial chemical fields. It is also used extensively in chemistry research.

Liquid chromatography



Preparative HPLC apparatus

Liquid chromatography (LC) is a separation technique in which the mobile phase is a liquid. Liquid chromatography can be carried out either in a column or a plane. Present day liquid chromatography that generally utilizes very small packing particles and a relatively high pressure is referred to as high performance liquid chromatography (HPLC).

In the HPLC technique, the sample is forced through a column that is packed with irregularly or spherically shaped particles, a porous monolithic layer (stationary phase) or a porous membrane by a liquid (mobile phase) at high pressure. HPLC is historically

divided into two different sub-classes based on the polarity of the mobile and stationary phases. Methods in which the stationary phase is more polar than the mobile phase (e.g. toluene as the mobile phase, silica as the stationary phase) are termed normal phase liquid chromatography (NPLC) and the opposite (e.g. water-methanol mixture as the mobile phase and C18 = octadecylsilyl as the stationary phase) is termed reversed phase liquid chromatography (RPLC). Ironically the "normal phase" has fewer applications and RPLC is therefore used considerably more.

Specific techniques which come under this broad heading are listed below. It should also be noted that the following techniques can also be considered fast protein liquid chromatography if no pressure is used to drive the mobile phase through the stationary phase.

Affinity chromatography

Affinity chromatography is based on selective non-covalent interaction between an analyte and specific molecules. It is very specific, but not very robust. It is often used in biochemistry in the purification of proteins bound to tags. These fusion proteins are labeled with compounds such as His-tags, biotin or antigens, which bind to the stationary phase specifically. After purification, some of these tags are usually removed and the pure protein is obtained.

Affinity chromatography often utilizes a biomolecule's affinity for a metal (Zn, Cu, Fe, etc.). Columns are often manually prepared. Traditional affinity columns are used as a preparative step to flush out unwanted biomolecules.

However, HPLC techniques exist that do utilize affinity chromatogaphy properties. Immobilized Metal Affinity Chromatography (IMAC) is useful to separate aforementioned molecules based on the relative affinity for the metal (I.e. Dionex IMAC) . Often these columns can be loaded with different metals to create a column with a targeted affinity.

Supercritical fluid chromatography

Supercritical fluid chromatography is a separation technique in which the mobile phase is a fluid above and relatively close to its critical temperature and pressure.

Techniques by separation mechanism

Ion exchange chromatography

Ion exchange chromatography uses ion exchange mechanism to separate analytes. It is usually performed in columns but can also be useful in planar mode. Ion exchange chromatography uses a charged stationary phase to separate charged compounds including amino acids, peptides, and proteins. In conventional methods the stationary

phase is an ion exchange resin that carries charged functional groups which interact with oppositely charged groups of the compound to be retained. Ion exchange chromatography is commonly used to purify proteins using FPLC.

Size-exclusion chromatography

Size-exclusion chromatography (SEC) is also known as **gel permeation chromatography** (GPC) or **gel filtration chromatography** and separates molecules according to their size (or more accurately according to their hydrodynamic diameter or hydrodynamic volume). Smaller molecules are able to enter the pores of the media and, therefore, molecules are trapped and removed from the flow of the mobile phase. The average residence time in the pores depends upon the effective size of the analyte molecules. However, molecules that are larger than the average pore size of the packing are excluded and thus suffer essentially no retention; such species are the first to be eluted. It is generally a low-resolution chromatography technique and thus it is often reserved for the final, "polishing" step of a purification. It is also useful for determining the tertiary structure and quaternary structure of purified proteins, especially since it can be carried out under native solution conditions.

Special techniques

Reversed-phase chromatography

Reversed-phase chromatography is an elution procedure used in liquid chromatography in which the mobile phase is significantly more polar than the stationary phase.

Two-dimensional chromatography

In some cases, the chemistry within a given column can be insufficient to separate some analytes. It is possible to direct a series of unresolved peaks onto a second column with different physico-chemical (Chemical classification) properties. Since the mechanism of retention on this new solid support is different from the first dimensional separation, it can be possible to separate compounds that are indistinguishable by one-dimensional chromatography. The sample is spotted at one corner of a square plate,developed, air-dried, then rotated by 90° and usually redeveloped in a second solvent system.

Simulated moving-bed chromatography

Pyrolysis gas chromatography

Fast protein liquid chromatography

Fast protein liquid chromatography (FPLC) is a term applied to several chromatography techniques which are used to purify proteins. Many of these techniques are identical to those carried out under high performance liquid chromatography, however use of FPLC techniques are typically for preparing large scale batches of a purified product.

Countercurrent chromatography

Countercurrent chromatography (CCC) is a type of liquid-liquid chromatography, where both the stationary and mobile phases are liquids. It involves mixing a solution of liquids, allowing them to settle into layers and then separating the layers.

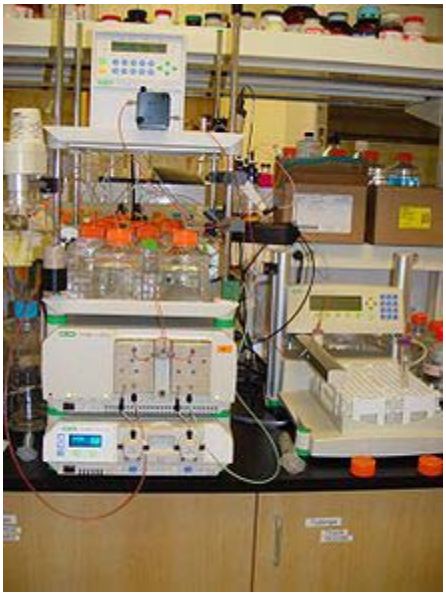
Chiral chromatography

Chiral chromatography involves the separation of stereoisomers. In the case of enantiomers, these have no chemical or physical differences apart from being three-dimensional mirror images. Conventional chromatography or other separation processes are incapable of separating them. To enable chiral separations to take place, either the mobile phase or the stationary phase must themselves be made chiral, giving differing affinities between the analytes. Chiral chromatography HPLC columns (with a chiral stationary phase) in both normal and reversed phase are commercially available.

Chapter- 5

Size-Exclusion Chromatography

Size-exclusion chromatography



Equipment for running size-exclusion chromatography. The buffer is pumped through the column (right) by a computer-controlled device

Acronym	SEC
Classification	Chromatography
Analytes	macromolecules synthetic polymers biomolecules

Other techniques

Related	High performance liquid chromatography Aqueous Normal Phase Chromatography Ion exchange chromatography Micellar liquid chromatography

Size-exclusion chromatography (SEC) is a chromatographic method in which molecules in solution are separated by their size, not by molecular weight. It is usually applied to large molecules or macromolecular complexes such as proteins and industrial polymers. Typically, when an aqueous solution is used to transport the sample through the column, the technique is known as **gel-filtration chromatography**, versus the name **Gel permeation chromatography**, which is used when an organic solvent is used as a mobile phase. SEC is a widely used polymer characterization method because of its ability to provide good Mw results for polymers.

Applications

The main application of gel-filtration chromatography is the fractionation of proteins and other water-soluble polymers, while gel permeation chromatography is used to analyze the molecular weight distribution of organic-soluble polymers. Either technique should not be confused with gel electrophoresis, where an electric field is used to "pull" or "push" molecules through the gel depending on their electrical charges.

SEC is a widely used technique for the purification and analysis of synthetic and biological polymers, such as proteins, polysaccharides and nucleic acids. Biologists and biochemists typically use a gel medium — usually polyacrylamide, dextran or agarose — and filter under low pressure. Polymer chemists typically use either a silica or crosslinked polystyrene medium under a higher pressure. These media are known as the **stationary phase**.

Advantages

The advantages of this method include good separation of large molecules from the small molecules with a minimal volume of eluate, and that various solutions can be applied without interfering with the filtration process, all while preserving the biological activity of the particles to be separated. The technique is generally combined with others that further separate molecules by other characteristics, such as acidity, basicity, charge, and affinity for certain compounds. With size exclusion chromatography, there are short and well-defined separation times and narrow bands, which lead to good sensitivity. There is also no sample loss because solutes do not interact with the stationary phase. Disadvantages are, for example, that only a limited number of bands can be

accommodated because the time scale of the chromatogram is short, and, in general, there has to be a 10% difference in molecular mass to have a good resolution

Discovery

The technique was invented by Grant Henry Lathe and Colin R Ruthven, working at Queen Charlotte's Hospital, London. They later received the John Scott Award for this invention. While Lathe and Ruthven used starch gels as the matrix, Jerker Porath and Per Flodin later introduced dextran gels; other gels with size fractionation properties include agarose and polyacrylamide. A short review of these developments has appeared.

There were also attempts to fractionate synthetic high polymers; however, it was not until 1964, when J. C. Moore of the Dow Chemical Company published his work on the preparation of Gel Permeation Chromatography (GPC) columns based on cross-linked polystyrene with controlled pore size, that a rapid increase of research activity in this field began. It was recognized almost immediately that with proper calibration, GPC was capable to provide molar mass and molar mass distribution information for synthetic polymers. Because the latter information was difficult to obtain by other methods, GPC came rapidly into extensive use.

Theory and method

One requirement for SEC is that the analyte does not interact with the surface of the stationary phases. Differences in elution time are based solely on the volume the analyte "sees". Thus, a small molecule that can penetrate every corner of the pore system of the stationary phase "sees" the entire pore volume and the interparticle volume, and will elute late (when the pore- and interparticle volume has passed through the column ~80% of the column volume). On the other extreme, a very large molecule that cannot penetrate the pore system "sees" only the interparticle volume (~35% of the column volume) and will elute earlier when this volume of mobile phase has passed through the column. The underlying principle of SEC is that particles of different sizes will elute (filter) through a stationary phase at different rates. This results in the separation of a solution of particles based on size. Provided that all the particles are loaded simultaneously or near-simultaneously, particles of the same size should elute together.

However, as there are various measure of the size of a macromolecule (for instance, the radius of gyration and the hydrodynamic radius), a fundamental problem in the theory of SEC has been the choice of a proper molecular size parameter by which molecules of different kinds are separated. Experimentally, Benoit and co-workers found an excellent correlation between elution volume and a dynamically based molecular size, the hydrodynamic volume, for several different chain architecture and chemical compositions. The observed correlation based on the hydrodynamic volume became accepted as the basis of universal SEC calibration.

Still, the use of the hydrodynamic volume, a size based on dynamical properties, in the interpretation of SEC data is not fully understood. This is because SEC is typically run under low flow rate conditions where hydrodynamic factor should have little effect on the separation. In fact, both theory and computer simulations assume a thermodynamic separation principle: the separation process is determined by the equilibrium distribution (partitioning) of solute macromolecules between two phases --- a dilute bulk solution phase located at the interstitial space and confined solution phases within the pores of column packing material. Based on this theory, it has been shown that the relevant size parameter to the partitioning of polymers in pores is the mean span dimension (mean maximal projection onto a line). Although this issue has not been fully resolved, it is likely that the mean span dimension and the hydrodynamic volume are strongly correlated.

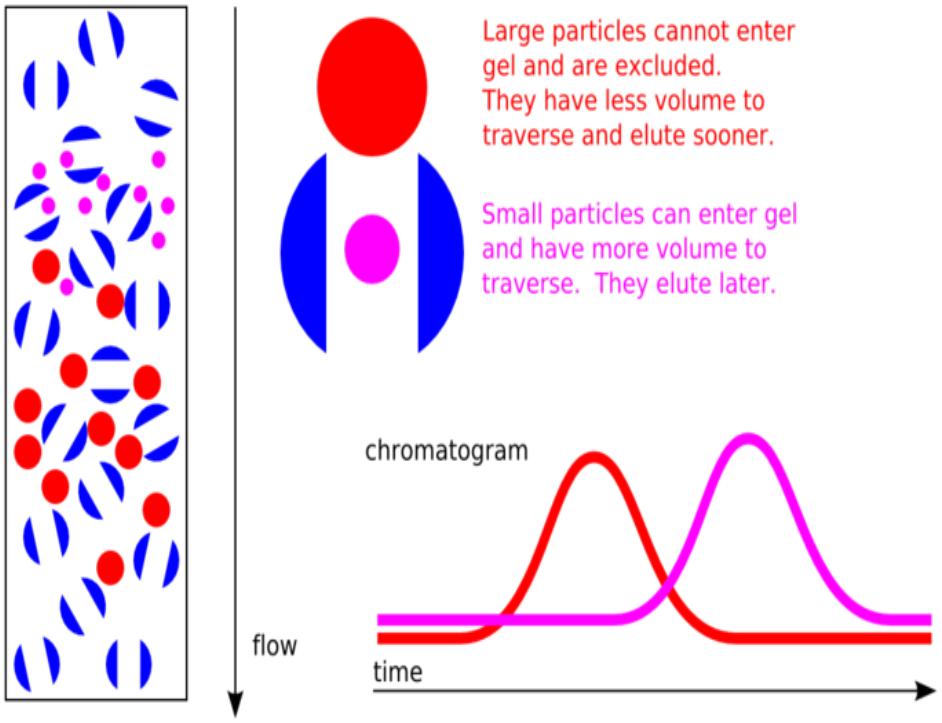
Each size exclusion column has a range of molecular weights that can be separated. The exclusion limit defines the molecular weight at the upper end of this range and is where molecules are too large to be trapped in the stationary phase. The permeation limit defines the molecular weight at the lower end of the range of separation and is where molecules of a small enough size can penetrate into the pores of the stationary phase completely and all molecules below this molecular mass are so small that they elute as a single band

A size exclusion column.

This is usually achieved with an apparatus called a column, which consists of a hollow tube tightly packed with extremely small porous polymer beads designed to have pores of different sizes. These pores may be depressions on the surface or channels through the bead. As the solution travels down the column some particles enter into the pores. Larger particles cannot enter into as many pores. The larger the particles, the faster the elution.

The filtered solution that is collected at the end is known as the **eluate**. The **void volume** includes any particles too large to enter the medium, and the solvent volume is known as the **column volume**.

Factors affecting filtration



A cartoon illustrating the theory behind **size exclusion chromatography**

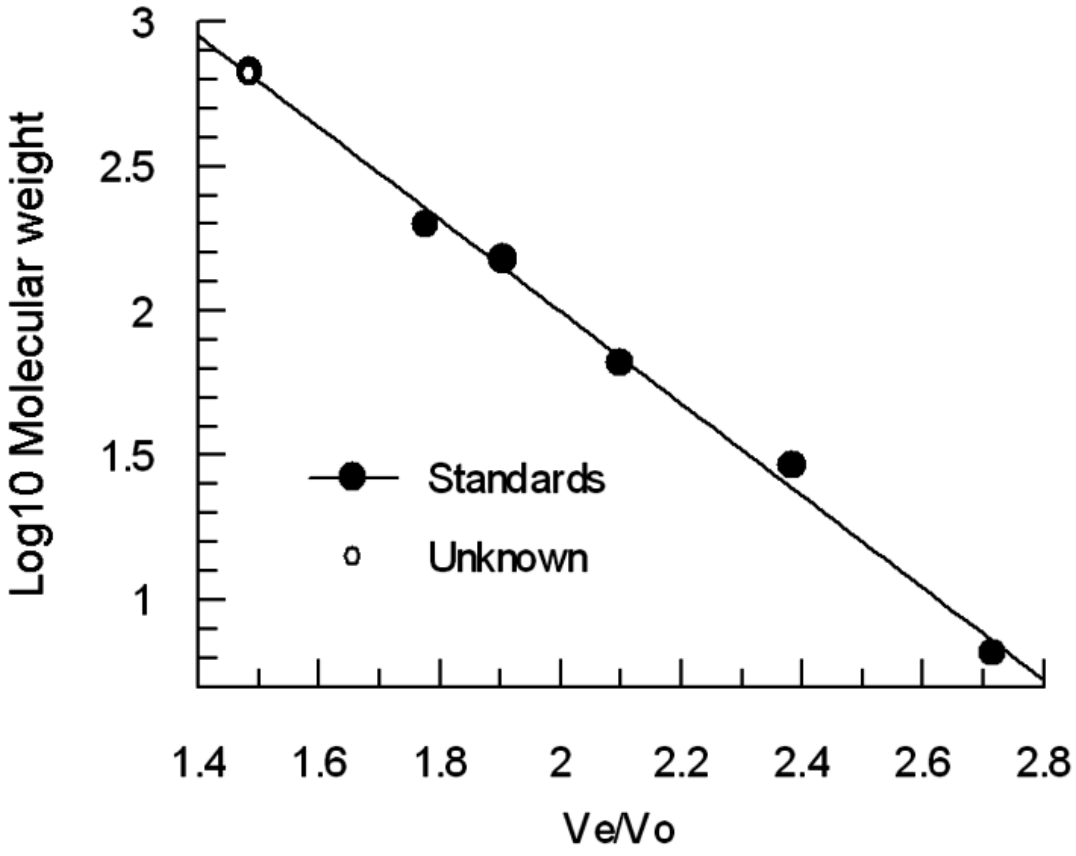
In real-life situations, particles in solution do not have a fixed size, resulting in the probability that a particle that would otherwise be hampered by a pore passing right by it. Also, the stationary-phase particles are not ideally defined; both particles and pores may vary in size. Elution curves, therefore, resemble Gaussian distributions. The stationary phase may also interact in undesirable ways with a particle and influence retention times, though great care is taken by column manufacturers to use stationary phases that are inert and minimize this issue.

Like other forms of chromatography, increasing the column length will enhance the resolution, and increasing the column diameter increases the capacity of the column. Proper column packing is important to maximize resolution: An overpacked column can collapse the pores in the beads, resulting in a loss of resolution. An underpacked column can reduce the relative surface area of the stationary phase accessible to smaller species, resulting in those species spending less time trapped in pores. Unlike affinity chromatography techniques, a solvent head at the top of the column can drastically

diminish resolution as the sample diffuses prior to loading, broadening the downstream elution.

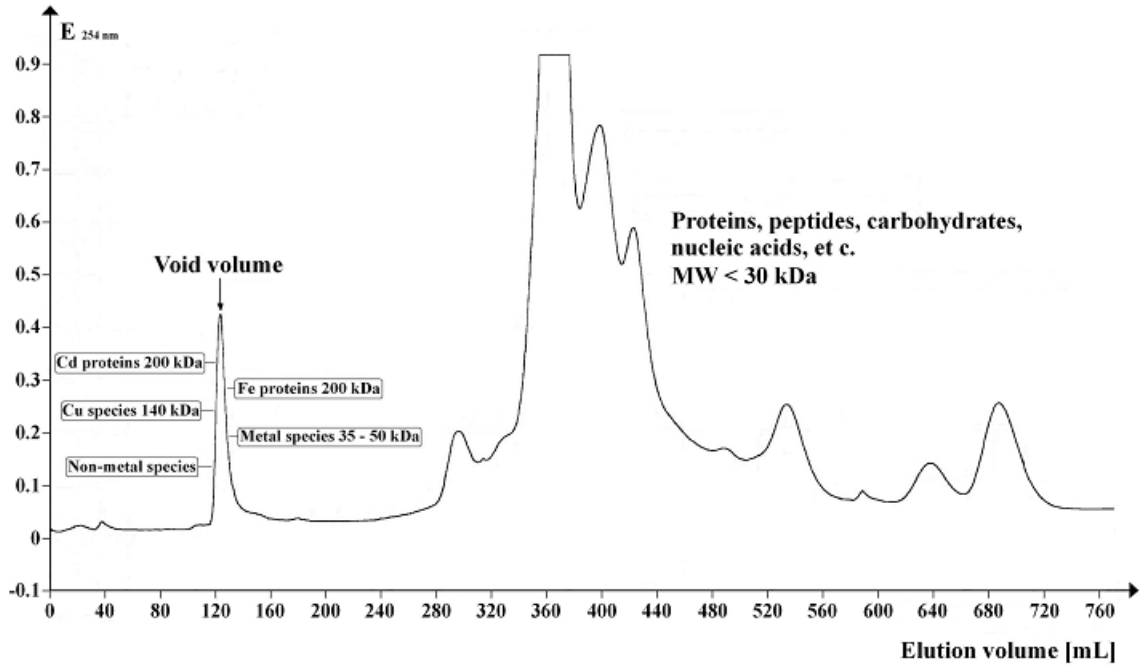
Analysis

In simple manual columns, the eluent is collected in constant volumes, known as fractions. The more similar the particles are in size the more likely they will be in the same fraction and not detected separately. More advanced columns overcome this problem by constantly monitoring the eluent.



Standardization of a size exclusion column.

The collected fractions are often examined by spectroscopic techniques to determine the concentration of the particles eluted. Common spectroscopy detection techniques are refractive index (RI)and ultraviolet (UV). When eluting spectroscopically similar species (such as during biological purification), other techniques may be necessary to identify the contents of each fraction. It is also possible to analyse the eluent flow continuously with RI, LALLS, Multi-Angle Laser Light Scattering MALS, UV, and/or viscosity measurements.



SEC Chromatogram of a biological sample.

The elution volume (Ve) decreases roughly linearly with the logarithm of the molecular hydrodynamic volume. Columns are often calibrated using 4-5 standard samples (e.g., folded proteins of known molecular weight), and a sample containing a very large molecule such as thyroglobulin to determine the void volume. (Blue dextran is not recommended for Vo determination because it is heterogeneous and may give variable results) The elution volumes of the standards are divided by the elution volume of the thyroglobulin (Ve/Vo) and plotted against the log of the standards' molecular weights.

Applications

Biochemical applications

In general, SEC is considered a low resolution chromatography as it does not discern similar species very well, and is therefore often reserved for the final "polishing" step of a purification. The technique can determine the quaternary structure of purified proteins that have slow exchange times, since it can be carried out under native solution conditions, preserving macromolecular interactions. SEC can also assay protein tertiary structure, as it measures the hydrodynamic volume (not molecular weight), allowing folded and unfolded versions of the same protein to be distinguished. For example, the

apparent hydrodynamic radius of a typical protein domain might be 14 Å and 36 Å for the folded and unfolded forms, respectively. SEC allows the separation of these two forms, as the folded form will elute much later due to its smaller size.

Polymer synthesis

SEC can be used as a measure of both the size and the polydispersity of a synthesised polymer, that is, the ability to be able to find the distribution of the sizes of polymer molecules. If standards of a known size are run previously, then a calibration curve can be created to determine the sizes of polymer molecules of interest in the solvent chosen for analysis (often THF). In alternative fashion, techniques such as light scattering and/or viscometry can be used online with SEC to yield absolute molecular weights that do not rely on calibration with standards of known molecular weight. Due to the difference in size of two polymers with identical molecular weights, the absolute determination methods are, in general, more desirable. A typical SEC system can quickly (in about half an hour) give polymer chemists information on the size and polydispersity of the sample. The preparative SEC can be used for polymer fractionation on an analytical scale. .

Drawback

In SEC, mass is not measured so much as the hydrodynamic volume of the polymer molecules, that is, how much space a particular polymer molecule takes up when it is in solution. However, the approximate molecular weight can be calculated from SEC data because the exact relationship between molecular weight and hydrodynamic volume for polystyrene can be found. For this, polystyrene is used as a standard. But the relationship between hydrodynamic volume and molecular weight is not the same for all polymers, so only an approximate measurement can be arrived at. Another drawback is the possibility of interaction between the stationary phase and the analyte. Any interaction leads to a later elution time and thus mimics a smaller analyte size.

Absolute size-exclusion chromatography

Absolute size-exclusion chromatography (ASEC) is a technique that couples a dynamic light scattering (DLS) instrument to a size exclusion chromatography system for absolute size measurements of proteins and macromolecules as they elute from the chromatography system.

The definition of absolute used here is that it does not require calibration to obtain hydrodynamic size, often referred to as hydrodynamic diameter (D_H in units of nm). The sizes of the macromolecules are measured as they elute into the flow cell of the DLS instrument from the size exclusion column set. It should be noted that the hydrodynamic size of the molecules or particles are measured and not their molecular weights. For proteins a Mark-Houwink type of calculation can be used to estimate the molecular weight from the hydrodynamic size.

A big advantage of DLS coupled with SEC is the ability to obtain enhanced DLS resolution. Batch DLS is quick and simple and provides a direct measure of the average size but the baseline resolution of DLS is 3 to 1 in diameter. Using SEC, the proteins and protein oligomers are separated, allowing oligomeric resolution. Aggregation studies can also be done using ASEC although the aggregate concentration may not be calculated, the size of the aggregate will be measured only to be limited by the maximum size eluting from the SEC columns.

Limitations of ASEC include flow-rate, concentration, and precision. Because a correlation function requires anywhere from 3–7 seconds to properly build, a limited number of data points can be collected across the peak.

Chapter- 6

Ion and Affinity Chromatography

Ion chromatography

Ion exchange chromatography

Acronym	IC, IEC
Classification	Chromatography
Related	**Other techniques** High performance liquid chromatography Aqueous Normal Phase Chromatography Size exclusion chromatography Micellar liquid chromatography

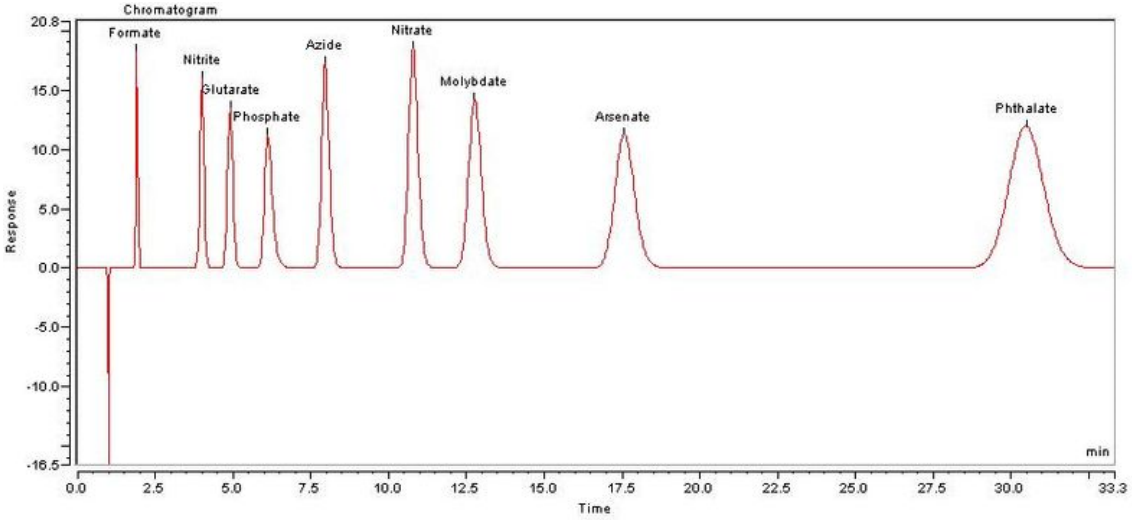
Ion-exchange chromatography (or *ion chromatography*) is a process that allows the separation of ions and polar molecules based on their charge. It can be used for almost any kind of charged molecule including large proteins, small nucleotides and amino acids. The solution to be injected is usually called a *sample*, and the individually separated components are called *analytes*. It is often used in protein purification, water analysis, and quality control.

History

Ion methods have been in use since 1850, when H. Thompson and J. T. Way, researchers in England, treated various clays with ammonium sulfate or carbonate in solution to extract the ammonia and release calcium. In 1927, the first zeolite mineral column was used to remove interfering calcium and magnesium ions from solution to determine the sulfate content of water. The modern version of IEC was developed during the wartime Manhattan Project. A technique was required to separate and concentrate the radioactive

elements needed to make the atom bomb. Researchers chose adsorbents that would latch onto charged transuranium elements, which could then be differentially eluted. Ultimately, once declassified, these techniques would use new IE resins to develop the systems that are often used today for specific purification of biologicals and inorganics. In the early 1970s, ion chromatography was developed by Hamish Small and co-workers at Dow Chemical Company as a novel method of IEC usable in automated analysis. This later led to the formation of Dionex Corp (Dow -Ion Exchange). IC uses weaker ionic resins for its stationary phase and an additional neutralizing stripper, or suppressor, column to remove background eluent ions. It is a powerful technique for determining low concentrations of ions and is especially useful in environmental and water quality studies, among other applications.

Principle



Ion Chromatogram

Ion exchange chromatography retains analyte molecules on the column based on coulombic (ionic) interactions. The stationary phase surface displays ionic functional groups (R-X) that interact with analyte ions of opposite charge. This type of chromatography is further subdivided into cation exchange chromatography and anion exchange chromatography. The ionic compound consisting of the cationic species M+ and the anionic species B- can be retained by the stationary phase.

Cation exchange chromatography retains positively charged cations because the stationary phase displays a negatively charged functional group:

$$\mathrm{R\text{-}X^-C^+ + M^+B^- \rightleftarrows R\text{-}X^-M^+ + C^+ + B^-}$$

Anion exchange chromatography retains anions using positively charged functional group:

$$\mathrm{R\text{-}X^+A^- + M^+B^- \rightleftarrows R\text{-}X^+B^- + M^+ + A^-}$$

Note that the ion strength of either C+ or A- in the mobile phase can be adjusted to shift the equilibrium position and thus retention time.

The ion chromatogram shows a typical chromatogram obtained with an anion exchange column.

Typical technique

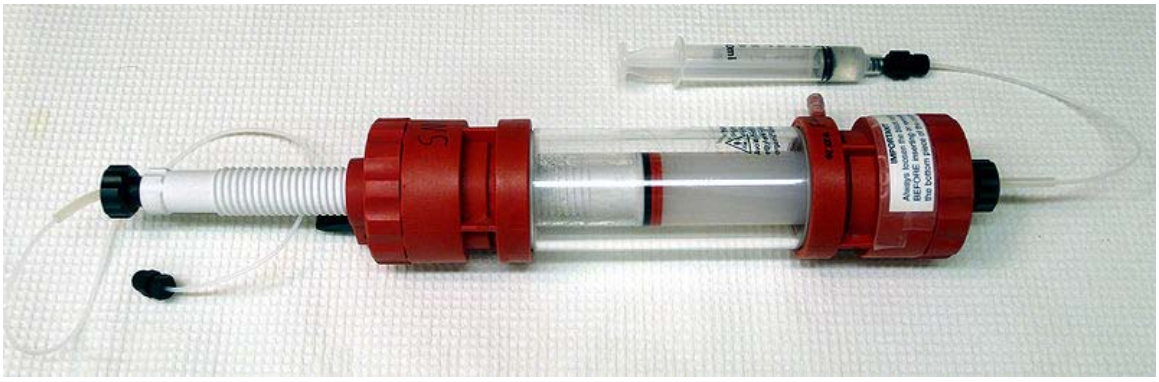
Metrohm 850 Ion chromatography system

A sample is introduced, either manually or with an autosampler, into a sample loop of known volume. A buffered aqueous solution known as the mobile phase carries the sample from the loop onto a column that contains some form of stationary phase material. This is typically a resin or gel matrix consisting of agarose or cellulose beads with covalently bonded charged functional groups. The target analytes (anions or cations) are retained on the stationary phase but can be eluted by increasing the concentration of a similarly charged species that will displace the analyte ions from the stationary phase. For example, in cation exchange chromatography, the positively charged analyte could be displaced by the addition of positively charged sodium ions. The analytes of interest must

then be detected by some means, typically by conductivity or UV/Visible light absorbance.

In order to control an IC system, a chromatography data system (CDS) is usually needed. In addition to IC systems, some of these CDSs can also control gas chromatography (GC) and HPLC

Separating proteins



Preparative-scale ion exchange column used for protein purification.

Proteins have numerous functional groups that can have both positive and negative charges. Ion exchange chromatography separates proteins according to their net charge, which is dependent on the composition of the mobile phase. By adjusting the pH or the ionic concentration of the mobile phase, various protein molecules can be separated. For example, if a protein has a net positive charge at pH 7, then it will bind to a column of negatively-charged beads, whereas a negatively charged protein would not. By changing the pH so that the net charge on the protein is negative, it too will be eluted.

Elution by changing the ionic strength of the mobile phase is a more subtle effect - it works as ions from the mobile phase will interact with the immobilized ions in preference over those on the stationary phase. This "shields" the stationary phase from the protein, (and vice versa) and allows the protein to elute.

Affinity chromatography

Affinity chromatography is a method of separating biochemical mixtures and based on a highly specific biological interaction such as that between antigen and antibody, enzyme and substrate, or receptor and ligand. Affinity chromatography combines the size fractionation capability of gel permeation chromatography with the ability to design a chromatography that reversibly binds to a known subset of molecules. The method was discovered and developed by Cuatrecasas P, Wilchek M,and Meir Wilchek for which the Wolf Prize in Medicine was awarded in 1987. Due to its interdisciplinary nature, affinity

chromatography has been the means by which many scientists from different disciplines have been introduced into the fields of modern biology.

Uses

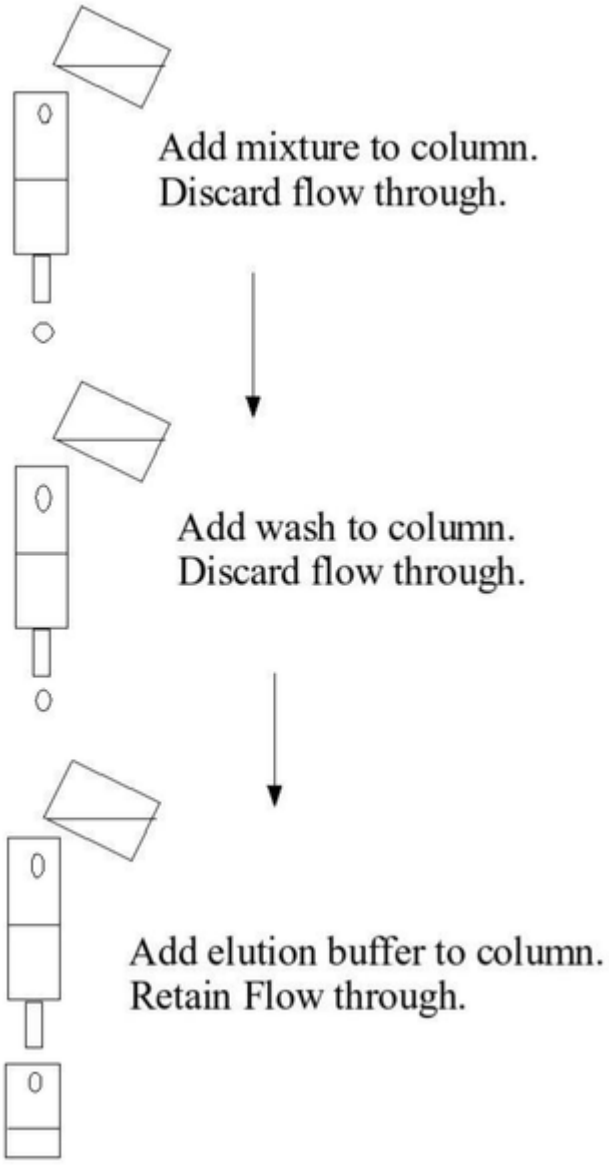
Affinity chromatography can be used to:

- Purify and concentrate a substance from a mixture into a buffering solution
- Reduce the amount of a substance in a mixture
- Discern what biological compounds bind to a particular substance, such as drugs
- Purify and concentrate an enzyme solution.

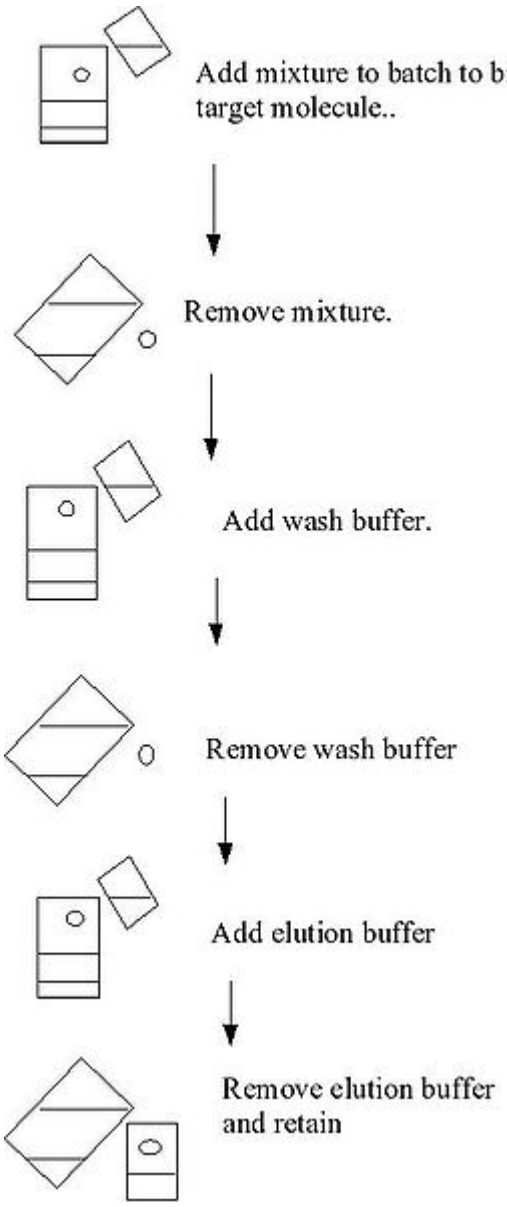
Principle

The immobile phase is typically a gel matrix, often of agarose; a linear sugar molecule derived from algae. Usually the starting point is an undefined heterogeneous group of molecules in solution, such as a cell lysate, growth medium or blood serum. The molecule of interest will have a well known and defined property which can be exploited during the affinity purification process. The process itself can be thought of as an entrapment, with the target molecule becoming trapped on a solid or stationary phase or medium. The other molecules in solution will not become trapped as they do not possess this property. The solid medium can then be removed from the mixture, washed and the target molecule released from the entrapment in a process known as elution. Possibly the most common use of affinity chromotography is for the purification of recombinant proteins.

Batch and column setup



Column chromatography



Batch chromatography

Binding to the solid phase may be achieved by column chromatography whereby the solid medium is packed onto a column, the initial mixture run through the column to allow setting, a wash buffer run through the column and the elution buffer subsequently applied to the column and collected. These steps are usually done at ambient pressure. Alternatively binding may be achieved using a batch treatment, by adding the initial mixture to the solid phase in a vessel, mixing, separating the solid phase (for example), removing the liquid phase, washing, re-centrifuging, adding the elution buffer, re-centrifuging and removing the eluate.

Sometimes a hybrid method is employed, the binding is done by the batch method, then the solid phase with the target molecule bound is packed onto a column and washing and elution are done on the column.

A third method,expanded bed adsorption, which combines the advantages of the two methods mentioned above, has also been developed. The solid phase particles are placed in a column where liquid phase is pumped in from the bottom and exits at the top. The gravity of the particles ensure that the solid phase does not exit the column with the liquid phase.

Specific uses

Affinity chromatography can be used in a number of applications, including nucleic acid purification, protein purification from cell free extracts, and purification from blood.

Immunoaffinity

Another use for the procedure is the affinity purification of antibodies from blood serum. If serum is known to contain antibodies against a specific antigen (for example if the serum comes from an organism immunized against the antigen concerned) then it can be used for the affinity purification of that antigen. This is also known as Immunoaffinity Chromatography. For example if an organism is immunised against a GST-fusion protein it will produce antibodies against the fusion-protein, and possibly antibodies against the GST tag as well. The protein can then be covalently coupled to a solid support such as agarose and used as an affinity ligand in purifications of antibody from immune serum.

For thoroughness the GST protein and the GST-fusion protein can each be coupled separately. The serum is initially allowed to bind to the GST affinity matrix. This will remove antibodies against the GST part of the fusion protein. The serum is then separated from the solid support and allowed to bind to the GST-fusion protein matrix. This allows any antibodies that recognize the antigen to be captured on the solid support. Elution of the antibodies of interest is most often achieved using a low pH buffer such as glycine pH 2.8. The eluate is collected into a neutral tris or phosphate buffer, to neutralize the low pH elution buffer and halt any degradation of the antibody's activity. This is a nice example as affinity purification is used to purify the initial GST-fusion protein, to remove the undesirable anti-GST antibodies from the serum and to purify the target antibody.

A simplified strategy is often employed to purify antibodies generated against peptide antigens. When the peptide antigens are produced synthetically, a terminal cysteine residue is added at either the N- or C-terminus of the peptide. This cysteine residue contains a sulfhydryl functional group which allows the peptide to be easily conjugated to a carrier protein (e.g.Keyhole Limpet Hemocyanin (KLH)). The same cysteine-containing peptide is also immobilized onto an agarose resin through the cysteine residue and is then used to purify the antibody.

Immobilized metal ion affinity chromatography

Immobilized metal ion affinity chromatography (IMAC) is based on the specific coordinate covalent bond of amino acids, particularly histidine, to metals. This technique works by allowing proteins with an affinity for metal ions to be retained in a column containing immobilized metal ions, such as cobalt, nickel, copper for the purification of histidine containing proteins or peptides, iron,zinc or gallium for the purification of phosphorylated proteins or peptides. Many naturally occurring proteins do not have an affinity for metal ions, therefore recombinant DNA technology can be used to introduce such a protein tag into the relevant gene. Methods used to elute the protein of interest include changing the pH, or adding a competitive molecule, such as imidazole.

Recombinant proteins

Possibly the most common use of affinity chromatography is for the purification of recombinant proteins. Proteins with a known affinity are protein tagged in order to aid their purification. The protein may have been genetically modified so as to allow it to be selected for affinity binding, this is known as a fusion protein. Tags include glutathione-S-transferase, hexahistidine (his), and maltose binding protein (MBP). His tags have an affinity for nickel or cobalt ions which are coordinate covalent bond with a chelator for the purposes of solid medium entrapment. For elution, an excess amount of a compound able to act as a metal ion ligand, such as imidazole, is used. GST has an affinity for glutathione which is commercially available immobilized as glutathione agarose. During elution, excess glutathione is used to displace the tagged protein.

Lectins

Lectin affinity chromatography is a form of affinity chromatography where lectins are used to separate components within the sample. Lectins, such as Concanavalin A are proteins which can bind specific carbohydrate (sugar) molecules. The most common application is to separate proteins based on their Glycosylation groups.

Chapter- 7

Liquid-Liquid Extraction

Liquid-liquid extraction, also known as **solvent extraction** and **partitioning**, is a method to separate compounds based on their relative solubilities in two different immiscible liquids, usually water and an organic solvent. It is an extraction of a substance from one liquid phase into another liquid phase. Liquid-liquid extraction is a basic technique in chemical laboratories, where it is performed using a separatory funnel. This type of process is commonly performed after a chemical reaction as part of the work-up.

The term *partitioning* is commonly used to refer to the underlying chemical and physical processes involved in *liquid-liquid extraction* but may be fully synonymous. The term *solvent extraction* can also refer to the separation of a substance from a mixture by preferentially dissolving that substance in a suitable solvent. In that case, a soluble compound is separated from an insoluble compound or a complex matrix.

Solvent extraction is used in nuclear reprocessing, ore processing, the production of fine organic compounds, the processing of perfumes, the production of vegetable oils and biodiesel, and other industries.

Liquid-liquid extraction is possible in non-aqueous systems: In a system consisting of a molten metal in contact with molten salt, metals can be extracted from one phase to the other. This is related to a mercury electrode where a metal can be reduced, the metal will often then dissolve in the mercury to form an amalgam that modifies its electrochemistry greatly. For example, it is possible for sodium cations to be reduced at a mercury cathode to form sodium amalgam, while at an inert electrode (such as platinum) the sodium cations are not reduced. Instead, water is reduced to hydrogen. A detergent or fine solid can be used to stabilize an emulsion, or third phase.

Measures of effectiveness

Distribution ratio

In solvent extraction, a distribution ratio is often quoted as a measure of how well-extracted a species is. The distribution ratio (D) is equal to the concentration of a solute

in the organic phase divided by its concentration in the aqueous phase. Depending on the system, the distribution ratio can be a function of temperature, the concentration of chemical species in the system, and a large number of other parameters.

Note that D is related to the ΔG of the extraction process.

Sometimes, the distribution ratio is referred to as the partition coefficient, which is often expressed as the logarithm. Note that a distribution ratio for uranium and neptunium between two inorganic solids (zirconolite and perovskite) has been reported. In solvent extraction, two immiscible liquids are shaken together. The more polar solutes dissolve preferentially in the more polar solvent, and the less polar solutes in the less polar solvent. In this experiment, the nonpolar halogens preferentially dissolve in the nonpolar mineral oil.

Separation factors

The separation factor is one distribution ratio divided by another; it is a measure of the ability of the system to separate two solutes. For instance, if the distribution ratio for nickel (D_{Ni}) is 10 and the distribution ratio for silver (D_{Ag}) is 100, then the silver/nickel separation factor ($SF_{Ag/Ni}$) is equal to $D_{Ag}/D_{Ni} = SF_{Ag/Ni} = 10$.

Decontamination factor

This is used to express the ability of a process to remove a contaminant from a product. For instance, if a process is fed with a mixture of 1:9 cadmium to indium, and the product is a 1:99 mixture of cadmium and indium, then the decontamination factor (for the removal of cadmium) of the process is 0.1 / 0.01 = 10.

Slopes of graphs

The easy way to work out the extraction mechanism is to draw graphs and measure the slopes. If for an extraction system the D value is proportional to the square of the concentration of a reagent (Z) then the slope of the graph of $\log_{10}(D)$ against $\log_{10}([[Z]])$ will be two.

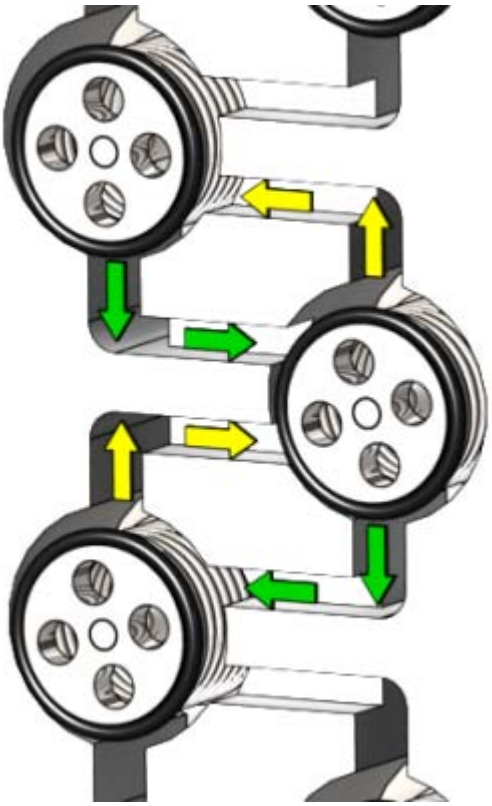
Techniques

Batchwise single stage extractions

This is commonly used on the small scale in chemical labs. It is normal to use a separating funnel. For instance, if a chemist were to extract anisole from a mixture of water and 5% acetic acid using ether, then the anisole will enter the organic phase. The two phases would then be separated.

The acetic acid can then be scrubbed (removed) from the organic phase by shaking the organic extract with sodium bicarbonate. The acetic acid reacts with the sodium bicarbonate to form sodium acetate, carbon dioxide, and water.

Multistage countercurrent continuous processes



Coflore Continuous Counter Current Extractor

These are commonly used in industry for the processing of metals such as the lanthanides; because the separation factors between the lanthanides are so small many extraction stages are needed. In the multistage processes, the aqueous raffinate from one extraction unit is fed to the next unit as the aqueous feed, while the organic phase is moved in the opposite direction. Hence, in this way, even if the separation between two metals in each stage is small, the overall system can have a higher decontamination factor.

Multistage countercurrent arrays have been used for the separation of lanthanides. For the design of a good process, the distribution ratio should be not too high (>100) or too low (<0.1) in the extraction portion of the process. It is often the case that the process will have a section for scrubbing unwanted metals from the organic phase, and finally a stripping section to obtain the metal back from the organic phase.

Multistage Podbielniak contactor centrifuges produce three to five stages of theoretical extraction in a single countercurrent pass, and are used in fermentation-based pharmaceutical and food additive production facilities.

Extraction without chemical change

Some solutes such as noble gases can be extracted from one phase to another without the need for a chemical reaction. This is the simplest type of solvent extraction. When a solvent is extracted, two immiscible liquids are shaken together. The more polar solutes dissolve preferentially in the more polar solvent, and the less polar solutes in the less polar solvent. Some solutes that do not at first sight appear to undergo a reaction during the extraction process do not have distribution ratio that is independent of concentration. A classic example is the extraction of carboxylic acids (**HA**) into nonpolar media such as benzene. Here, it is often the case that the carboxylic acid will form a dimer in the organic layer so the distribution ratio will change as a function of the acid concentration (measured in either phase).

For this case, the extraction constant k is described by $k = [[\mathbf{HA}_{\text{organic}}]]^2/[[\mathbf{HA}_{\text{aqueous}}]]$

Solvation mechanism

Using solvent extraction it is possible to extract uranium, plutonium, or thorium from acid solutions. One solvent used for this purpose is the organophosphate tri-n-butyl phosphate. The PUREX process that is commonly used in nuclear reprocessing uses a mixture of tri-n-butyl phosphate and an inert hydrocarbon (kerosene), the uranium(VI) are extracted from strong nitric acid and are back-extracted (stripped) using weak nitric acid. An organic soluble uranium complex $[UO_2(TBP)_2(NO_3)_2]$ is formed, then the organic layer bearing the uranium is brought into contact with a dilute nitric acid solution; the equilibrium is shifted away from the organic soluble uranium complex and towards the free TBP and uranyl nitrate in dilute nitric acid. The plutonium(IV) forms a similar complex to the uranium(VI), but it is possible to strip the plutonium in more than one way; a reducing agent that converts the plutonium to the trivalent oxidation state can be added. This oxidation state does not form a stable complex with TBP and nitrate unless the nitrate concentration is very high (circa 10 mol/L nitrate is required in the aqueous phase). Another method is to simply use dilute nitric acid as a stripping agent for the plutonium. This PUREX chemistry is a classic example of a solvation extraction.

Here in this case $D_U = k\ TBP^2[[NO_3]]^2$

Ion exchange mechanism

Another extraction mechanism is known as the ion exchange mechanism. Here, when an ion is transferred from the aqueous phase to the organic phase, another ion is transferred in the other direction to maintain the charge balance. This additional ion is often a hydrogen ion; for ion exchange mechanisms, the distribution ratio is often a function of

pH. An example of an ion exchange extraction would be the extraction of americium by a combination of terpyridine and a carboxylic acid in *tert*-butyl benzene. In this case

$D_{Am} = k$ terpyridine^{1}carboxylic acid^{3}H+$^{-3}$

Another example is the extraction of zinc, cadmium, or lead by a dialkyl phosphinic acid (R_2PO_2H) into a nonpolar diluent such as an alkane. A non-polar diluent favours the formation of uncharged non-polar metal complexes.

Some extraction systems are able to extract metals by both the solvation and ion exchange mechanisms; an example of such a system is the americium (and lanthanide) extraction from nitric acid by a combination of 6,6'-*bis*-(5,6-dipentyl-1,2,4-triazin-3-yl)-2,2'-bipyridine and 2-bromohexanoic acid in *tert*-butyl benzene. At both high- and low-nitric acid concentrations, the metal distribution ratio is higher than it is for an intermidate nitric acid concentration.

Ion pair extraction

It is possible by careful choice of counterion to extract a metal. For instance, if the nitrate concentration is high, it is possible to extract americium as an anionic nitrate complex if the mixture contains a lipophilic quaternary ammonium salt.

An example that is more likely to be encountered by the *'average'* chemist is the use of a phase transfer catalyst. This is a charged species that transfers another ion to the organic phase. The ion reacts and then forms another ion, which is then transferred back to the aqueous phase.

For instance, the 31.1 kJ mol^{-1} is required to transfer an acetate anion into nitrobenzene, while the energy required to transfer a chloride anion from an aqueous phase to nitrobenzene is 43.8 kJ mol^{-1}. Hence, if the aqueous phase in a reaction is a solution of sodium acetate while the organic phase is a nitrobenzene solution of benzyl chloride, then, when a phase transfer catalyst, the acetate anions can be transferred from the aqueous layer where they react with the benzyl chloride to form benzyl acetate and a chloride anion. The chloride anion is then transferred to the aqueous phase. The transfer energies of the anions contribute to that given out by the reaction.

A 43.8 to 31.1 kJ mol^{-1} = 12.7 kJ mol^{-1} of additional energy is given out by the reaction when compared with energy if the reaction had been done in nitrobenzene using one equivalent weight of a tetraalkylammonium acetate.

Aqueous two-phase extraction

Using an aqueous two-phase system, it is possible to generate two immiscible water phases. This can then be used to extract proteins, which would denature if exposed to organic solvents.

Kinetics of extraction

It is important to investigate the rate at which the solute is transferred between the two phases, in some cases by an alteration of the contact time it is possible to alter the selectivity of the extraction. For instance, the extraction of palladium or nickel can be very slow because the rate of ligand exchange at these metal centers is much lower than the rates for iron or silver complexes.

Aqueous complexing agents

If a complexing agent is present in the aqueous phase then it can lower the distribution ratio. For instance, in the case of iodine being distributed between water and an inert organic solvent such as carbon tetrachloride then the presence of iodide in the aqueous phase can alter the extraction chemistry.

Instead of D_{I+2} being a constant it becomes $D_{I+2} = k[[I_{2\cdot Organic}]]/[I_{2\cdot Aqueous}]\ [[I^-_{\cdot Aqueous}]]$

This is because the iodine reacts with the iodide to form I_3^-. The I_3^- anion is an example of a polyhalide anion that is quite common.

Industrial process design

In a typical scenario, an industrial process will use an extraction step in which solutes are transferred from the aqueous phase to the organic phase; this is often followed by a scrubbing stage in which unwanted solutes are removed from the organic phase, then a stripping stage in which the wanted solutes are removed from the organic phase. The organic phase may then be treated to make it ready for use again.

After use, the organic phase may be subjected to a cleaning step to remove any degradation products; for instance, in PUREX plants, the used organic phase is washed with sodium carbonate solution to remove any dibutyl hydrogen phosphate or butyl dihydrogen phosphate that might be present.

Equipment

Two layers separating during a liquid-liquid extraction.

An organic MTBE solution is extracted with aqueous sodium bicarbonate solution. This base removes benzoic acid as benzoate but leaves non-acidic benzil (yellow) behind in the upper organic phase.

While solvent extraction is often done on a small scale by synthetic lab chemists using a separatory funnel or Craig apparatus, it is normally done on the industrial scale using machines that bring the two liquid phases into contact with each other. Such machines include centrifugal contactors, thin layer extractors, spray columns, pulsed columns, and mixer-settlers.

Extraction of metals

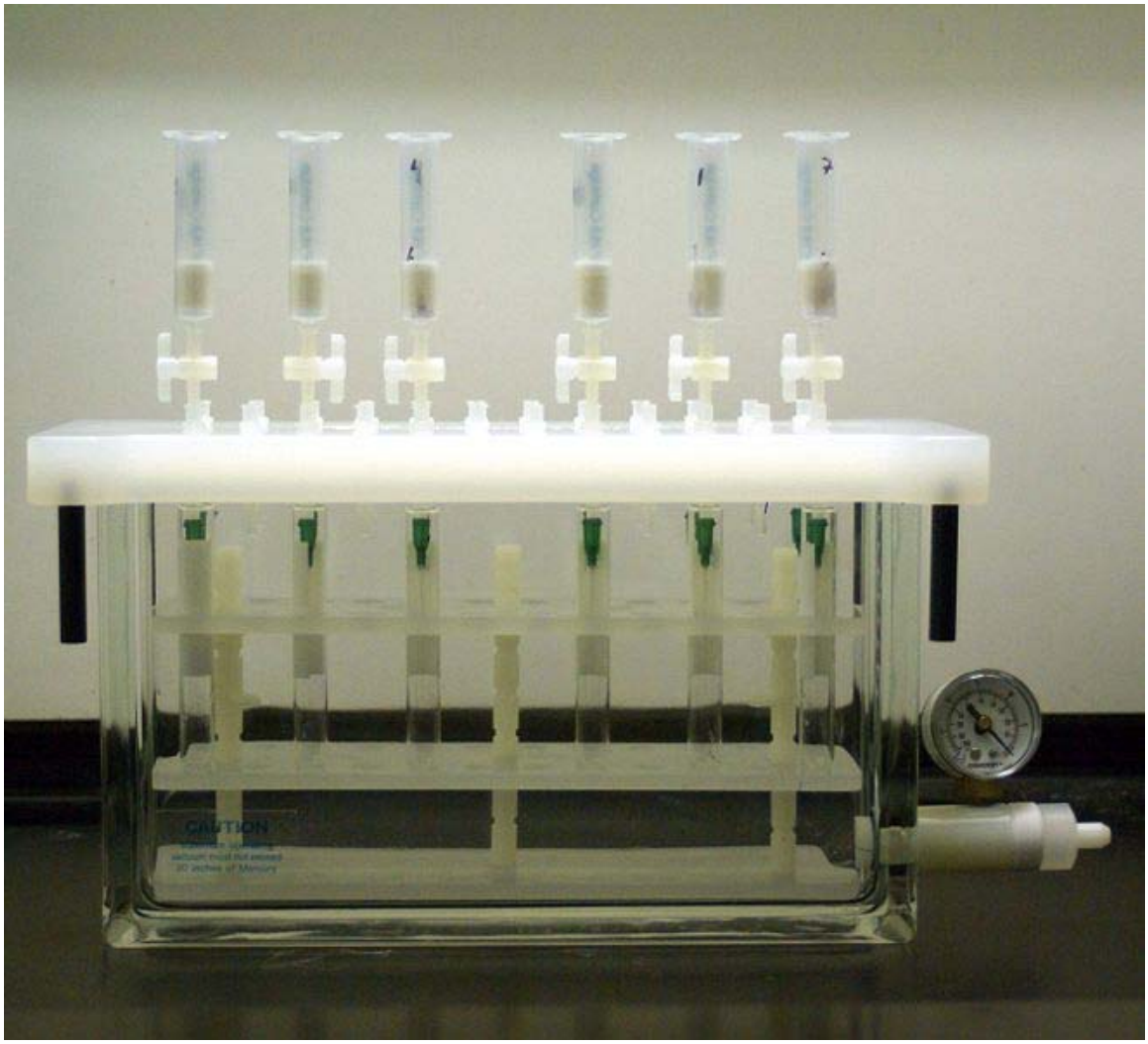
The extraction methods for a range of metals include:

- Cobalt - The extraction of cobalt from hydrochloric acid using alamine 336 in *meta*-xylene. Cobalt can be extracted also using Cyanex 272 {*bis*-(2,4,4-trimethylpentyl) phosphinic acid}.
- Copper - Copper can be extracted using hydroxyoximes as extractants, a recent paper describes an extractant that has a good selectivity for copper over cobalt and nickel.
- Neodymium - This rare earth is extracted by di(2-ethyl-hexyl)phosphoric acid into hexane by an ion exchange mechanism.
- Nickel - Nickel can be extracted using di(2-ethyl-hexyl)phosphoric acid and tributyl phosphate in a hydrocarbon diluent (Shellsol).
- Palladium and platinum - Dialkyl sulfides, tributyl phosphate and alkyl amines have been used for extracting these metals.

- Zinc and cadmium - The zinc and cadmium are both extracted by an ion exchange process, the *N,N,N′,N′*-tetrakis(2-pyridylmethyl)ethylenediamine (TPEN) acts as a masking agent for the zinc and an extractant for the cadmium. In the modified Zincex process, zinc is separated from most divalent ions by solvent extraction. D2EHPA (Di (2) ethyl hexyl phosphoric acid) is used for this. A zinc ion replaces the proton from two D2EHPA molecules. To strip the zinc from the D2EHPA, sulfuric acid is used, at a concentration of above 170g/l (typically 240-265g/l).

Chapter- 8

Solid Phase Extraction



A typical solid phase extraction manifold. The cartridges drip into the chamber below, where tubes collect the effluent. A vacuum port with gauge is used to control the vacuum applied to the chamber.

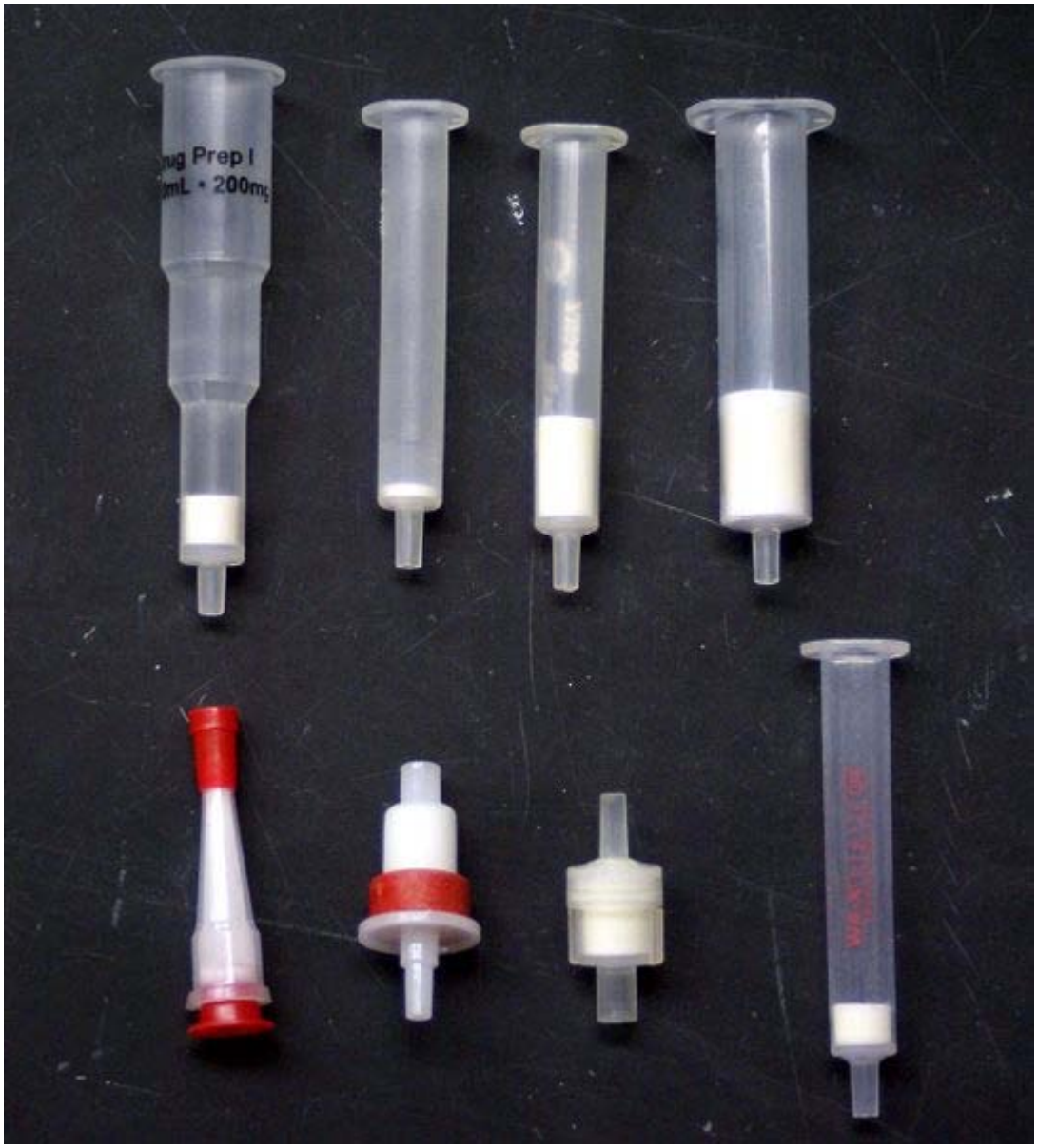
Solid-phase extraction (**SPE**) is a separation process by which compounds that are dissolved or suspended in a liquid mixture are separated from other compounds in the mixture according to their physical and chemical properties. Analytical laboratories use solid phase extraction to concentrate and purify samples for analysis. Solid phase extraction can be used to isolate analytes of interest from a wide variety of matrices, including urine, blood, water, beverages, soil, and animal tissue.

SPE uses the affinity of solutes dissolved or suspended in a liquid (known as the mobile phase) for a solid through which the sample is passed (known as the stationary phase) to separate a mixture into desired and undesired components. The result is that either the desired analytes of interest or undesired impurities in the sample are retained on the stationary phase. The portion that passes through the stationary phase is collected or discarded, depending on whether it contains the desired analytes or undesired impurities. If the portion retained on the stationary phase includes the desired analytes, they can then be removed from the stationary phase for collection in an additional step, in which the stationary phase is rinsed with an appropriate eluent.

The stationary phase comes in the form of a packed syringe-shaped cartridge, a 96 well plate or a 47- or 90-mm flat disk, each of which can be mounted on its specific type of extraction manifold. The manifold allows multiple samples to be processed by holding several SPE media in place and allowing for an equal number of samples to pass through them simultaneously. A typical cartridge SPE manifold can accommodate up to 24 cartridges, while a typical disk SPE manifold can accommodate 6 disks. Most SPE manifolds are equipped with a vacuum port. Application of vacuum speeds up the extraction process by pulling the liquid sample through the stationary phase. The analytes are collected in sample tubes inside or below the manifold after they pass through the stationary phase.

Solid phase extraction cartridges and disks are available with a variety of stationary phases, each of which can separate analytes according to different chemical properties. Most stationary phases are based on silica that has been bonded to a specific functional group. Some of these functional groups include hydrocarbon chains of variable length (for reversed phase SPE), quaternary ammonium or amino groups (for anion exchange), and sulfonic acid or carboxyl groups (for cation exchange).

Normal Phase SPE procedure



A selection of solid phase extraction cartridges, available in many sizes, shapes, and types of stationary phase.

A typical solid phase extraction involves four basic steps. First, the cartridge is equilibrated with a non-polar solvent or slightly polar, which wets the surface and penetrates the bonded phase. Then water, or buffer of the same composition as the sample, is typically washed through the column to wet the silica surface. The sample is then added to the cartridge. As the sample passes through the stationary phase, the analytes in the sample will interact and retain on the sorbent while the solvent, salts, and other impurities pass through the cartridge. After the sample is loaded, the cartridge is washed with buffer or solvent to remove further impurities. Then, the analyte is eluted with a non-polar solvent or a buffer of the appropriate pH.

A stationary phase of polar functionally bonded silicas with short carbons chains frequently makes up the solid phase. This stationary phase will adsorb polar molecules which can be collected with a more polar solvent.

Reversed phase SPE

Reversed phase SPE separates analytes based on their polarity. The stationary phase of a reversed phase SPE cartridge is derivatized with hydrocarbon chains, which retain compounds of mid to low polarity due to the hydrophobic effect. The analyte can be eluted by washing the cartridge with a non-polar solvent, which disrupts the interaction of the analyte and the stationary phase.

A stationary phase of silicon with carbon chains is commonly used. Relying on mainly non-polar, hydrophobic interactions, only non-polar or very weakly polar compounds will adsorb to the surface.

Ion exchange SPE

Ion exchange sorbents separate analytes based on electrostatic interactions between the analyte of interest and the positively charged groups on the stationary phase. For ion exchange to occur, both the stationary phase and sample must be at a pH where both are charged.

Anion exchange

Anion exchange sorbents are derivatized with positively charged functional groups that interact and retain negatively charged anions, such as acids. Strong anion exchange sorbents contain quaternary ammonium groups that have a permanent positive charge in aqueous solutions, and weak anion exchange sorbents use amine groups which are charged when the pH is below about 9. Strong anion exchange sorbents are useful because any strongly acidic impurities in the sample will bind to the sorbent and usually will not be eluted with the analyte of interest; to recover a strong acid a weak anion exchange cartridge should be used. To elute the analyte from either the strong or weak sorbent, the stationary phase is washed with a solvent that neutralizes the charge of either the analyte, the stationary phase, or both. Once the charge is neutralized, the electrostatic interaction between the analyte and the stationary phase no longer exists and the analyte will elute from the cartridge.

Cation Exchange

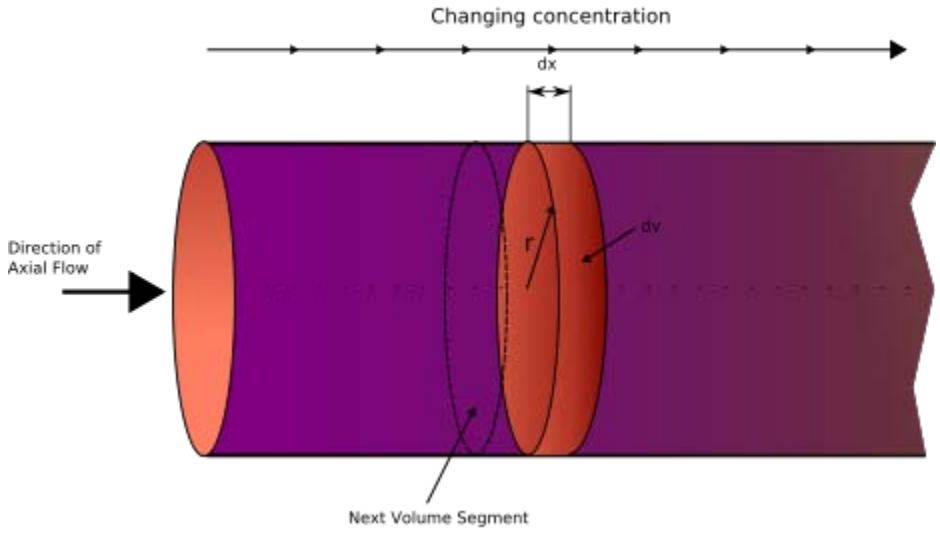
Cation exchange sorbents are derivatized with functional groups that interact and retain positively charged cations, such as bases. Strong cation exchange sorbents contain aliphatic sulfonic acid groups that are always negatively charged in aqueous solution, and weak cation exchange sorbents contain aliphatic carboxylic acids, which are charged when the pH is above about 5. Strong cation exchange sorbents are useful because any

strongly basic impurities in the sample will bind to the sorbent and usually will not be eluted with the analyte of interest; to recover a strong base a weak cation exchange cartridge should be used. To elute the analyte from either the strong or weak sorbent, the stationary phase is washed with a solvent that neutralizes ionic interaction between the analyte and the stationary phase.

Chapter- 9

Plug Flow Reactor Model

The **plug flow reactor** (**PFR**) model is used to describe chemical reactions in continuous, flowing systems. The PFR model is used to predict the behaviour of chemical reactors, so that key reactor variables, such as the dimensions of the reactor, can be estimated. PFR's are also sometimes called Continuous Tubular Reactors (CTR's).



Schematic diagram of a Plug Flow Reactor (PFR)

Fluid going through a PFR may be modeled as flowing through the reactor as a series of infinitely thin coherent "plugs", each with a uniform composition, traveling in the axial direction of the reactor, with each plug having a different composition from the ones before and after it. The key assumption is that as a plug flows through a PFR, the fluid is perfectly mixed in the radial direction but not in the axial direction (forwards or backwards). Each plug of differential volume is considered as a separate entity, effectively an infinitesimally small batch reactor, limiting to zero volume. As it flows down the tubular PFR, the residence time (τ) of the plug is a function of its position in the

reactor. In the ideal PFR, the residence time distribution is therefore a Dirac delta function with a value equal to τ.

PFR modeling

PFRs are frequently referred to as piston flow reactors, or sometimes as continuous tubular reactors. They are governed by ordinary differential equations, the solution for which can be calculated providing that appropriate boundary conditions are known.

The PFR model works well for many fluids: liquids, gases, and slurries. Although turbulent flow and axial diffusion cause a degree of mixing in the axial direction in real reactors, the PFR model is appropriate when these effects are sufficiently small that they can be ignored.

In the simplest case of a PFR model, several key assumptions must be made in order to simplify the problem, some of which are outlined below. Note that not all of these assumptions are necessary, however the removal of these assumptions does increase the complexity of the problem. The PFR model can be used to model multiple reactions as well as reactions involving changing temperatures, pressures and densities of the flow. Although these complications are ignored in what follows, they are often relevant to industrial processes.

Assumptions:

- plug flow
- steady state
- constant density (reasonable for some liquids but a 20% error for polymerizations; valid for gases only if there is no pressure drop, no net change in the number of moles, nor any large temperature change)
- constant tube diameter
- single reaction

A material balance on the differential volume of a fluid element, or plug, on species *i* of axial length *dx* between *x* and *x* + *dx* gives

[accumulation] = [in] - [out] + [generation] - [consumption]

1. $F_i(x) - F_i(x + dx) + A_t dx \nu_i r = 0$.

When linear velocity, *u*, and molar flow rate relationships, F_i, $u = \frac{\dot{v}}{A_t} = \frac{4\dot{v}}{\pi D^2}$ and $F_i = A_t u C_i$, are applied to Equation 1 the mass balance on *i* becomes

2. $A_t u[C_i(x) - C_i(x + dx)] + A_t dx \nu_i r = 0$.

When like terms are canceled and the limit $dx \to 0$ is applied to Equation 2 the mass balance on species *i* becomes

3. $$u\frac{dC_i}{dx} = \nu_i r,$$

where $C_i(x)$ is the molar concentration of species *i* at position *x*, A_t the cross-sectional area of the tubular reactor, *dx* the differential thickness of fluid plug, and ν_i stoichiometric coefficient. The reaction rate, *r*, can be figured by using the Arrhenius temperature dependence. Generally, as the temperature increases so does the rate at which the reaction occurs. Residence time, τ, is the average amount of time a discrete quantity of reagent spends inside the tank.

Assume:

- isothermal conditions, or constant temperature (k is constant)
- single, irreversible reaction ($\nu_A = -1$)
- first-order reaction ($r = kC_A$)

After integration of Equation 3 using the above assumptions, solving for $C_A(L)$ we get an explicit equation for the output concentration of species *A*,

4. $$C_A(V) = C_{Ao}e^{-k\tau},$$

where C_{Ao} is the inlet concentration of species *A*.

Operation and uses

PFRs are used to model the chemical transformation of compounds as they are transported in systems resembling "pipes". The "pipe" can represent a variety of engineered or natural conduits through which liquids or gases flow. (e.g. rivers, pipelines, regions between two mountains, etc.)

An ideal plug flow reactor has a fixed residence time: Any fluid (plug) that enters the reactor at time *t* will exit the reactor at time $t + \tau$, where τ is the residence time of the reactor. The residence time distribution function is therefore a dirac delta function at τ. A real plug flow reactor has a residence time distribution that is a narrow pulse around the mean residence time distribution.

A typical plug flow reactor could be a tube packed with some solid material (frequently a catalyst). Typically these types of reactors are called packed bed reactors or PBR's. Sometimes the tube will be a tube in a shell and tube heat exchanger.

Advantages and disadvantages

CSTRs (Continuous Stirred Tank Reactor) and PFRs have fundamentally different equations, so the kinetics of the reaction being undertaken will to some extent determine which system should be used. However there are a few general comments that can be made with regards to PFRs compared to other reactor types.

Plug flow reactors have a high volumetric unit conversion, run for long periods of time without maintenance, and the heat transfer rate can be optimized by using more, thinner tubes or fewer, thicker tubes in parallel. Disadvantages of plug flow reactors are that temperatures are hard to control and can result in undesirable temperature gradients. PFR maintenance is also more expensive than CSTR maintenance.

Through a recycle loop a PFR is able to approximate a CSTR in operation. This occurs due to a decrease in the concentration change due to the smaller fraction of the flow determined by the feed; in the limiting case of total recycling, infinite recycle ratio, the PFR perfectly mimics a CSTR.

Applications

Plug flow reactors are used for some of the following applications:

- Large-scale reactions
- Fast reactions
- Homogeneous or heterogeneous reactions
- Continuous production
- High-temperature reactions

Chapter- 10

Artificial Membrane

An **artificial membrane**, or **synthetic membrane**, is a synthetically created membrane which is usually intended for separation purposes in laboratory or in industry. Synthetic membranes have been successfully used for small and large-scale industrial processes since the middle of twentieth century. A wide variety of synthetic membranes is known. They can be produced from organic materials such as polymers and liquids, as well as inorganic materials. The most of commercially utilized synthetic membranes in separation industry are made of polymeric structures. They can be classified based on their surface chemistry, bulk structure, morphology, and production method. The chemical and physical properties of synthetic membranes and separated particles as well as a choice of driving force define a particular membrane separation process. The most commonly used driving forces of a membrane process in industry are pressure and concentration gradients. The respective membrane process is therefore known as filtration. Synthetic membranes utilized in a separation process can be of different geometry and the respective flow configuration. They can be also categorized based on their application and separation regime. The best known synthetic membrane separation processes include water purification, reverse osmosis, dehydrogenation of natural gas, removal of cell particles by microfiltration and ultrafiltration, removal of microorganisms from dairy products, and dialysis.

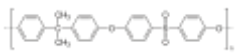
Membrane types and structure

Synthetic membrane can be fabricated from a large number of different materials. It can be made from organic or inorganic materials including solids such as metal or ceramic, homogenous films (polymers), heterogeneous solids (polymeric mixes, mixed glasses), and liquids. Ceramic membranes are produced from inorganic materials such as aluminium oxides, silicon carbide, and zirconium oxide. Ceramic membranes are very resistant to the action of aggressive media (acids, strong solvents). They are very stable chemically, thermally, mechanically, and biologically inert. Even though ceramic membranes have a high weight and substantial production costs, they are ecologically friendly and have long working life. Ceramic membranes are generally made as monolithic shapes of tubular capillaries.

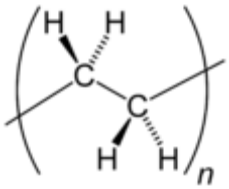
Liquid membrane refers to synthetic membranes made of non-rigid material. Several types of liquid membranes can be encountered in industry: emulsion liquid membranes,

immobilized (supported) liquid membranes, molten salts, and hollow-fiber contained liquid membranes. Liquid membranes are being extensively studied but have limited commercial applications.

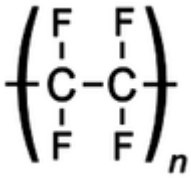
Polymeric membranes lead the membrane separation industry market because they are very competitive in performance and economics. Many polymers are available, but the choice of membrane polymer is not a trivial task. A polymer has to have appropriate characteristics for the intended application. The polymer sometimes has to offer a low binding affinity for separated molecules (as in the case of biotechnology applications) , and has to withstand the harsh cleaning conditions. It has to be compatible with chosen membrane fabrication technology. The polymer has to be a suitable membrane former in terms of its chains rigidity, chain interactions, stereoregularity, and polarity of its functional groups. The polymers can form amorphous and semicrystalline structures (can also have different glass transition temperatures), affecting the membrane performance characteristics. The polymer has to be obtainable and reasonably priced to comply with the low cost criteria of membrane separation process. Many membrane polymers are grafted, custom-modified, or produced as copolymers to improve their properties. The most common polymers in membrane synthesis are cellulose acetates, nitrates, and esters (CA, CN, and CE), polysulfone (PS), polyether sulfone(PES), polyacrilonitrile (PAN), polyamide, polyimide, polyethylene and polypropylene (PE and PP), polytetrafluoroethylene (PTFE), polyvinylidene fluoride (PVDF), polyvinylchloride (PVC).



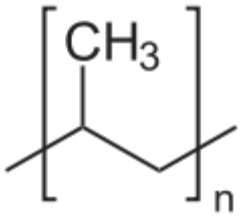
Polysulfone (PS)



Polyethylene (PE)

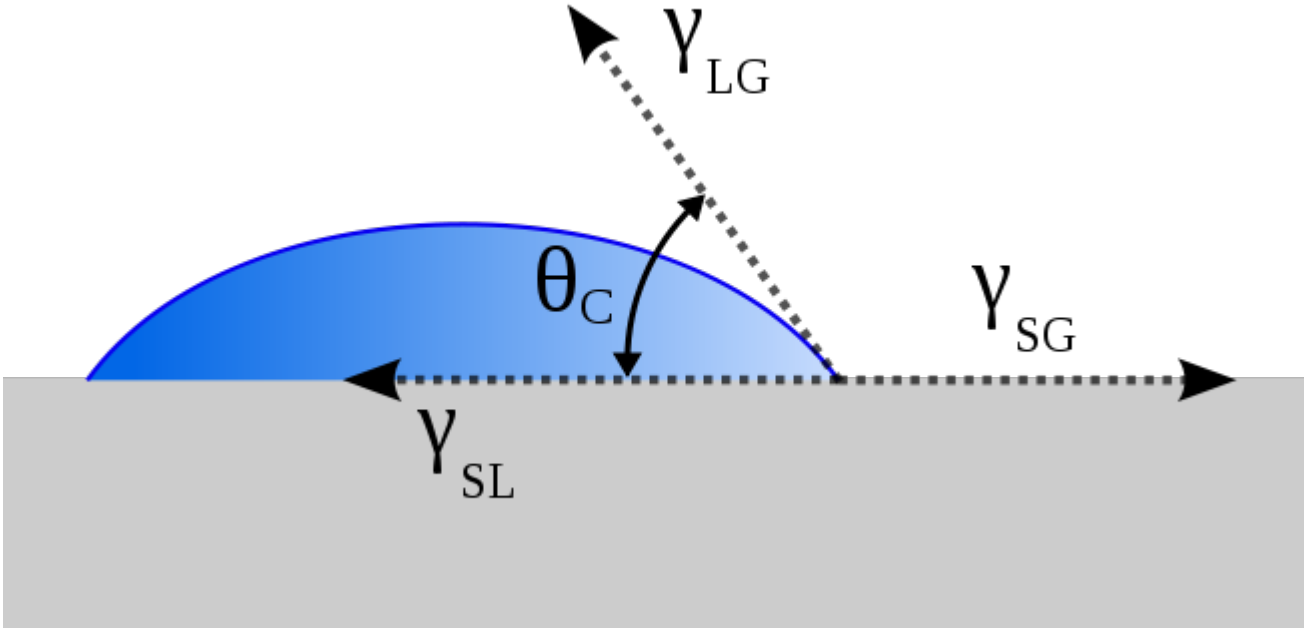


Polytetrafluoroethylene (PTFE)



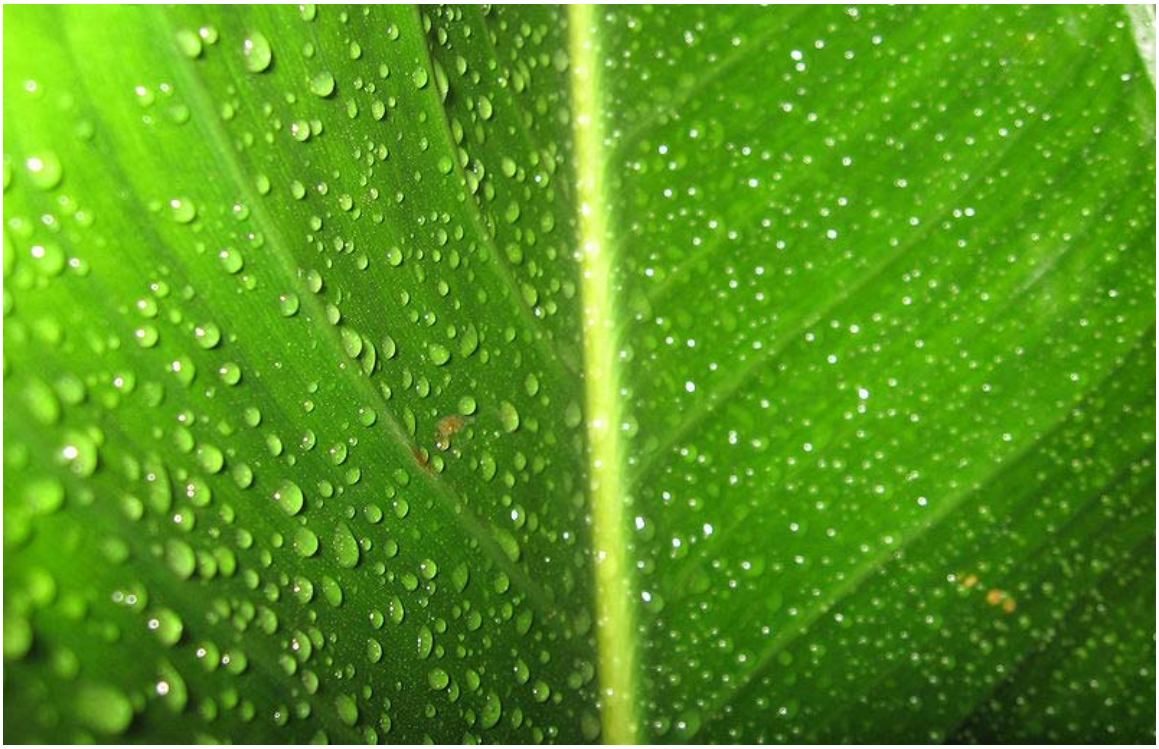
Polypropylene (PP)

Surface chemistry



Contact angle of a liquid droplet wetted to a rigid solid surface.Young's equation: $\gamma_{LG} \cdot \cos\theta + \gamma_{SL} = \gamma_{SG}$.

One of the critical characteristics of a synthetic membrane is its chemistry. Synthetic membrane chemistry usually refers to the chemical nature and composition of the surface in contact with a separation process stream. The chemical nature of a membrane's surface can be quite different from its bulk composition. This difference can result from material partitioning at some stage of the membrane's fabrication, or from an intended surface postformation modification. Membrane surface chemistry creates very important properties such as hydrophilicity or hydrophobicity (related to surface free energy), presence of ionic charge, membrane chemical or thermal resistance, binding affinity for particles in a solution, and biocompatibility (in case of bioseparations). Hydrophilicity and hydrophobicity of membrane surfaces can be expressed in terms of water (liquid) contact angle θ. Hydrophilic membrane surfaces have a contact angle in the range of $0^o<\theta<90^o$ (closer to 0^o), where hydrophobic materials have θ in the range of $90^o<\theta<180^o$.



Wetting of a leaf.

The contact angle is determined by solving the Young's equation for the interfacial force balance. At equilibrium three interfacial tensions corresponding to solid/gas (γ_{SG}), solid/liquid (γ_{SL}), and liquid/gas (γ_{LG}) interfaces are counterbalanced. The consequence of the contact angle's magnitudes is known as wetting phenomena, which is important to characterize the capillary (pore) intrusion behavior. Degree of membrane surface wetting is determined by the contact angle. The surface with smaller contact angle has better wetting properties ($\theta=0^{o}$-perfect wetting). In some cases low surface tension liquids such as alcohols or surfactant solutions are used to enhance wetting of non-wetting membrane surfaces. The membrane surface free energy (and related hydrophilicity/hydrophobicity) influences membrane particle adsorption or fouling phenomena. In most membrane separation processes (especially bioseparations), higher surface hydrophilicity corresponds to the lower fouling. Synthetic membrane fouling impairs membrane performance. As a consequence, a wide variety of membrane cleaning techniques have been developed. Sometimes fouling is irreversible, and the membrane needs to be replaced. Another feature of membrane surface chemistry is surface charge. The presence of the charge changes the properties of the membrane-liquid interface. The membrane surface may develop an electrochemical potential and induce the formation of layers of solution particles which tend to neutralize the charge.

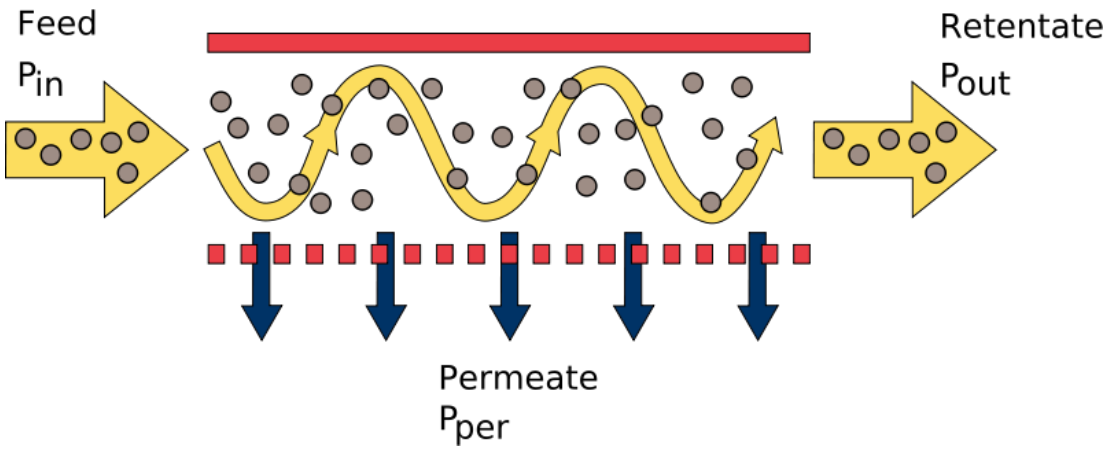
Membrane morphology

Synthetic membranes can be also categorized based on their structure (morphology). Three such types of synthetic membranes are commonly used in separation industry:

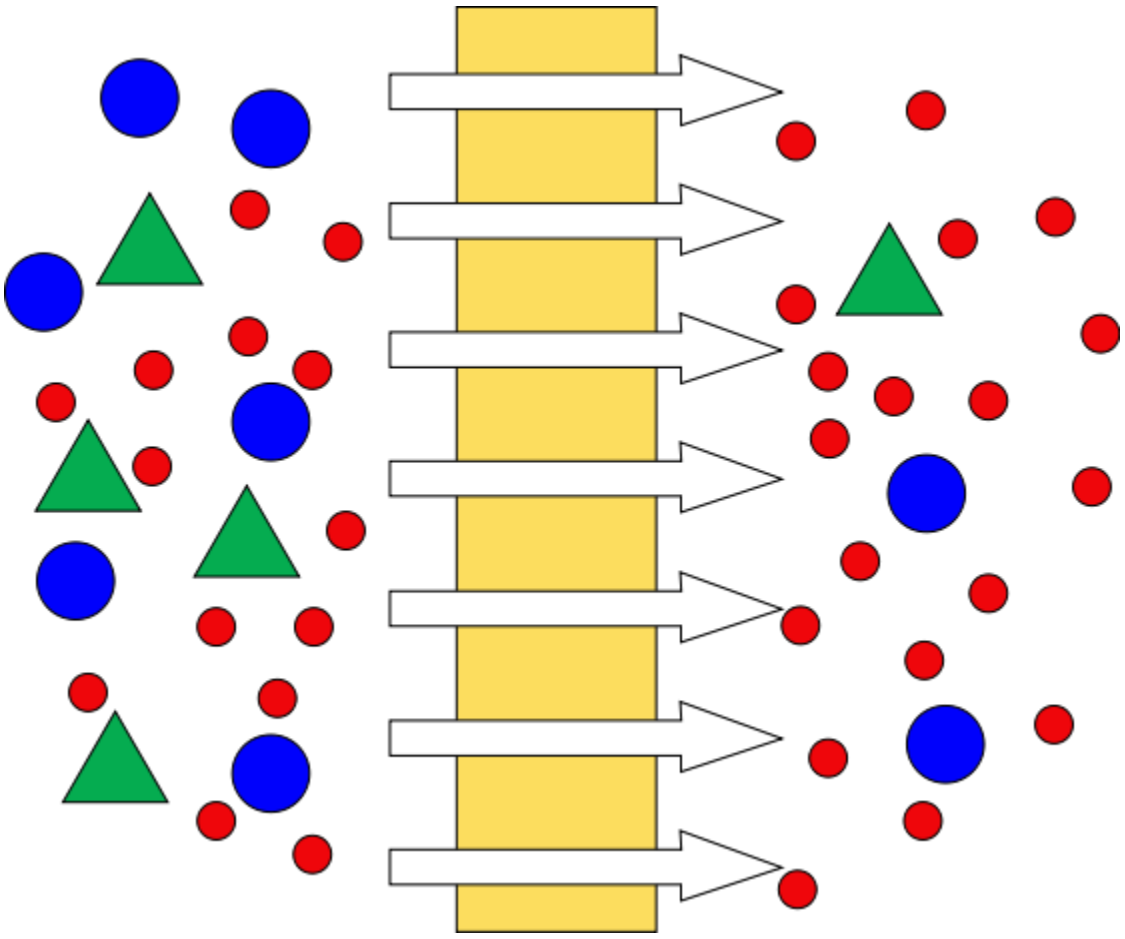
dense membranes, porous membranes, and asymmetric membranes. Dense and porous membranes are distinct from each other based on the size of separated molecules. Dense membrane is usually a thin layer of dense material utilized in the separation processes of small molecules (usually in gas or liquid phase). Dense membranes are widely used in industry for gas separations and reverse osmosis applications.

Dense membranes can be synthesized as amorphous or heterogeneous structures. Polymeric dense membranes such as polytetrafluoroethylene and cellulose esters are usually fabricated by compression molding, solvent casting, and spraying of a polymer solution. The membrane structure of a dense membrane can be in a rubbery or a glassy state at a given temperature depending on its glass transition temperature . Porous membranes are intended on separation of larger molecules such as solid colloidal particles, large biomolecules (proteins, DNA, RNA) and cells from the filtering media. Porous membranes find use in the microfiltration, ultrafiltration, and dialysis applications. There is some controversy in defining a “membrane pore”. The most commonly used theory assumes a cylindrical pore for simplicity. This model assumes that pores have the shape of parallel, nonintersecting cylindrical capillaries. But in reality a typical pore is a random network of the unevenly shaped structures of different sizes. The formation of a pore can be induced by the dissolution of a "better" solvent into a "poorer" solvent in a polymer solution. Other types of pore structure can be produced by stretching of crystalline structure polymers. The structure of porous membrane is related to the characteristics of the interacting polymer and solvent, components concentration, molecular weight, temperature, and storing time in solution. The thicker porous membranes sometimes provide support for the thin dense membrane layers, forming the asymmetric membrane structures. The latter are usually produced by a lamination of dense and porous membranes.

Membrane shapes and flow geometries



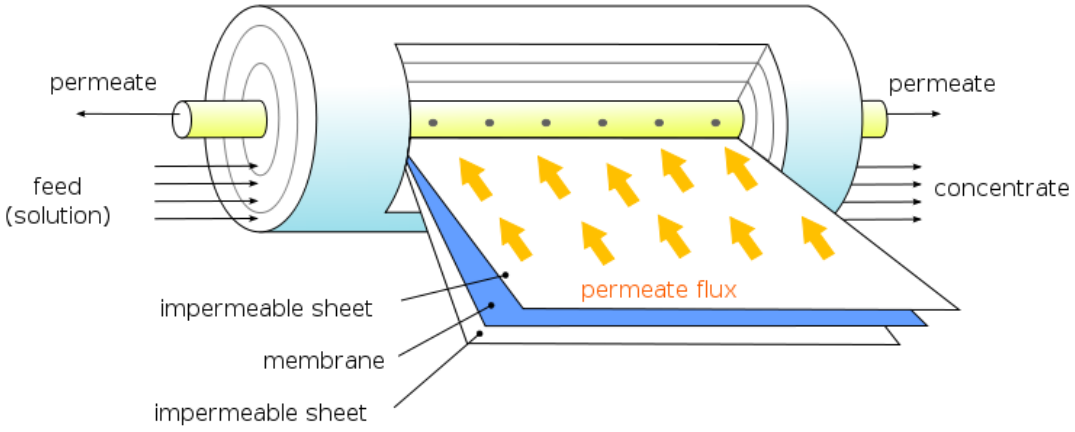
Cross-flow geometry.



Dead-end geometry.

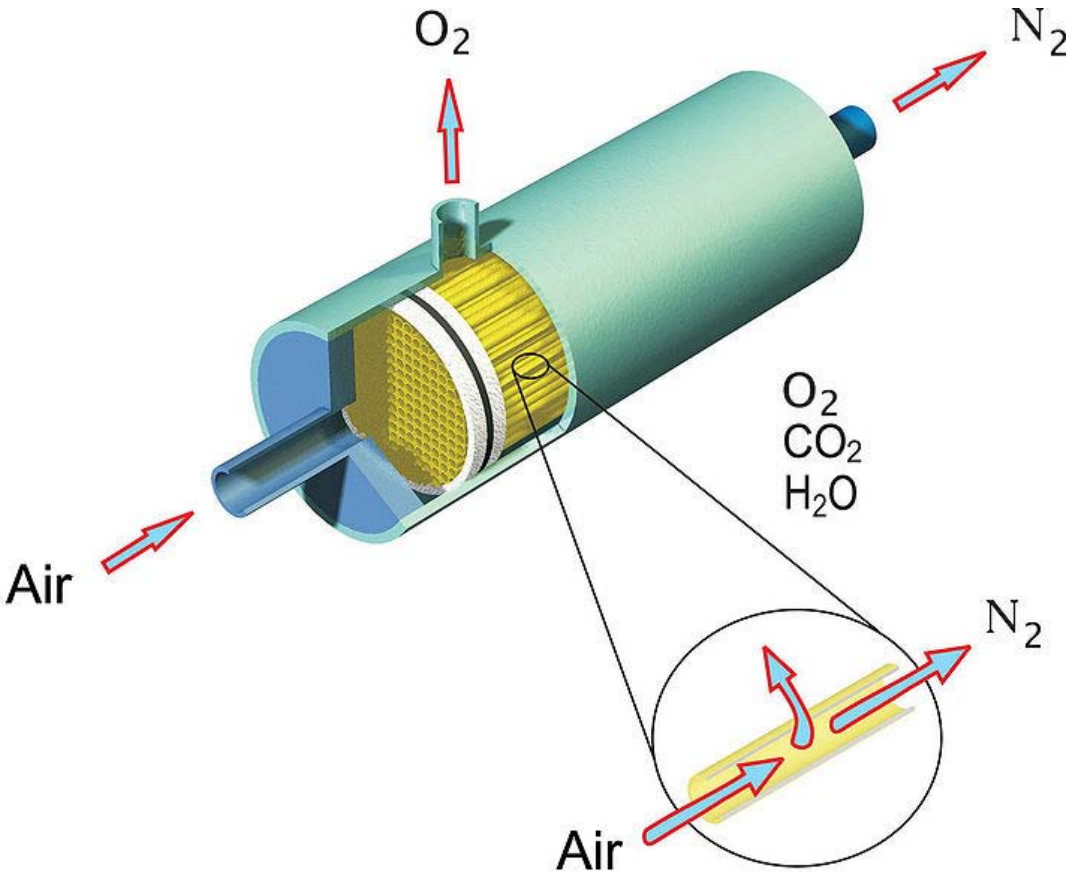
There are two main flow configurations of membrane processes: cross-flow and dead-end filtrations. In cross-flow filtration the feed flow is tangential to the surface of membrane, retentate is removed from the same side further downstream, whereas the permeate flow is tracked on the other side. In dead-end filtration the direction of the fluid flow is normal to the membrane surface. Both flow geometries offer some advantages and disadvantages. The dead-end membranes are relatively easy to fabricate which reduces the cost of the separation process. The dead-end membrane separation process is easy to implement and the process is usually cheaper than cross-flow membrane filtration. The dead-end filtration process is usually a batch-type process, where the filtering solution is loaded (or slowly fed) into membrane device, which then allows passage of some particles subject to the driving force. The main disadvantage of a dead end filtration is the extensive membrane fouling and concentration polarization. The fouling is usually induced faster at the higher driving forces. Membrane fouling and particle retention in a feed solution also builds up a concentration gradients and particle backflow (concentration polarization). The tangential flow devices are more cost and labor intensive, but they are less susceptible to fouling due to the sweeping effects and high shear rates of the passing flow. The most commonly used synthetic membrane devices (modules) are flat plates, spiral wounds, and hollow fibers.

Flat plates are usually constructed as circular thin flat membrane surfaces to be used in dead-end geometry modules. Spiral wounds are constructed from similar flat membranes but in a form of a “pocket” containing two membrane sheets separated by a highly porous support plate. Several such pockets are then wound around a tube to create a tangential flow geometry and to reduce membrane fouling. Hollow fiber modules consist of an assembly of self-supporting fibers with a dense skin separation layers, and more open matrix helping to withstand pressure gradients and maintain structural integrity. The hollow fiber modules can contain up to 10,000 fibers ranging from 200 to 2500 μm in diameter; The main advantage of hollow fiber modules is very large surface area within an enclosed volume, increasing the efficiency of the separation process.



Spiral wound membrane module.

Hollow fiber membrane module.



Separation of air in oxygen and nitrogen through a membrane

Membrane performance and governing equations

The selection of synthetic membranes for a targeted separation process is usually based on few requirements. Membranes have to provide enough mass transfer area to process large amounts of feed stream. The selected membrane has to have high selectivity (rejection) properties for certain particles; it has to resist fouling and to have high mechanical stability. It also needs to be reproducible and to have low manufacturing costs. The main modeling equation for the dead-end filtration at constant pressure drop is represented by Darcy's law:

$$\frac{dV_p}{dt} = Q = \frac{\Delta p}{\mu} A \left(\frac{1}{R_m + R} \right)$$

where V_p and Q are the volume of the permeate and its volumetric flow rate respectively (proportional to same characteristics of the feed flow), μ is dynamic viscosity of permeating fluid, A is membrane area, R_m and R are the respective resistances of membrane and growing deposit of the foulants. R_m can be interpreted as a membrane

resistance to the solvent (water) permeation. This resistance is a membrane intrinsic property and expected to be fairly constant and independent of the driving force, Δp. R is related to the type of membrane foulant, its concentration in the filtering solution, and the nature of foulant-membrane interactions. Darcy's law allows to calculate the membrane area for a targeted separation at given conditions. The solute sieving coefficient is defined by the equation:

$$S = \frac{C_p}{C_f}$$

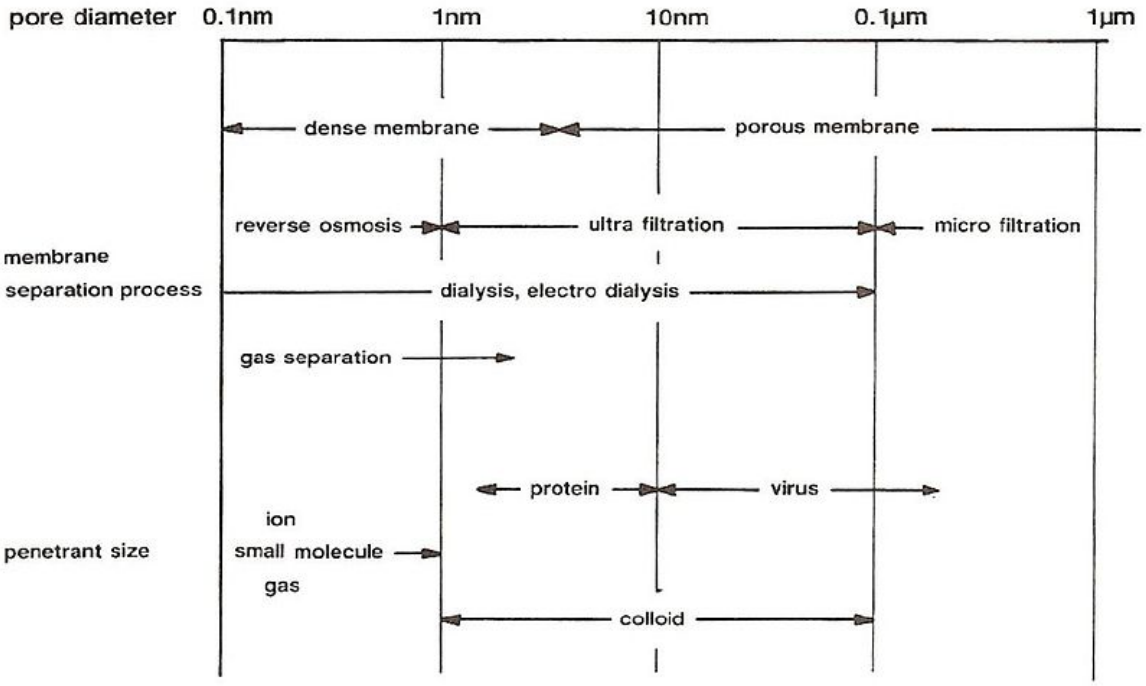
where C_f and C_p are the solute concentrations in feed and permeate respectively. Hydraulic permeability is defined as the inverse of resistance and is represented by the equation:

$$L_p = \frac{J}{\Delta p}$$

where J is the permeate flux which is the volumetric flow rate per unit of membrane area. The solute sieving coefficient and hydraulic permeability allow the quick assessment of the synthetic membrane performance.

Membrane separation processes

Membrane separation processes have very important role in separation industry. Nevertheless, they were not considered technically important until mid-1970. Membrane separation processes differ based on separation mechanisms and size of the separated particles. The widely used membrane processes include microfiltration, ultrafiltration, nanofiltration, reverse osmosis, electrolysis, dialysis, electrodialysis, gas separation, vapor permeation, pervaporation, membrane distillation, and membrane contactors. All processes except for pervaporation involve no phase change. All processes except (electro)dialysis are pressure driven. Microfltration and ultrafiltration is widely used in food and beverage processing (beer microfiltration, apple juice ultrafiltration), biotechnological applications and pharmaceutical industry (antibiotic production, protein purification), water purification and wastewater treatment, microelectronics industry, and others. Nanofiltration and reverse osmosis membranes are mainly used for water purification purposes. Dense membranes are utilized for gas separations (removal of CO_2 from natural gas, separating N_2 from air, organic vapor removal from air or nitrogen stream) and sometimes in membrane distillation. The later process helps in separating of azeotropic compositions reducing the costs of distillation processes.



Ranges of membrane based separations.

Chapter- 11

Gas Chromatography

Gas chromatography

A gas chromatograph with a headspace sampler

Acronym	GLC, GC
Classification	chromatography
Analytes	organic inorganic must be volatile
Manufacturers	Agilent Bruker PerkinElmer Shimadzu

	Thermo Fisher Scientific
	LECO Corporation
	Alpha MOS - Perichrom
	Vernier Software & Technology
	Other techniques
Related	Thin layer chromatography
	High performance liquid chromatography
Hyphenated	Gas chromatography-mass spectrometry

Gas chromatography (GC), is a common type of chromatography used in analytic chemistry for separating and analyzing compounds that can be vaporized without decomposition. Typical uses of GC include testing the purity of a particular substance, or separating the different components of a mixture (the relative amounts of such components can also be determined). In some situations; GC may help in identifying a compound. In preparative chromatography, GC can be used to prepare pure compounds from a mixture.

In gas chromatography, the *moving phase* (or "mobile phase") is a carrier gas, usually an inert gas such as helium or an unreactive gas such as nitrogen. The *stationary phase* is a microscopic layer of liquid or polymer on an inert solid support, inside a piece of glass or metal tubing called a column (a homage to the fractionating column used in distillation). The instrument used to perform gas chromatography is called a *gas chromatograph* (or "aerograph", "gas separator").

The gaseous compounds being analyzed interact with the walls of the column, which is coated with different stationary phases. This causes each compound to elute at a different time, known as the *retention time* of the compound. The comparison of retention times is what gives GC its analytical usefulness.

Gas chromatography is in principle similar to column chromatography (as well as other forms of chromatography, such as HPLC, TLC), but has several notable differences. Firstly, the process of separating the compounds in a mixture is carried out between a liquid stationary phase and a gas moving phase, whereas in column chromatography the stationary phase is a solid and the moving phase is a liquid. (Hence the full name of the procedure is "Gas-liquid chromatography", referring to the mobile and stationary phases, respectively.) Secondly, the column through which the gas phase passes is located in an oven where the temperature of the gas can be controlled, whereas column chromatography (typically) has no such temperature control. Thirdly, the concentration of a compound in the gas phase is solely a function of the vapor pressure of the gas.

Gas chromatography is also similar to fractional distillation, since both processes separate the components of a mixture primarily based on boiling point (or vapor pressure)

differences. However, fractional distillation is typically used to separate components of a mixture on a large scale, whereas GC can be used on a much smaller scale (i.e. microscale).

Gas chromatography is also sometimes known as **vapor-phase chromatography** (VPC), or **gas-liquid partition chromatography** (GLPC). These alternative names, as well as their respective abbreviations, are frequently found in scientific literature. Strictly speaking, GLPC is the most correct terminology, and is thus preferred by many authors.

History

Chromatography dates to 1903 in the work of the Russian scientist, Mikhail Semenovich Tswett. German graduate student Fritz Prior developed solid state gas chromatography in 1947. Archer John Porter Martin, who was awarded the Nobel Prize for his work in developing liquid-liquid (1941) and paper (1944) chromatography, laid the foundation for the development of gas chromatography and he later produced liquid-gas chromatography (1950). Erika Cremer laid the groundwork, and oversaw much of Prior's work.

GC analysis

A **gas chromatograph** is a chemical analysis instrument for separating chemicals in a complex sample. A gas chromatograph uses a flow-through narrow tube known as the *column*, through which different chemical constituents of a sample pass in a gas stream (carrier gas, *mobile phase*) at different rates depending on their various chemical and physical properties and their interaction with a specific column filling, called the *stationary phase*. As the chemicals exit the end of the column, they are detected and identified electronically. The function of the stationary phase in the column is to separate different components, causing each one to exit the column at a different time (*retention time*). Other parameters that can be used to alter the order or time of retention are the carrier gas flow rate, and the temperature.

In a GC analysis, a known volume of gaseous or liquid analyte is injected into the "entrance" (head) of the column, usually using a microsyringe (or, solid phase microextraction fibers, or a gas source switching system). As the carrier gas sweeps the analyte molecules through the column, this motion is inhibited by the adsorption of the analyte molecules either onto the column walls or onto packing materials in the column. The rate at which the molecules progress along the column depends on the strength of adsorption, which in turn depends on the type of molecule and on the stationary phase materials. Since each type of molecule has a different rate of progression, the various components of the analyte mixture are separated as they progress along the column and reach the end of the column at different times (retention time). A detector is used to monitor the outlet stream from the column; thus, the time at which each component reaches the outlet and the amount of that component can be determined. Generally,

substances are identified (qualitatively) by the order in which they emerge (elute) from the column and by the retention time of the analyte in the column.

Physical components

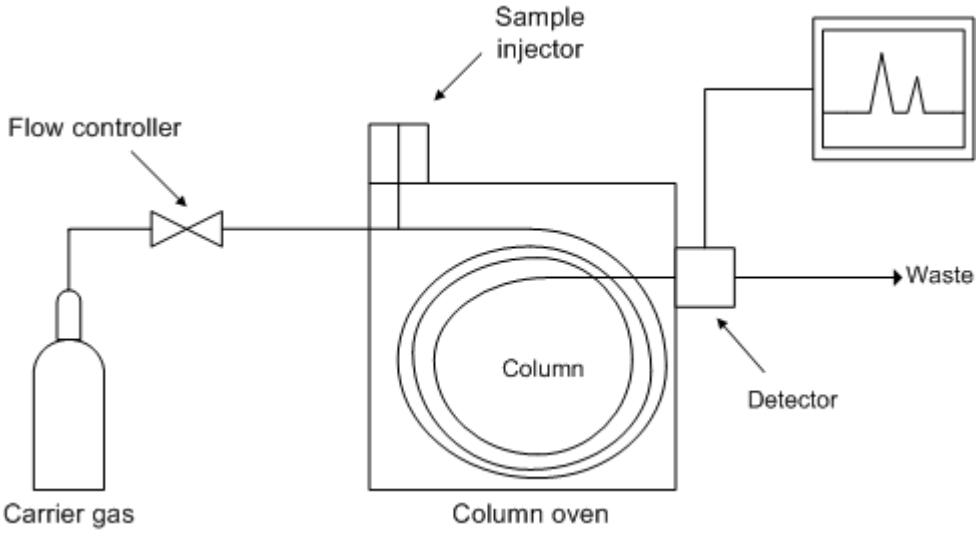


Diagram of a gas chromatograph.

Autosamplers

The autosampler provides the means to introduce a sample automatically into the inlets. Manual insertion of the sample is possible but is no longer common. Automatic insertion provides better reproducibility and time-optimization.

Different kinds of autosamplers exist. Autosamplers can be classified in relation to sample capacity (auto-injectors vs. autosamplers, where auto-injectors can work a small number of samples), to robotic technologies (XYZ robot vs. rotating robot – the most common), or to analysis:

- **Liquid**
- **Static head-space by syringe technology**
- **Dynamic head-space by transfer-line technology**
- **Solid phase microextraction (SPME)**

a complete range of autosamplers. Historically, the countries most active in autosampler technology development are the United States, Italy and Switzerland.

Inlets

The **column inlet** (or *injector*) provides the means to introduce a sample into a continuous flow of carrier gas. The inlet is a piece of hardware attached to the *column head*.

Common inlet types are:

- **S/SL (Split/Splitless) injector**; a sample is introduced into a heated small chamber via a syringe through a *septum* - the heat facilitates volatilization of the sample and sample matrix. The carrier gas then either sweeps the entirety (*splitless* mode) or a portion (*split* mode) of the sample into the column. In split mode, a part of the sample/carrier gas mixture in the injection chamber is exhausted through the *split vent*. Split injection is preferred when working with samples with high analyte concentrations (>0.1%) whereas splitless injection is best suited for trace analysis with low amounts of analytes. (<0.01%)
- **On-column inlet**; the sample is here introduced in its entirety without heat.
- **PTV injector**; Temperature-programmed sample introduction was first described by Vogt in 1979. Originally Vogt developed the technique as a method for the introduction of large sample volumes (up to 250 µL) in capillary GC. Vogt introduced the sample into the liner at a controlled injection rate. The temperature of the liner was chosen slightly below the boiling point of the solvent. The low-boiling solvent was continuously evaporated and vented through the split line. Based on this technique, Poy developed the Programmed Temperature Vaporising injector; PTV. By introducing the sample at a low initial liner temperature many of the disadvantages of the classic hot injection techniques could be circumvented.
- **Gas source inlet** or **gas switching valve**; gaseous samples in collection bottles are connected to what is most commonly a six-port *switching valve*. The carrier gas flow is not interrupted while a sample can be expanded into a previously evacuated *sample loop*. Upon switching, the contents of the sample loop are inserted into the carrier gas stream.
- **P/T (Purge-and-Trap) system**; An inert gas is bubbled through an aqueous sample causing insoluble volatile chemicals to be purged from the matrix. The volatiles are 'trapped' on an absorbent column (known as a trap or concentrator) at ambient temperature. The trap is then heated and the volatiles are directed into the carrier gas stream. Samples requiring preconcentration or purification can be introduced via such a system, usually hooked up to the S/SL port.
- **SPME** (solid phase microextraction) offers a convenient, low-cost alternative to P/T systems with the versatility of a syringe and simple use of the S/SL port.

Columns

Two types of columns are used in GC:

- **Packed columns** are 1.5 – 10 m in length and have an internal diameter of 2 – 4 mm. The tubing is usually made of stainless steel or glass and contains a *packing* of finely divided, inert, solid support material (e.g. diatomaceous earth) that is coated with a liquid or solid stationary phase. The nature of the coating material determines what type of materials will be most strongly adsorbed. Thus numerous columns are available that are designed to separate specific types of compounds.
- **Capillary columns** have a very small internal diameter, on the order of a few tenths of millimeters, and lengths between 25–60 meters are common. The inner column walls are coated with the active materials (WCOT columns), some columns are quasi solid filled with many parallel micropores (PLOT columns). Most capillary columns are made of fused-silica (FSOT columns) with a polyimide outer coating. These columns are flexible, so a very long column can be wound into a small coil.
- **New developments** are sought where stationary phase incompatibilities lead to geometric solutions of parallel columns within one column. Among these new developments are:
    - Internally heated *microFAST* columns, where two columns, an internal heating wire and a temperature sensor are combined within a common column sheath (microFAST);
    - *Micropacked columns* (1/16" OD) are column-in-column packed columns where the outer column space has a packing different from the inner column space, thus providing the separation behaviour of two columns in one. They can easily fit to inlets and detectors of a capillary column instrument.

The **temperature-dependence** of molecular adsorption and of the rate of progression along the column necessitates a careful control of the column temperature to within a few tenths of a degree for precise work. Reducing the temperature produces the greatest level of separation, but can result in very long elution times. For some cases temperature is ramped either continuously or in steps to provide the desired separation. This is referred to as a **temperature program**. Electronic pressure control can also be used to modify flow rate during the analysis, aiding in faster run times while keeping acceptable levels of separation.

The **choice of carrier gas** (*mobile phase*) is important, with hydrogen being the most efficient and providing the best separation. However, helium has a larger range of flowrates that are comparable to hydrogen in efficiency, with the added advantage that helium is non-flammable, and works with a greater number of detectors. Therefore, helium is the most common carrier gas used.

Detectors

A number of detectors are used in gas chromatography. The most common are the flame ionization detector (FID) and the thermal conductivity detector (TCD). Both are sensitive to a wide range of components, and both work over a wide range of concentrations.

While TCDs are essentially universal and can be used to detect any component other than the carrier gas (as long as their thermal conductivities are different from that of the carrier gas, at detector temperature), FIDs are sensitive primarily to hydrocarbons, and are more sensitive to them than TCD. However, an FID cannot detect water. Both detectors are also quite robust. Since TCD is non-destructive, it can be operated in-series before an FID (destructive), thus providing complementary detection of the same analytes.

Other detectors are sensitive only to specific types of substances, or work well only in narrower ranges of concentrations. They include:

- discharge ionization detector (DID), which uses a high-voltage electric discharge to produce ions.
- electron capture detector (ECD), which uses a radioactive Beta particle (electron) source to measure the degree of electron capture.
- flame photometric detector (FPD)
- flame ionization detector (FID)
- Hall electrolytic conductivity detector (ElCD)
- helium ionization detector (HID)
- Nitrogen Phosphorus Detector (NPD)
- Infrared Detector (IRD)
- mass selective detector (MSD)
- photo-ionization detector (PID)
- pulsed discharge ionization detector (PDD)
- thermal energy(conductivity) analyzer/detector (TEA/TCD)

Some gas chromatographs are connected to a mass spectrometer which acts as the detector. The combination is known as GC-MS. Some GC-MS are connected to an NMR spectrometer which acts as a backup detector. This combination is known as GC-MS-NMR. Some GC-MS-NMR are connected to an infrared spectrophotometer which acts as a backup detector. This combination is known as GC-MS-NMR-IR. It must, however, be stressed this is very rare as most analyses needed can be concluded via purely GC-MS.

Methods

The **method** is the collection of conditions in which the GC operates for a given analysis. **Method development** is the process of determining what conditions are adequate and/or ideal for the analysis required.

Conditions which can be varied to accommodate a required analysis include inlet temperature, detector temperature, column temperature and temperature program, carrier gas and carrier gas flow rates, the column's stationary phase, diameter and length, inlet type and flow rates, sample size and injection technique. Depending on the detector(s) (see below) installed on the GC, there may be a number of detector conditions that can also be varied. Some GCs also include valves which can change the route of sample and carrier flow. The timing of the opening and closing of these valves can be important to method development.

This image above shows the interior of a GeoStrata Technologies Eclipse Gas Chromatograph that runs continuously in three minute cycles. Two valves are used to switch the test gas into the sample loop. After filling the sample loop with test gas, the valves are switched again applying carrier gas pressure to the sample loop and forcing the sample through the Column for separation.

Carrier gas selection and flow rates

Typical carrier gases include helium, nitrogen, argon, hydrogen and air. Which gas to use is usually determined by the detector being used, for example, a DID requires helium as the carrier gas. When analyzing gas samples, however, the carrier is sometimes selected based on the sample's matrix, for example, when analyzing a mixture in argon, an argon carrier is preferred, because the argon in the sample does not show up on the chromatogram. Safety and availability can also influence carrier selection, for example, hydrogen is flammable, and high-purity helium can be difficult to obtain in some areas of the world.

The purity of the carrier gas is also frequently determined by the detector, though the level of sensitivity needed can also play a significant role. Typically, purities of 99.995% or higher are used. Trade names for typical purities include "Zero Grade," "Ultra-High Purity (UHP) Grade," "4.5 Grade" and "5.0 Grade."

The carrier gas flow rate affects the analysis in the same way that temperature does (see above). The higher the flow rate the faster the analysis, but the lower the separation between analytes. Selecting the flow rate is therefore the same compromise between the level of separation and length of analysis as selecting the column temperature.

With GCs made before the 1990s, carrier flow rate was controlled indirectly by controlling the carrier inlet pressure, or "column head pressure." The actual flow rate was measured at the outlet of the column or the detector with an electronic flow meter, or a bubble flow meter, and could be an involved, time consuming, and frustrating process. The pressure setting was not able to be varied during the run, and thus the flow was essentially constant during the analysis. The relation between flow rate and inlet pressure is calculated with Poiseuille's equation for compressible fluids.

Many modern GCs, however, electronically measure the flow rate, and electronically control the carrier gas pressure to set the flow rate. Consequently, carrier pressures and flow rates can be adjusted during the run, creating pressure/flow programs similar to temperature programs.

Stationary compound selection

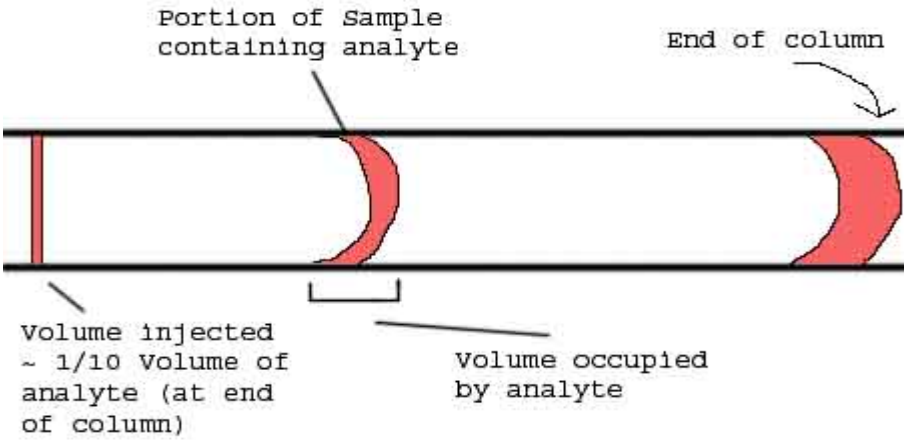
The polarity of the solute is crucial for the choice of stationary compound, which in an optimal case would have a similar polarity than the solute. Common stationary phases in open tubular columns are cyanopropylphenyl dimethyl polysiloxane, carbowax polyethyleneglycol, biscyanopropyl cyanopropylphenyl polysiloxane and diphenyl dimethyl polysiloxane. For packed columns there are more options available.

Inlet types and flow rates

The choice of inlet type and injection technique depends on if the sample is in liquid, gas, adsorbed, or solid form, and on whether a solvent matrix is present that has to be vaporized. Dissolved samples can be introduced directly onto the column via a COC injector, if the conditions are well known; if a solvent matrix has to be vaporized and partially removed, a S/SL injector is used (most common injection technique); gaseous samples (e.g., air cylinders) are usually injected using a gas switching valve system; adsorbed samples (e.g., on adsorbent tubes) are introduced using either an external (on-line or off-line) desorption apparatus such as a purge-and-trap system, or are desorbed in the S/SL injector (SPME applications).

Sample size and injection technique

Sample injection



The rule of ten in gas chromatography

The real chromatographic analysis starts with the introduction of the sample onto the column. The development of capillary gas chromatography resulted in many practical problems with the injection technique. The technique of on-column injection, often used with packed columns, is usually not possible with capillary columns. The injection system, in the capillary gas chromatograph, should fulfil the following two requirements:

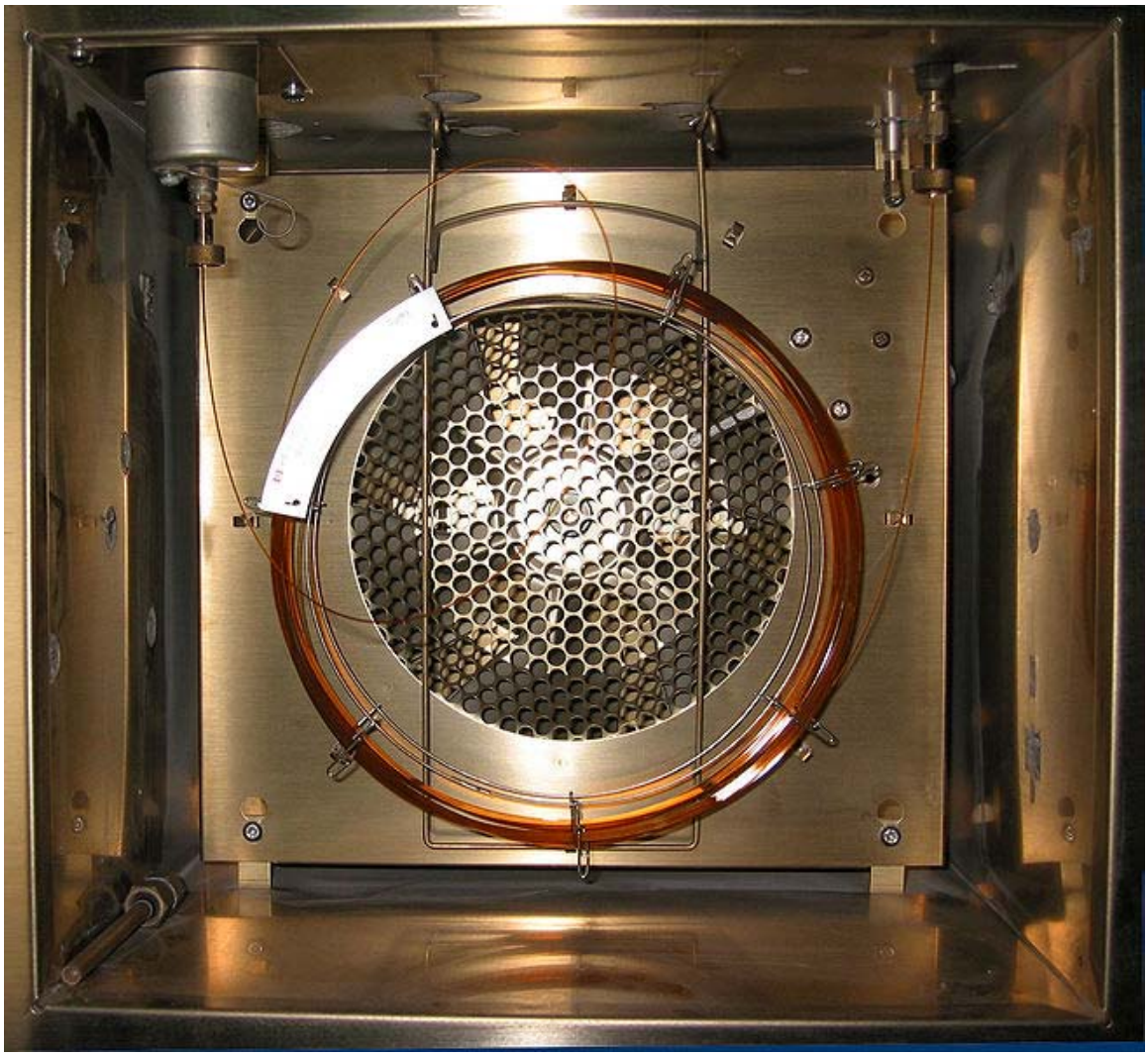
1. The amount injected should not overload the column.
2. The width of the injected plug should be small compared to the spreading due to the chromatographic process. Failure to comply with this requirement will reduce the separation capability of the column. As a general rule, the volume injected, V_{inj}, and the volume of the detector cell, V_{det}, should be about 1/10 of the volume occupied by the portion of sample containing the molecules of interest (analytes) when they exit the column.

Some general requirements, which a good injection technique should fulfill, are:

- It should be possible to obtain the column's optimum separation efficiency.
- It should allow accurate and reproducible injections of small amounts of representative samples.
- It should induce no change in sample composition. It should not exhibit discrimination based on differences in boiling point, polarity, concentration or thermal/catalytic stability.
- It should be applicable for trace analysis as well as for undiluted samples.

Column selection

Column temperature and temperature program



A gas chromatography oven, open to show a capillary column

The column(s) in a GC are contained in an oven, the temperature of which is precisely controlled electronically. (When discussing the "temperature of the column," an analyst is technically referring to the temperature of the column oven.)

The rate at which a sample passes through the column is directly proportional to the temperature of the column. The higher the column temperature, the faster the sample moves through the column. However, the faster a sample moves through the column, the less it interacts with the stationary phase, and the less the analytes are separated.

In general, the column temperature is selected to compromise between the length of the analysis and the level of separation.

A method which holds the column at the same temperature for the entire analysis is called "isothermal." Most methods, however, increase the column temperature during the analysis, the initial temperature, rate of temperature increase (the temperature "ramp") and final temperature is called the "**temperature program**."

A temperature program allows analytes that elute early in the analysis to separate adequately, while shortening the time it takes for late-eluting analytes to pass through the column.

Data reduction and analysis

Qualitative analysis:

Generally chromatographic data is presented as a graph of detector response (y-axis) against retention time (x-axis), which is called a chromatogram. This provides a spectrum of peaks for a sample representing the analytes present in a sample eluting from the column at different times. Retention time can be used to identify analytes if the method conditions are constant. Also, the pattern of peaks will be constant for a sample under constant conditions and can identify complex mixtures of analytes. In most modern applications however the GC is connected to a mass spectrometer or similar detector that is capable of identifying the analytes represented by the peaks.

Quantitative analysis:

The area under a peak is proportional to the amount of analyte present in the chromatogram. By calculating the area of the peak using the mathematical function of integration, the concentration of an analyte in the original sample can be determined. Concentration can be calculated using a calibration curve created by finding the response for a series of concentrations of analyte, or by determining the relative response factor of an analyte. The relative response factor is the expected ratio of an analyte to an internal standard (or external standard) and is calculated by finding the response of a known amount of analyte and a constant amount of internal standard (a chemical added to the sample at a constant concentration, with a distinct retention time to the analyte).

In most modern GC-MS systems, computer software is used to draw and integrate peaks, and match MS spectra to library spectra.

Application

In general, substances that vaporize below ca. 300 °C (and therefore are stable up to that temperature) can be measured quantitatively. The samples are also required to be salt-free; they should not contain ions. Very minute amounts of a substance can be measured, but it is often required that the sample must be measured in comparison to a sample containing the pure, suspected substance.

Various temperature programs can be used to make the readings more meaningful; for example to differentiate between substances that behave similarly during the GC process.

Professionals working with GC analyze the content of a chemical product, for example in assuring the quality of products in the chemical industry; or measuring toxic substances in soil, air or water. GC is very accurate if used properly and can measure picomoles of a substance in a 1 ml liquid sample, or parts-per-billion concentrations in gaseous samples.

In practical courses at colleges, students sometimes get acquainted to the GC by studying the contents of Lavender oil or measuring the ethylene that is secreted by *Nicotiana benthamiana* plants after artificially injuring their leaves. These GC analyses hydrocarbons (C2-C40+). In a typical experiment, a packed column is used to separate the light gases, which are then detected with a TCD. The hydrocarbons are separated using a capillary column and detected with an FID. A complication with light gas analyses that include H_2 is that He, which is the most common and most sensitive inert carrier (sensitivity is proportional to molecular mass) has an almost identical thermal conductivity to hydrogen (it is the difference in thermal conductivity between two separate filaments in a Wheatstone Bridge type arrangement that shows when a component has been eluted). For this reason, dual TCD instruments are used with a separate channel for hydrogen that uses nitrogen as a carrier are common. Argon is often used when analysing gas phase chemistry reactions such as F-T synthesis so that a single carrier gas can be used rather than 2 separate ones. The sensitivity is less but this is a tradeoff for simplicity in the gas supply.

GCs in popular culture

Movies, books and TV shows tend to misrepresent the capabilities of gas chromatography and the work done with these instruments.

In the U.S. TV show CSI, for example, GCs are used to rapidly identify unknown samples. "This is gasoline bought at a Chevron station in the past two weeks," the analyst will say fifteen minutes after receiving the sample.

In fact, a typical GC analysis takes much more time; sometimes a single sample must be run more than an hour according to the chosen program; and even more time is needed to "heat out" the column so it is free from the first sample and can be used for the next. Equally, several runs are needed to confirm the results of a study - a GC analysis of a single sample may simply yield a result per chance.

Also, GC does not positively identify most samples; and not all substances in a sample will necessarily be detected. All a GC truly tells you is at which relative time a component eluted from the column and that the detector was sensitive to it. To make results meaningful, analysts need to know which components at which concentrations are to be expected; and even then a small amount of a substance can hide itself behind a substance having both a higher concentration and the same relative elution time. Last but

not least it is often needed to check the results of the sample against a GC analysis of a reference sample containing only the suspected substance.

A GC-MS can remove much of this ambiguity, since the mass spectrometer will identify the component's molecular weight. But this still takes time and skill to do properly.

Similarly, most GC analyses are not push-button operations. You cannot simply drop a sample vial into an auto-sampler's tray, push a button and have a computer tell you everything you need to know about the sample. According to the substances one expects to find the operating program must be carefully chosen.

A push-button operation can exist for running similar samples repeatedly, such as in a chemical production environment or for comparing 20 samples from the same experiment to calculate the mean content of the same substance. However, for the kind of investigative work portrayed in books, movies and TV shows this is clearly not the case.