**Freeze-drying,** also known as **lyophilisation,**  **lyophilization,**  or **cryodesiccation,** is a [dehydration](http://en.wikipedia.org/wiki/Drying_(food)) process typically used to [preserve](http://en.wikipedia.org/wiki/Food_preservation) a perishable material or make the material more convenient for transport.

Freeze-drying works by [freezing](http://en.wikipedia.org/wiki/Freezing) the material and then reducing the surrounding [pressure](http://en.wikipedia.org/wiki/Pressure) to allow the frozen water in the material to [sublimate](http://en.wikipedia.org/wiki/Sublimation_(chemistry)) directly from the solid phase to the gas phase.

## The origins of freeze drying

The [Andean civilizations](http://en.wikipedia.org/wiki/Andean_civilizations) preserved potatoes using a freeze drying process. They called this foodstuff Chuño

Freeze-drying was actively developed during World War II. [Serum](http://en.wikipedia.org/wiki/Serum_(blood)) being sent to Europe from the US for medical treatment of the wounded required refrigeration, but because of the lack of simultaneous refrigeration and transport, many serum supplies were spoiling before reaching their intended recipients.

The freeze-drying process was developed as a commercial technique that enabled serum to be rendered chemically stable and viable without having to be refrigerated. Shortly thereafter, the freeze-dry process was applied to penicillin and bone, and lyophilization became recognized as an important technique for preservation of biologicals. Since that time, freeze-drying has been used as a preservation or processing technique for a wide variety of products.

**These applications include the following but are not limited to:**

1. the processing of food,
2. pharmaceuticals,
3. diagnostic kits;
4. the restoration of water damaged documents;
5. the manufacturing of ceramics used in the semiconductor industry;
6. the production of synthetic skin; the restoration of historic/reclaimed boat hulls.

**The freeze-drying process**

There are four stages in the complete drying process: pretreatment, freezing, primary drying, and secondary drying.

**Pretreatment**

This may include concentrating the product, formulation revision (i.e., addition of components to increase stability and/or improve processing), decreasing a high vapor pressure solvent or increasing the surface area.

Methods of pretreatment include: Freeze concentration, Solution phase concentration, Formulation to Preserve Product Appearance, Formulation to Stabilize Reactive Products, Formulation to Increase the Surface Area, and Decreasing High Vapor Pressure Solvents

**Freezing**

In a lab, this is often done by placing the material in a freeze-drying flask and rotating the flask in a bath, called a shell freezer, which is cooled by mechanical refrigeration, [dry ice](http://en.wikipedia.org/wiki/Dry_ice) and [methanol](http://en.wikipedia.org/wiki/Methanol), or [liquid nitrogen](http://en.wikipedia.org/wiki/Liquid_nitrogen).

On a larger scale, freezing is usually done using a freeze-drying machine. In this step, it is important to cool the material below its [triple point](http://en.wikipedia.org/wiki/Triple_point), the lowest temperature at which the solid and liquid phases of the material can coexist. This ensures that sublimation rather than melting will occur in the following steps.

Usually, the freezing temperatures are between −50 °C and −80 °C. The freezing phase is the most critical in the whole freeze-drying process, because the product can be spoiled if badly done.

**Primary drying**

During the primary drying phase, the pressure is lowered and enough heat is supplied to the material for the water to [sublime](http://en.wikipedia.org/wiki/Sublimation_(chemistry)). The amount of heat necessary can be calculated using the sublimating molecules’ [latent heat of sublimation](http://en.wikipedia.org/wiki/Latent_heat). In this initial drying phase, about 95% of the water in the material is sublimated. This phase may be slow (can be several days in the industry), because, if too much heat is added, the material’s structure could be altered.

In this phase, pressure is controlled through the application of [partial vacuum](http://en.wikipedia.org/wiki/Vacuum). The vacuum speeds up the sublimation, making it useful as a deliberate drying process. Furthermore, a cold condenser chamber and/or condenser plates provide a surface(s) for the water vapour to re-solidify on. This condenser plays no role in keeping the material frozen; rather, it prevents water vapor from reaching the vacuum pump, which could degrade the pump's performance. Condenser temperatures are typically below −50 °C (−60 °F).

It is important to note that, in this range of pressure, the heat is brought mainly by conduction or radiation; the convection effect is negligible, due to the low air density.

**Secondary drying**

The secondary drying phase aims to remove unfrozen water molecules, since the ice was removed in the primary drying phase. This part of the freeze-drying process is governed by the material’s [adsorption isotherms](http://en.wikipedia.org/wiki/Adsorption_isotherm). In this phase, the temperature is raised higher than in the primary drying phase, and can even be above 0 °C, to break any physico-chemical interactions that have formed between the water molecules and the frozen material. Usually the pressure is also lowered in this stage to encourage desorption (typically in the range of microbars, or fractions of a [pascal](http://en.wikipedia.org/wiki/Pascal_(unit)" \o "Pascal (unit))). However, there are products that benefit from increased pressure as well.

After the freeze-drying process is complete, the vacuum is usually broken with an inert gas, such as nitrogen, before the material is sealed.

At the end of the operation, the final residual water content in the product is extremely low, around 1% to 4%.

**[](http://en.wikipedia.org/wiki/File:Freeze-Dried-Ice-Cream.jpg)Properties of freeze-dried products**

Freeze dried [ice cream](http://en.wikipedia.org/wiki/Ice_cream)

1. If a freeze-dried substance is sealed to prevent the reabsorption of moisture, the substance may be stored at [room temperature](http://en.wikipedia.org/wiki/Room_temperature) without refrigeration, and be protected against spoilage for many years. Preservation is possible because the greatly reduced water content inhibits the action of [microorganisms](http://en.wikipedia.org/wiki/Microorganism" \o "Microorganism)and [enzymes](http://en.wikipedia.org/wiki/Enzyme) that would normally [spoil](http://en.wikipedia.org/wiki/Decomposition) or degrade the substance.
2. Freeze-drying also causes less damage to the substance than other [dehydration](http://en.wikipedia.org/wiki/Dehydration) methods using higher temperatures.
3. Freeze-drying does not usually cause shrinkage or toughening of the material being dried.
4. In addition, flavours, smells and nutritional content generally remain unchanged, making the process popular for preserving food

**Applications of freeze-drying**

**Pharmaceutical and biotechnology**

Pharmaceutical companies often use freeze-drying to increase the shelf life of products, such as vaccines and other injectables. By removing the water from the material and sealing the material in a vial, the material can be easily stored, shipped, and later reconstituted to its original form for injection.

**Food industry**

Freeze-drying is used to preserve [food](http://en.wikipedia.org/wiki/Food), the resulting product being very lightweight. The process has been popularized in the forms of [freeze-dried ice cream](http://en.wikipedia.org/wiki/Freeze-dried_ice_cream), an example of [astronaut food](http://en.wikipedia.org/wiki/Space_food).

It is also widely used to produce essences or flavourings to add to food. Because of its light weight per volume of reconstituted food, freeze dried product is also popular and convenient for [hikers](http://en.wikipedia.org/wiki/Hiking).

[Instant coffee](http://en.wikipedia.org/wiki/Instant_coffee) is sometimes freeze-dried, despite the high costs of the freeze-driers used. The coffee is often dried by vaporization in a hot air flow, or by projection onto hot metallic plates.

Freeze-dried fruits are used in some breakfast cereal or sold as a [snack](http://en.wikipedia.org/wiki/Snack), and are an especially popular snack choice among [toddlers](http://en.wikipedia.org/wiki/Toddlers), [preschoolers](http://en.wikipedia.org/wiki/Preschoolers) and [dieters](http://en.wikipedia.org/wiki/Dieting), as well as being used by some pet owners as a treat for [pet](http://en.wikipedia.org/wiki/Pet) [birds](http://en.wikipedia.org/wiki/Birds). Culinary herbs are also freeze-dried, although air-dried herbs are far more common and less expensive.

**Technological industry**

In bioseparations, freeze-drying can be used also as a late-stage purification procedure, because it can effectively remove solvents. Furthermore, it is capable of concentrating substances with low molecular weights that are too small to be removed by a [filtration](http://en.wikipedia.org/wiki/Filtration) membrane.

Freeze-drying is a relatively expensive process. The equipment is about three times as expensive as the equipment used for other separation processes, and the high energy demands lead to high energy costs.

Furthermore, freeze-drying also has a long process time, because the addition of too much heat to the material can cause melting or structural deformations. Therefore, freeze-drying is often reserved for materials that are heat-sensitive, such as [proteins](http://en.wikipedia.org/wiki/Protein), [enzymes](http://en.wikipedia.org/wiki/Enzyme), [microorganisms](http://en.wikipedia.org/wiki/Microorganism), and [blood plasma](http://en.wikipedia.org/wiki/Blood_plasma). The low [operating temperature](http://en.wikipedia.org/wiki/Operating_temperature) of the process leads to minimal damage of these heat-sensitive products

In [bacteriology](http://en.wikipedia.org/wiki/Bacteriology) freeze-drying is used to conserve special [strains](http://en.wikipedia.org/wiki/Strain_(biology)).

Freeze drying is also used for floral preservation. Wedding [bouquet](http://en.wikipedia.org/wiki/Flower_bouquet) preservation has become very popular with brides who want to preserve their wedding day flowers[[3]](http://en.wikipedia.org/wiki/Freeze-drying" \l "cite_note-3)

Fast freezing by high pressure or other methods, freeze substitution (FS) is the most common way to process whole cells, tissues or organisms for electron microscopy. Freeze substitution acts to dehydrate then chemically fix samples at low temperatures in preparation for various treatments including embedding in resins.

Embedding permits cutting of thin sections, which is the most familiar way for most EM researchers to evaluate cell ultrastructure. By dehydrating and fixing cells at low temperature, some of the distortions that are common to conventional room temperature processing are avoided. However, as with conventional methods, different cells and tissues may require different FS processing methods to give optimal preservation of structure.

**The main variables to consider in designing a freeze subsitution protocol include:**

1. Which equipment to use for processing. The usual options are

1) and automatic freeze substitution (AFS) device. The virtue of the AFS machines is that they can be programmed for any temperatures and rates of warming the operator desires. Several vendors offer these.

2) Low temperature freezers (LTF). This option is probably the least used. While freezers are common enough in most labs, you may need 2 or 3 dedicated LTFs at different temperatures to process samples. Also, most labs prefer not to mix volatile fixatives with the antibodies and other proteins that are typically stored in LTFs.

3)Dry ice in an insulated box. This is the low-cost option and is suitable for a wide variety of samples, but it lacks the reproducibility of the AFS and LTF approaches.

2. The initial temperature and time. Many FS methods start with a temperature of -90˚C if a liquid nitrogen cooling system is used, or -78˚C if dry ice is the coolant. How long the material is held at these initial temperatures varies from a few hours to several days or more. Some researchers [1] like to start the procedure and -155˚C and warm slowly to the initial holding temperature of -90˚C.

3. Choice of organic solvent and additives. The most common FS fixative is 1-2% osmium tetroxide in acetone. A small percentage (0.1 – 0.5%) of uranyl acetate is often added. However, other solvents such as methanol may also be used [2-4]. Recently, the work of Walther and Ziegler [5] has promoted the addition of a small amount (3=5%) of water to the FS cocktail. For some cells, such as yeast, this water addition can have dramatic results on the visualization of

4. Warm-up rates between temperatures. Warm up rates between starting and ending (or intermediate) temperatures are typically in the range of 1-10˚C per hour.

**Freeze-substitution**

Freeze-substitution is a physicochemical process in which biological specimens are immobilized and stabilized for microscopy. Water frozen within cells is replaced by organic solvents at subzero temperatures. Freeze-substitution is widely used for ultrastructural and immunocytochemical analyses of cells by transmission and scanning electron microscopy.

Less well recognized is its superiority over conventional chemical fixation in preserving labile and rare tissue antigens for immunocytochemistry by light microscopy. In the postgenome era, the focus of molecular genetics will shift from analyzing DNA sequence structure to elucidating the function of gene networks, the intercellular effects of polygenetic diseases, and the conformational rearrangements of proteins in situ.

Novel strategies will be needed to integrate knowledge of chemical structures of normal and abnormal macromolecules with the physiology and developmental biology of cells and tissues from whole organisms. This review summarizes the progress and future prospects of freeze-substitution for such explorations.