Banishing the mysteries of evaporation and concentration

Part I of this two-part article explains the basic theories of evaporation and concentration, and reviews some of the commonly used techniques. The second part of the article will review the wide variety of hardware used in these applications, including pumps, cold traps and centrifuges.

True evaporation is the vaporisation of the solvent without it boiling. In many 'evaporators', including vacuum concentrators, the solvent is actually boiling rather than evaporating. Systems are generically referred to as evaporators as a convenient shorthand.

Vacuum concentrators rely on the vacuum in the system to cause the liquids to boil at lower temperatures than they would at atmospheric pressure. For example, water boils at 100°C at room temperature and pressure. By reducing the pressure, the temperature at which water boils drops, eg at 10mbar water boils at +7.5°C. In a freeze dryer with a very strong vacuum pump, the pressure is taken well below 0.5mbar, and at this pressure pure water boils at -31°C and therefore will freeze. In a freeze dryer, the solvent neither boils nor evaporates, but it sublimes, ie it passes from the solid phase to vapour phase without passing through the liquid phase.

Method of choice

The broad range of applications requiring solvent removal has led to the development of a wide range of commercial evaporation systems. Different applications require the use of specific sample formats and solvents, and it is not surprising that no one solvent removal technique or system offers a universal solution. This article reviews the most common methods of concentration and evaporation to provide scientists with a better understanding of the different techniques to enable them to make informed choices when sourcing equipment.

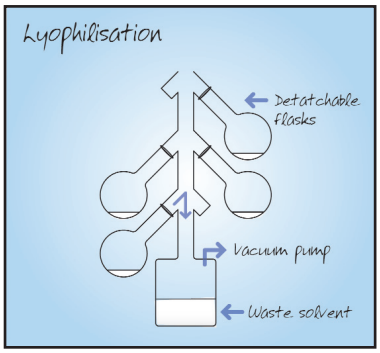
Freeze drying: Freeze dryers sublime solvent from the frozen samples to leave the product as a loose 'fluffy' powder. They often employ a deep vacuum and, due to the large surface area of the product, achieve a very high level of dryness.

There are two basic types of freeze dryers, those that actively freeze samples, and those that do not. Systems that actively freeze samples have chilled shelves, similar to a standard laboratory freezer, on which the samples are placed. Passive systems usually have a 'tree' to which samples in flasks are attached. The samples are loaded into the flasks in small vials, or as a liquid directly into the flask.

Freeze dryers have a refrigerated cold trap to collect the solvents but they are poor at preventing 'solvent bumping' which leads to loss of sample and cross-contamination of samples. Samples may be pre-frozen to help prevent bumping, however, preventing bumping by freezing alone can be demanding. If samples contain volatile solvents then they must be actively frozen at very low temperatures, eg acetonitrile freezes at -47°C, methanol freezes at -96°C.

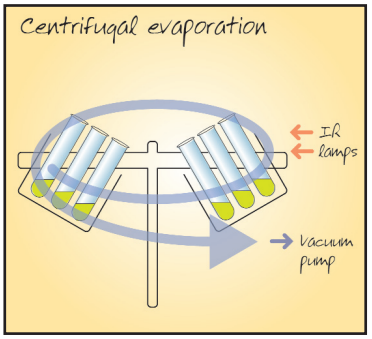
Centrifugal concentrators: Centrifugal concentrators are very similar to freeze dryers, they often have cold traps to catch the solvent and have a vacuum pump to enable boiling at low temperature. The crucial difference is that they generally do not freeze the sample (unless high vacuum is used when concentrating water-containing samples), and this means that they are much faster than a freeze dryer.

The centrifuge is crucial to prevent sample loss, and to prevent cross-contamination of samples. It ensures that the solvent boils from the surface level of the sample downwards. Solvent at the surface of the liquid will be at the pressure in the evaporator, whereas solvent below this level will be additionally subject to the extra weight of solvent multiplied by the g-force of the spinning rotor pushing on it, and therefore will be at a higher pressure. This means that the solvent at the surface boils first and boiling over is prevented. Systems with very high rotor speeds of 500g or more are proven to prevent bumping. More advanced centrifugal concentrators can also be used to achieve rapid freeze drying by first concentrating the majority of the sample before freeze drying the last few millilitres.



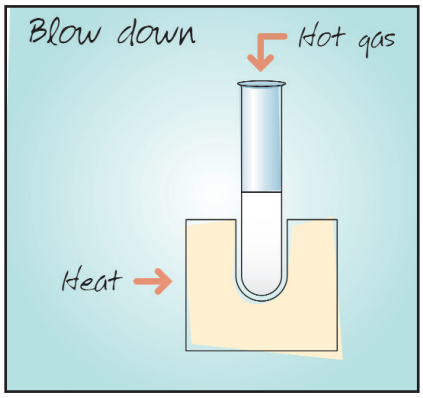
Freeze drying gives excellent dryness and easy-to-redissolve samples.

Blow down systems: In a blow down evaporator system, an inert gas, such as nitrogen, is blown down on to samples in tubes, vials or microplates through needles, creating a flow over the surface of the liquid. This alters the equilibrium between vapour phase and liquid phase to favour the vapour phase. Heat is normally applied to the samples to hasten evaporation, and often warmed gases are used. Blow down can be quick if the solvents are volatile, but is often slow with high-boiling-point or difficult-to-evaporate samples. Typically blow down achieves poor final dryness and does little to prevent cross-contamination between samples. Blow down is commonly used for concentrating a large volume to a few millilitres, specialised teat-ended tubes are available for this process, and some manufacturers have systems that detect when the sample is concentrated into the teat, and stop the process automatically.



Centrifugal evaporation induces boiling at low temperature by drawing vacuum on the sample.

Vortex evaporators: Vortex evaporators are based upon boiling of



Blow down evaporation is a fast method for concentrating volatile solvents.

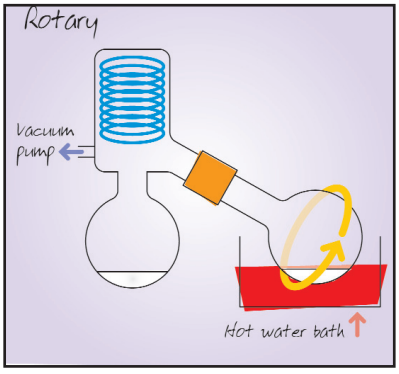
solvents aided by swirling the sample tubes to create a vortex. The vortex is beneficial in aiding drying as it increases the surface area of the sample. However, on the downside, the resultant dried sample is deposited all over the sides of the tube. While swirling tubes helps prevent bumping, it is by no means a foolproof system because of the insufficient g-force generated. Some vortexing systems have heating lamps that shine directly into the sample tubes, enabling very rapid evaporation. Such systems are, however, prone to overheating the sample (or portion of sample) which is dry. A rotary evaporator is essentially a form of vortex evaporator for a single sample contained in a flask.

The difference between temperature and heat

The terms temperature and heat, although directly linked, relate to quite different things and are often confused with one another. Heat refers to heat energy and is measured in Watts. Temperature is a measure of the level of heat energy within the object being measured. Commonly researchers state that they have heat-sensitive samples and are concerned about heating of the sample. In actuality they have temperature-sensitive samples and are concerned about samples going above a specific temperature threshold. The reality is that a majority of samples can be heated without degradation but the overall temperature must be kept within bounds.

The relationship between heat and temperature is demonstrated using specific heat, in the following equation:

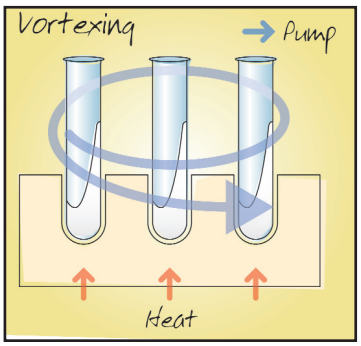
$Q = cm\Delta T$, where Q is heat added; c is the specific heat of the object being heated; m is the mass of the object; and ΔT is the change in temperature. Therefore, ΔT can be expressed in terms of heat added as follows: $\Delta T = Q/cm$. These equations hold when all other parameters remain the same. When a phase change occurs, eg boiling, the heat added does not change the temperature but is used to change the phase. With truly evaporative systems, ie those that do not boil the sample, the sample will be at the temperature of the system that controls it. By comparison, in freeze dryers which actively freeze the product, the sample will be at the temperature at which it is frozen.



Rotary evaporation is an excellent method for use with single samples in flasks.

Vacuum evaporators that rely on boiling the solvent, and freeze dryers that do not actively freeze, are in a dynamic state. When a sample is wet and boiling, the sample is at the boiling temperature of the liquid. When all of the solvent is removed the sample will warm up to the temperature of the system. Therefore, while samples are wet, it is possible to heat the system up to very high temperatures and the samples will not reach this temperature until the solvent is completely removed.

When handling temperature-sensitive samples it is important to make sure that the system being used can accurately control or limit the temperature the sample reaches. Temperature control over the sample holder will give ultimate protection for the sample as it cannot be at a higher temperature than the holder, unless it is being heated directly and independently of the holder. This normally necessitates using solid metal holders constructed from materials such as aluminium.



Vortex evaporation is similar to rotary evaporation and uses vacuum and swirls to increase area and speed up drying.

What controls the speed of concentration?

When solvents are boiling, the faster the operator can supply energy (in the form of heat), the faster solvents will boil. As well as heat energy, the faster the operator can remove vapours, the faster the solvents will boil. While samples are wet they are at the boiling temperature of the solvent. Therefore, the better able a system is at getting heat into boiling samples, the faster will concentration occur, and the better able a system will be at removing vapours by condensing them in a cold trap, or removing them with a pump, or both. Generally, it is not true to say that a higher vacuum level gives faster evaporation.

With evaporative systems, the principle of more heat equalling faster evaporation holds. However, samples will be at the set temperature, unlike samples that are boiling. Also, the greater the surface area achieved, the faster evaporation occurs. A blow down system speeds up evaporation of the solvents by blowing gas, sometimes pre-warmed, on to the samples in solution. This increases the rate of evaporation by shifting the equilibrium to favour the solvent existing in the vapour phase, rather than in the liquid phase. However, if the flow rate is too high, splashing may occur. In a freeze dryer, vapour flow rate is the controlling factor - the faster the vapour can be removed, the faster the samples dry. This is usually most affected by pump rate. sp^2

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