Drying

Drying is a mass transfer process consisting of the removal of water or another solvent by evaporation from a solid, semi-solid or liquid. This process is often used as a final production step before selling or packaging products. To be considered "dried", the final product must be solid, in the form of a continuous sheet (e.g., paper), long pieces (e.g., wood), particles (e.g., cereal grains or corn flakes) or powder (e.g., sand, salt, washing powder, milk powder). A source of heat and an agent to remove the vapor produced by the process are often involved. In bioproducts like food, grains, and pharmaceuticals like vaccines, the solvent to be removed is almost invariably water. Desiccation may be synonymous with drying or considered an extreme form of drying.

In the most common case, a gas stream, e.g., air, applies the heat by convection and carries away the vapor as humidity. Other possibilities are vacuum drying, where heat is supplied by conduction or radiation (or microwaves), while the vapor thus produced is removed by the vacuum system. Another indirect technique is drum drying (used, for instance, for manufacturing potato flakes), where a heated surface is used to provide the energy, and aspirators draw the vapor outside the room. In contrast, the mechanical extraction of the solvent, e.g., water, by centrifugation, is not considered "drying" but rather "draining".

Drying mechanism

In some products having a relatively high initial moisture content, an initial linear reduction of the average product moisture content as a function of time may be observed for a limited time, often known as a "constant drying rate period". Usually, in this period, it is surface moisture outside individual particles that is being removed. The drying rate during this period is mostly dependent on the rate of heat transfer to the material being dried. Therefore, the maximum achievable drying rate is considered to be heat-transfer limited. If drying is continued, the slope of the curve, the drying rate, becomes less steep (falling rate period) and eventually tends to nearly horizontal at very long times. The product moisture content is then constant at the "equilibrium moisture content", where it is, in practice, in equilibrium with the dehydrating medium. In the falling-rate period, water migration from the product interior to the surface is mostly by molecular diffusion, i,e. the water flux is proportional to the moisture content gradient. This means that water moves from zones with higher moisture content to zones with lower values, a phenomenon explained by the second law of thermodynamics. If water removal is considerable, the products usually undergo shrinkage and deformation, except in a well-designed freeze-drying process. The drying rate in the falling-rate period is controlled by the rate of removal of moisture or solvent from the interior of the solid being dried and is referred to as being "mass-transfer limited". This is widely noticed in hygroscopic products such as fruits and vegetables, where drying occurs in the falling rate period with the constant drying rate period said to be negligible.

Methods of drying

In a typical phase diagram, the boundary between gas and liquid runs from the triple point to the critical point. Regular drying is the green arrow, while supercritical drying is the red arrow and freeze drying is the blue.

The following are some general methods of drying:

Application of hot air (convective or direct drying). Air heating increases the driving force for heat transfer and accelerates drying. It also reduces air relative humidity, further increasing the driving force for drying. In the falling rate period, as moisture content falls, the solids heat up and the higher temperatures speed up diffusion of water from the interior of the solid to the surface. However, product quality considerations limit the applicable rise to air temperature. Excessively hot air can almost completely dehydrate the solid surface, so that its pores shrink and almost close, leading to crust formation or "case hardening", which is usually undesirable. For instance in wood (timber) drying, air is heated (which speeds up drying) though some steam is also added to it (which hinders drying rate to a certain extent) in order to avoid excessive surface dehydration and product deformation owing to high moisture gradients across timber thickness. Spray drying belongs in this category.

Indirect or contact drying (heating through a hot wall), as drum drying, vacuum drying. Again, higher wall temperatures will speed up drying but this is limited by product degradation or case-hardening. Drum drying belongs in this category.

Dielectric drying (radiofrequency or microwaves being absorbed inside the material) is the focus of intense research nowadays. It may be used to assist air drying or vacuum drying. Researchers have found that microwave finish drying speeds up the otherwise very low drying rate at the end of the classical drying methods.

Freeze drying or lyophilization is a drying method where the solvent is frozen prior to drying and is then sublimed, i.e., passed to the gas phase directly from the solid phase, below the melting point of the solvent. It is increasingly applied to dry foods, beyond its already classical pharmaceutical or medical applications. It keeps biological properties of proteins, and retains vitamins and bioactive compounds. Pressure can be reduced by a high vacuum pump (though freeze drying at atmospheric pressure is possible in dry air). If using a vacuum pump, the vapor produced by sublimation is removed from the system by converting it into ice in a condenser, operating at very low temperatures, outside the freeze drying chamber.

Supercritical drying (superheated steam drying) involves steam drying of products containing water. This process is feasible because water in the product is boiled off, and joined with the drying medium, increasing its flow. It is usually employed in closed circuit and allows a proportion of latent heat to be recovered by recompression, a feature which is not possible with conventional air drying, for instance. The process has potential for use in foods if carried out at reduced pressure, to lower the boiling point.

Natural air drying takes place when materials are dried with unheated forced air, taking advantage of its natural drying potential. The process is slow and weather-dependent, so a wise strategy "fan off-fan on" must be devised considering the following conditions: Air temperature, relative humidity and moisture content and temperature of the material being dried. Grains are increasingly dried with this technique, and the total time (including fan off and on periods) may last from one week to various months, if a winter rest can be tolerated in cold areas.

Applications of drying

Drying of fish in Lofoten in the production of stockfish

Food

Foods are dried to inhibit microbial development and quality decay. However, the extent of drying depends on product end-use. Cereals and oilseeds are dried after harvest to the moisture content that allows microbial stability during storage. Vegetables are blanched before drying to avoid rapid darkening, and drying is not only carried out to inhibit microbial growth, but also to avoid browning during storage. Concerning dried fruits, the reduction of moisture acts in combination with its acid and sugar contents to provide protection against microbial growth. Products such as milk powder must be dried to very low moisture contents in order to ensure flowability and avoid caking. This moisture is lower than that required to ensure inhibition to microbial development. Other products as crackers are dried beyond the microbial growth threshold to confer a crispy texture, which is liked by consumers.

Non-food products

Among non-food products, those that require considerable drying are wood (as part of Timber processing), paper, flax, and washing powder. The first two, owing to their organic origins, may develop mold if insufficiently dried. Another benefit of drying is a reduction in volume and weight.

Chromatography

THEORY OF CHROMATOGRAPHY

Separation of two sample components in chromatography is based on their different distribution between two non-miscible phases. The one, the stationary phase, a liquid or solid, is fixed in the system. The other, the mobile phase, a fluid, is streaming through the chromatographic system. In gas chromatography the mobile phase is a gas, in liquid chromatography it is a liquid.

The molecules of the analytes are distributed between the mobile and the stationary phase. When present in the stationary phase, they are retained, and are not moving through the system. In contrast, they migrate with the velocity, v, of the mobile phase when being there. Due to the different distribution of the particular analytes the mean residence time in the stationary phase differs, too, resulting in a different net migration velocity. This is the principle of chromatographic separation.

Paper Partition Chromatography

Introduction: Chromatography is a simple and rapid method for the separation of a mixture of compounds in a solution and a sensitive method for identification of each compound. One or the basic principles underlying chromatography techniques is the very rapid partitioning of the various compounds (solutes) between two different immiscible phases: a **stationary** phase and a **mobile** phase.

For this paper chromatography exercise, the developing or irrigating solvent consists of a homogeneous mixture of ethyl acetate, formic acid, and water in a definite proportion (7:2:1). Ethyl acetate and water are immiscible with each other but the addition of formic acid allows the two liquids to become mutually soluble within limits. The chromatography paper is manufactured from nearly pure cellulose fibers. Cellulose is a polysaccharide of glucose. Other solvent systems such as 80% phenol/20% water are also excellent for amino acids.

When the chromatography paper comes into contact with the irrigating solvent, the water molecules will become attracted to the cellulose molecules. Why? This combination of cellulose and water is called the **stationary** phase. The remainder of the irrigating solvent (not the water) is the **mobile** phase. The components that are to be separated and identified will partition rapidly between the two phases. Compounds that are more nonpolar will tend to be more "soluble" in the nonpolar mobile phase.

Solutions containing the compounds to be separate are applied near one edge of the paper and allowed to dry. When the paper becomes wet with the irrigating solvent, the dry compounds must first dissolve in the solvent. The nonpolar compounds will dissolve more rapidly in the mobile phase and consequently migrate across the paper at a faster rate than the more polar compounds.

The rate of movement of compounds across the paper for a given irrigating solvent system is dependent not only on partitioning properties, but also on the size and charge of each molecule since chromatography also involves physical movement of solutes through molecular size channels of the paper.

PROCEDURE:

1. To avoid contaminating the chromatography paper, handle the paper near one corner whenever possible and never place the paper on bare table top but use paper towels.

2. Using a clean ruler and a pencil (no pen) draw a line about 2 cm from one of the longer edges of the paper. Starting from one end of the line, place lightly with a pencil, dots about 1-1.5 cm apart with the last dot at least 2 cm from the end of the line. Label each dot with appropriate amino acid abbreviations.

3. A solution of an amino acid (0.05M) or a mixture of amino acids (unknown) or purified tyrosine is applied as a small circle (5-6 mm diameter) with an L2 pipettor. Pipette 1 ul of amino acid into the pipettor tip, touch against the side of the container to remove excess liquid from the tip, and then carefully apply to the appropriately labeled dot. For solutions of known amino acids, only a single application will be required. For the unknown amino acid mixture or tyrosine, several applications of the solution may be necessary since these solutions may be less concentrated than the knowns.

4. To make multiple applications on the paper, the spot must be completely dry before the second, third, etc. application is to be made.

5. Put your name near the top edge of the paper.

6. A chromatography jar containing sufficient volume of the irrigation solvent to just cover the bottom is required. The irrigation solvent is prepared by mixing together 28 ml of ethyl acetate and 12 ml of dilute formic acid solution. Put any excess solvent into appropriate waste bottles.

7. When all of the spots are completely dry, the paper is stapled into a cylinder. Do not overlap the edges.

8. Place the paper cylinder carefully into the jar containing the solvent so that the cylinder does not touch the side of the jar. Be certain that the spots are above the solvent. Seal the jar making sure the lid has a plastic insert. The system must be vapor tight since the solvent migration is dependent upon a solvent vapor saturated environment.

9. The solvent "front" is allowed to migrate upward until it reaches about 1 cm from the top edge of the paper. Do not open the jar to check the location of the solvent front. Why? This upward solvent migration will take between 1 to 4 hours, depending upon the solvent system.

10. Remove the developed paper from the jar and hang it in the hood to dry.

11. In another fume hood, spray the dried paper with ninhydrin solution. Do not soak the paper. The paper should be dampened uniformly; to hasten the development of color, the sprayed paper may be placed in a 100°C oven for about 2 minutes. Do not lay the paper directly on the metal grid of the oven.

RESULTS:

1. After the colored spots are fully developed, carefully outline each spot with a pencil. In your laboratory notebook, note the shade, color, and relative size of each spot.

2. To compare the relative distance of migration between each amino acid, the following ratio, R_f, is measured:

$$R_f = \frac{\text{distance traveled by spot from center from point of application}}{\text{distance the solvent front has moved from point of application}}$$

3. Tabulate your results. Identify the amino acids in your unknown by comparisons with knowns.

4. Did all of the amino acids produce the same color with ninhydrin? Explain any differences. What general relationship can be concluded between amino acid structure and R_f value?

Size Exclusion (Gel Filtration) Chromatography Size exclusion chromatography is used for semi-preparative purifications and various analytical assays. It is a separation technique which takes the advantage of the difference in size and geometry of the molecules. The molecules are separated based on their size. Grant Henry Lathe and Colin R Ruthven was the pioneer of size exclusion chromatography who started this technique for separation of analytes of different size with starch gels as the matrix, later Jerker Porath and Per Flodin introduced dextran gels. Other gel filtration matrices include agarose and polyacrylamide. Note: Unlike ion exchange chromatography, gel filtration does not depend on any chemical interaction with protein, rather it is based on a physical property of the protein - that being the effective molecular radius (which relates to mass for most globular proteins).

Principle: Size exclusion chromatography (SEC) is the separation of mixtures based on the molecular size (more correctly, their hydrodynamic volume) of the components. Separation is achieved by the differential exclusion or inclusion of solutes as they pass through stationary phase consisting of heteroporous (pores of different sizes) cross linked polymeric gels or beads. The process is based upon different permeation rates of each solute molecule into the interior of gel particles. Size exclusion chromatography involves gentle interaction with the sample, enabling high retention of biomolecular activity. For the separation of biomolecules in aqueous systems, SEC is referred to as gel filtration chromatography (GFC), while the separation of organic polymers in non-aqueous systems is called gel permeation chromatography (GPC)

The basic principle of size exclusion chromatography is quite simple. A column of gel particles or porous matrix is in equilibrium with a suitable mobile phase for the molecules to be separated. Large molecules are completely excluded from the pores will pass through the space in between the gel particles or matrix and will come first in the effluent. Smaller molecules will get distributed in between the mobile phase of in and outside the molecular sieve and will then pass through the column at a slower rate, hence appear later in effluent

Gas chromatography

For more details on this topic, see 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 chromatographic separation is always carried out in a column, which is typically "packed" or "capillary". Packed columns are the routine work horses of gas chromatography, being cheaper and easier to use and often giving adequate performance. Capillary columns generally give far superior resolution and although more expensive are becoming widely used, especially for complex mixtures. Both types of column are made from non-adsorbent and chemically inert materials. Stainless steel and glass are the usual materials for packed columns and quartz or fused silica for capillary columns.

Gas chromatography is based on a partition equilibrium of analyte between a solid or viscous liquid 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 (commonly 0.53 – 0.18mm inside diameter) glass or fused-silica 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 denatures 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. It 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 HPLC the sample is forced by a liquid at high pressure (the mobile phase) through a column that is packed with a stationary phase composed of irregularly or spherically shaped particles, a porous monolithic layer, or a porous membrane. 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).

Specific techniques under this broad heading are listed below.

Affinity chromatography

For more details on this topic, see Affinity chromatography.

Affinity chromatography[8] 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 chromatography 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

For more details on this topic, see 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

For more details on this topic, see Ion exchange chromatography.

Ion exchange chromatography (usually referred to as ion chromatography) uses an ion exchange mechanism to separate analytes based on their respective charges. 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 anions, cations, amino acids, peptides, and proteins. In conventional methods the stationary phase is an ion exchange resin that carries charged functional groups that interact with oppositely charged groups of the compound to retain. Ion exchange chromatography is commonly used to purify proteins using FPLC.

Size-exclusion chromatography

For more details on this topic, see 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.

Expanded bed adsorption chromatographic separation

For more details on this topic, see Expanded bed adsorption.

Expanded bed adsorption (EBA) 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

Main article: Reversed-phase chromatography

Reversed-phase chromatography (RPC) is any liquid chromatography procedure in which the mobile phase is significantly more polar than the stationary phase. It is so named because in normal-phase liquid chromatography, the mobile phase is significantly less polar than the stationary phase. Hydrophobic molecules in the mobile phase tend to adsorb to the relatively hydrophobic stationary phase. Hydrophilic molecules in the mobile phase will tend to elute first. Separating columns typically comprise a C8 or C18 carbon-chain bonded to a silica particle substrate.

Hydrophobic interaction chromatography

Hydrophobic interactions between proteins and the chromatographic matrix can be exploited to purify the proteins. In hydrophobic interaction chromatography, the matrix material is lightly substituted with octyl or phenyl groups. At high salt concentrations, nonpolar groups on the surface on proteins "interact" with the hydrophobic groups; that is, both types of groups are excluded by the polar solvent (hydrophobic effects are augmented by increased ionic strength). The eluant is typically an aqueous buffer with decreasing salt concentrations, increasing concentrations of detergent (which disrupts hydrophobic interactions), or changes in pH.

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

For more details on this topic, see Simulated moving bed.

The simulated moving bed (SMB) technique is a variant of high performance liquid chromatography; it is used to separate particles and/or chemical compounds that would be difficult or impossible to resolve otherwise. This increased separation is brought about by a valve-and-column arrangement that is used to lengthen the stationary phase indefinitely. In the moving bed technique of preparative chromatography the feed entry and the analyte recovery are simultaneous and continuous, but because of practical difficulties with a continuously moving bed, simulated moving bed technique was proposed. In the simulated moving bed technique instead of moving the bed, the sample inlet and the analyte exit positions are moved continuously, giving the impression of a moving bed. True moving bed chromatography (TMBC) is only a theoretical concept. Its simulation, SMBC is achieved by the use of a multiplicity of columns in series and a complex valve arrangement, which provides for sample and solvent feed, and also analyte and waste takeoff at appropriate locations of any column, whereby it allows switching at regular intervals the sample entry in one direction, the solvent entry in the opposite direction, whilst changing the analyte and waste takeoff positions appropriately as well.

Pyrolysis gas chromatography

Pyrolysis gas chromatography mass spectrometry is a method of chemical analysis in which the sample is heated to decomposition to produce smaller molecules that are separated by gas chromatography and detected using mass spectrometry.

Pyrolysis is the thermal decomposition of materials in an inert atmosphere or a vacuum. The sample is put into direct contact with a platinum wire, or placed in a quartz sample tube, and rapidly heated to 600–1000 °C. Depending on the application even higher temperatures are used. Three different heating techniques are used in actual pyrolyzers: Isothermal furnace, inductive heating (Curie Point filament), and resistive heating using platinum filaments. Large molecules cleave at their weakest points and produce smaller, more volatile fragments. These fragments can be separated by gas chromatography. Pyrolysis GC chromatograms are typically complex because a wide range of different decomposition products is formed. The data can either be used as fingerprint to prove material identity or the GC/MS data is used to identify individual fragments to obtain structural information. To increase the volatility of polar fragments, various methylating reagents can be added to a sample before pyrolysis.

Besides the usage of dedicated pyrolyzers, pyrolysis GC of solid and liquid samples can be performed directly inside Programmable Temperature Vaporizer (PTV) injectors that provide quick heating (up to 30 °C/s) and high maximum temperatures of 600–650 °C. This is sufficient for some pyrolysis applications. The main advantage is that no dedicated instrument has to be purchased and pyrolysis can be performed as part of routine GC analysis. In this case quartz GC inlet liners have to be used. Quantitative data can be acquired, and good results of derivatization inside the PTV injector are published as well.

Fast protein liquid chromatography

For more details on this topic, see Fast protein liquid chromatography.

Fast protein liquid chromatography (FPLC), is a form of liquid chromatography that is often used to analyze or purify mixtures of proteins. As in other forms of chromatography, separation is possible because the different components of a mixture have different affinities for two materials, a moving fluid (the "mobile phase") and a porous solid (the stationary phase). In FPLC the mobile phase is an aqueous solution, or "buffer". The buffer flow rate is controlled by a positive-displacement pump and is normally kept constant, while the composition of the buffer can be varied by drawing fluids in different proportions from two or more external reservoirs. The stationary phase is a resin composed of beads, usually of cross-linked agarose, packed into a cylindrical glass or plastic column. FPLC resins are available in a wide range of bead sizes and surface ligands depending on the application.

Countercurrent chromatography

For more details on this topic, see Countercurrent chromatography.

An example of a HPCCC system

Countercurrent chromatography (CCC) is a type of liquid-liquid chromatography, where both the stationary and mobile phases are liquids. The operating principle of CCC equipment requires a column consisting of an open tube coiled around a bobbin. The bobbin is rotated in a double-axis gyratory motion (a cardioid), which causes a variable gravity (G) field to act on the column during each rotation. This motion causes the column to see one partitioning step per revolution and components of the sample separate in the column due to their partitioning coefficient between the two immiscible liquid phases used. There are many types of CCC available today. These include HSCCC (High Speed CCC) and HPCCC (High Performance CCC). HPCCC is the latest and best performing version of the instrumentation available currently.

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.