Concentration of Solutes: Options for the Removal of Solvents from Samples

Introduction

Solvent removal from solutions is commonly performed in laboratory and production processes. There are a number of different methods that can be used for solvent removal, including evaporation, vacuum concentration, lyophilization, reverse extraction, solute precipitation, and dialysis (solvent exchange). The objective of solvent removal is to preserve solutes or to concentrate solutes for analysis. It may also be used as a step in the synthesis or modification of solutes.

Below is a brief overview of different options for removing solvents.

Evaporation

Solutes that are not volatile can be concentrated by drawing the solvent into a gaseous head space. Two approaches can be used for solvent removal, one by applying heat or a vacuum, and the other by directing a stream of inert gas over the solvent. In the latter approach, the gas extracts solvent from the liquid phase by dissolving it into a gaseous stream, diluting it into the atmosphere. This is the basis of gas chromatography, a gaseous mobile phase dissolving solutes and solvents. As gas flows, decreased concentration of the vapor-phase solvent molecules shifts the vapor/liquid phase equilibrium. This process draws more liquid solvent into the vapor phase. By streaming the gas, the evaporation rate is increased. Evaporation systems, such as the Labconco RapidVap Evaporation Systems, direct a gas stream of of inert nitrogen or compressed air over the sample. The RapidVap Evaporators have the option of applying heat and vortex motion to the sample to help accelerate the evaporation process..

Vacuum Concentration

The removal of solvents can be effectively accomplished by boiling. Unfortunately, many solutes such as proteins are destroyed by the heat required to drive off solvents. Solvents can boil by either applying heat or by lowering the atmospheric pressure. In both cases, the energy of molecular motion is greater than the intermolecular forces holding the molecules in solution. When applying a vacuum, or heat, the force by which the molecules move from liquid to gas causes the solution to splatter, also called *bumping*. Bumping may cause loss and/or cross contamination between samples when multiple tubes are positioned together. In

vacuum concentration devices, such as the Labconco CentriVap Centrifugal Concentrators, a vacuum pump is attached to an airtight, low speed centrifuge that prevents bumping by forcing the liquid down into the tube. The system can run at high vacuum levels to speed solvent removal. The Labconco CentriVap Centrifugal Concentrators have the option of regulating chamber temperature, which is useful for regulating sample temperatures at lower vacuum pressures.

Lyophilization

The process of lyophilization uses a pre-frozen sample to remove moisture through sublimation vs. evaporation. The solution must be 100% pre-frozen before the vacuum is applied. Pre-freeze, primary drying and secondary drying. The first stage, pre-freezing, is critically important to the overall process. Slow freezing of a sample causes large ice crystals to form which makes freeze drying easier, but may denature many temperature sensitive proteins. Freezing a sample results in small ice crystals which can slow the freeze dry process. Many proteins retain activity when flash frozen. The second stage, primary drying, occurs when the sample temperature is raised sufficiently to allow heat to flow into the frozen solution and drive the sublimation process. During primary drying, if the temperature increases too much, the sample can thaw and collapse. Primary drying removes 90% or more of the solvent. Secondary drying, the third stage, is done by increasing sample temperature to drive off the additional bound moisture. Heat can be increased because the bulk of the solvent is removed during primary drying and the risk of melting is lessened. For instance, mannitol undergoes primary drying at temperatures below -23°C depending on the formulation, while secondary drying is as high as 40°C.

Lyophilizers, such as the Labconco FreeZone® Freeze Dry Systems, are an extremely effective tool for removing large volumes of solvents while retaining activity of sensitive solutes. Freeze drying is very effective for concentrating and preserving biologically active proteins.

Reverse Extraction

Though extraction is traditionally viewed as a method to transfer a solute from one liquid phase or solid, to another, it can also be used for solvent removal. The classic example of this approach is concentrating small volumes of DNA in aqueous buffer by adding dry n-butanol. Water is miscible with the alcohol while DNA is not. The net flow of water into the butanol phase results in a higher concentration of DNA in the remaining, original aqueous buffer.

Dialysis

Semi-permeable membranes have been used for decades for removing small solutes (primarily salts) and solvents from solutions. Centrifugal concentration through a semi-permeable membrane and dialyzing solvents by mass action are typical methods for concentrating solutes by dialysis. In both cases, membranes with controlled pore size allow low molecular weight solutes and solvents to pass through the membrane while retaining the larger molecules. Centrifugal concentrators use centrifugal force to push the solution through the membrane while dialysis utilizes diffusion. Solvents can be removed by dialysis against concentrated solutions containing large molecular weight compounds or against a substance in the solid phase miscible in the dialyzed solvent. For example, water can be removed from a solution housed in dialysis tubing by placing the tubing in starch. As long as the pore size in the dialysis tubing is too small for a starch molecule to pass through, the water will move from the sample and solubilize the starch.

Precipitation

Altering a solution to render solutes insoluble is a long used method for concentration. This approach is routinely used to concentrate DNA by precipitation with salt and ethanol, and *salting out* proteins from solution by adding ammonium sulfate. Precipitation is practical if the solute is inert to the precipitation conditions. However, there is the possibility that biologically active molecules with labile structural motifs may become denatured and lose activity. Precipitation is especially practical for concentrating robust biomolecules such as carbohydrates, nucleic acids and many structural proteins.

Conclusion

Each of these processes will remove solvents from your sample. Choosing the right one involves many factors. If your samples can tolerate heat, a vacuum concentrator or evaporator is a good option. If the samples need long term storage, a lyophilizer is the best option. Make sure you know your sample prior to choosing your concentration process.

LABCONCO CORPORATION
8811 Prospect Avenue
Kansas City, MO 64132
(800) 821-5525 | (816) 333-8811
labconco.com

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