

Understanding Evaporation and Concentration Technologies

Dr Induka Abeysena, Application Specialist & Rob Darrington, Product Manager, Genevac Ltd, Ipswich, UK.

This paper explains the basic principles of evaporation and concentration, and outlines some of the commonly used technologies. It also reviews the wide variety of systems used in evaporation, including pumps, cold traps and evaporators themselves.

Introduction

Solvent removal is an essential process across a broad range of applications in the pharmaceutical, chemical and biotechnology industries. A diversity of sample formats and solvents is used with no single technique for solvent removal providing a universal solution. Various commercial evaporation and concentration systems have been developed to accommodate the range of applications. These systems and associated hardware, vacuum pumps, cold traps and heating technologies, have recently benefited from exciting developments in freeze drying and centrifugal concentration technologies enabling enhanced evaporation performance and improved sample integrity. Further advances with new generation high-power cold traps are offering improved solvent recovery, thereby reducing the environmental impact of the evaporation / concentration process.

Having an up-to-date understanding of the processes of evaporation and concentration, their application at a practical level and using the latest equipment enables optimisation of protocols for improved and more rapid sample concentration.

Processes Involved With Solvent Removal

During solvent removal, energy is applied as heat such that the liquid is vaporised to gas, which is removed to leave a concentrated or solvent-free (dry) product. Many systems are referred to generically as 'evaporators'. However, true evaporation is vaporisation to gas at the liquid surface; for many "evaporators", boiling occurs rather than evaporation. The process of freeze drying involves neither evaporation nor boiling, but sublimation; that is, a shift from solid to vapour phase without a liquid phase.

The phase of a substance is determined by two major factors – heat and pressure – and the temperature at which boiling or vaporisation occurs is set by the pressure. Therefore, vacuum concentrators apply vacuum in the system to decrease a solvent's boiling point, such that liquid vaporisation occurs at lower temperatures, e.g. water boils at 7.5°C at 10 mbar pressure. Similarly, in freeze dryers, heat energy supplied to a frozen sample at low pressure transfers sufficient energy for thawing but the pressure is insufficient for liquid formation, and hence the solvent sublimates to gas. Generated vapour is removed by a cold trap or condenser, where solvent is recovered.

Heat and Temperature

Solvent removal systems use an input of heat energy to induce solvent vaporisation, various heating mechanisms are employed, such as electrical heating blocks, lamps or low-temperature steam. Heat and temperature, although linked, are different and distinguishing between them is important. Heat refers to heat energy measured in Joules, whereas temperature measures the level of heat energy, i.e. the hotness or coldness of an object. Samples referred to as heat-sensitive are typically temperature-sensitive and a majority of samples can be heated without degradation provided temperature remains within defined boundaries. Applying vacuum in a system decreases a solvent's boiling point, such that liquid vaporisation occurs at lower temperatures that are safe for the sample.

Heat and temperature are related by the equation $Q = mc\Delta T$, where Q is heat energy added, m is the mass of the object, c is the specific heat capacity of the heated object, and ΔT is the change in temperature. ΔT can be expressed in terms of heat added as $\Delta T = Q/mc$. These equations hold true when all other parameters remain the same. However, at a change in phase, added heat energy does not increase temperature since energy is required for the change in state, for example from liquid to gas. Therefore in true evaporative systems (no boiling), a sample is at the temperature of the system controlling it; whereas in a freeze dryer that actively freezes products, the sample is at the temperature at which it is frozen and then its temperature is governed by the ice sublimation temperature controlled by the vacuum level.

The opposite dynamic exists in vacuum concentrators that boil solvent. When a sample is wet and boiling, the sample is at the boiling temperature of the liquid. Figure 1 shows the relationship between boiling point and pressure for some common solvents. At this stage, it is possible to heat the system to high temperatures and a sample will not reach this temperature until the solvent is completely removed. Only when all solvent is removed will a sample warm to the temperature of the system. Accurate control and monitoring of sample temperature is

therefore essential. Typically control of the sample holder temperature offers effective protection, as the sample within cannot exceed this temperature unless the sample is heated directly and independently of the holder. To enable this necessitates using solid metal holders constructed from materials such as aluminium that can transmit the maximum amount of heat into the sample and are cooled by evaporating solvent. Real-time temperature monitoring is built into some systems, enabling accurate determination of when evaporation is complete thereby avoiding overheating of samples.

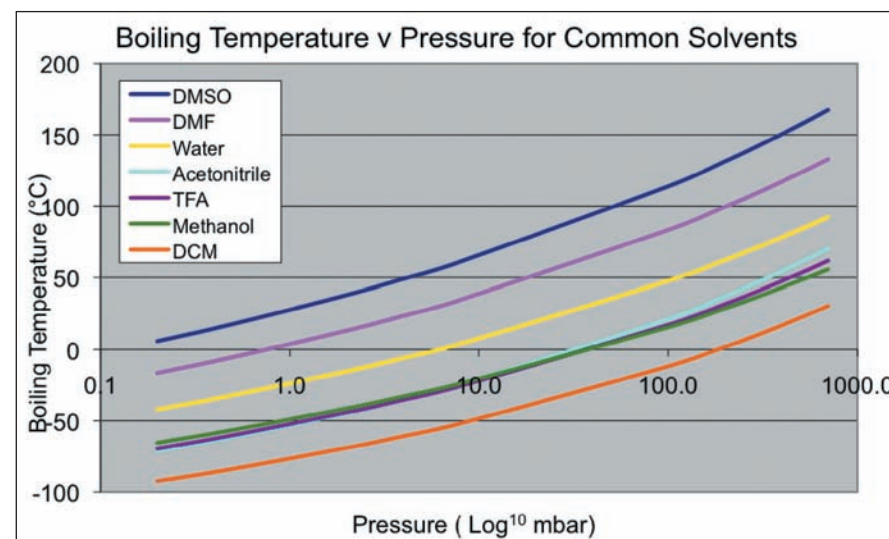
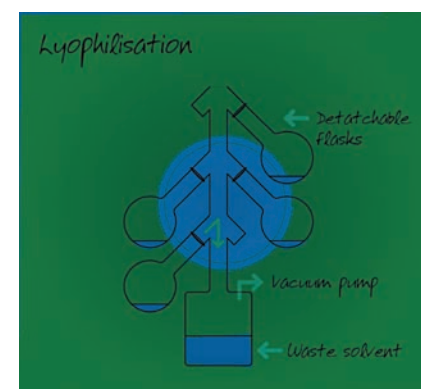


Figure 1. Relationship between pressure and boiling point for some common solvents

Different Drying Methodologies

Freeze Drying

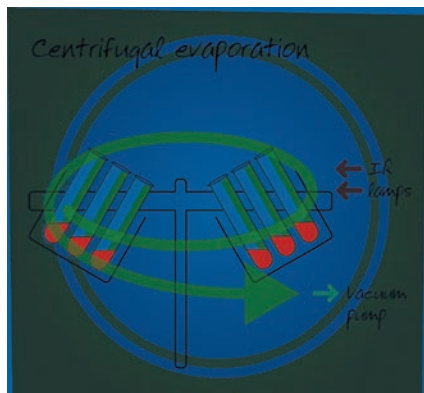
Freeze dryers are available as two basic types: one that actively freezes samples placed on chilled shelves similar to a laboratory freezer; the second type of (passive) systems do not actively freeze and instead utilise a manifold with attached flasks that contain sample either directly or within vials. A high vacuum is often employed such that samples remain frozen and hence well preserved as solvent sublimates and is collected in the cold trap. A typical freeze dried product is a diffuse 'fluffy' powder that has a very high level of dryness (due to the large surface area available for solvent removal) and is easy to weigh and redissolve. Some samples, such as DNA, may require careful handling during movement to avoid loss of the fine powder. Freeze drying is a comparatively slow batch process, although a range of configurations are available that can accommodate large batches of samples per cycle. Solvent bumping may occur, although this can be reduced by pre-freezing samples where feasible. The freezing process limits the technique to aqueous solutions or one of a few simple organic solvents that freeze easily e.g. tertiary butanol or 1,4-dioxane. Samples containing volatile solvents must be actively frozen at very low temperatures, which may demand vacuum control at very low pressure and be so cold that the condenser functions inefficiently.



Centrifugal Concentration

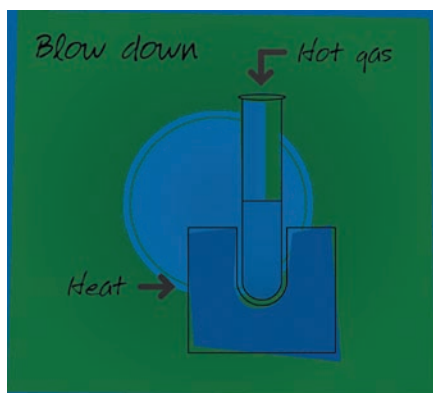
Centrifugal concentrators induce solvent boiling under vacuum and hence samples are cold but, in contrast to freeze dryers, not frozen, and so the process can be faster than freeze drying. Care has to be taken with centrifugal evaporation of aqueous samples that are prone to freezing. Centrifugal evaporators use cold traps to recover the vapourised solvent.

Centrifugation ensures that solvent boils from the sample surface downwards, thereby minimising boiling over and solvent bumping thereby preventing sample loss and cross-contamination. Solvent at the liquid surface is at the pressure of the equipment, whereas solvent below this level is at higher pressure due to the extra weight of solvent multiplied by the g force exerted by the centrifuge rotor. Systems with very high rotor speeds generating 500g or more are proven to prevent solvent bumping. The centrifugal evaporation technique accommodates a wide range of solvents and can concentrate, dry to a film or freeze dry samples.



Advanced centrifugal evaporation systems can achieve rapid freeze drying by concentrating the majority of a larger volume before freeze drying the final few millilitres of sample. Samples must be processed in batches although large numbers of small samples can be processed at the same time. Although early systems were viewed as slow, recent developments in high-energy sample heating using low-temperature low-pressure steam, such as employed by the Genevac Rocket™ Evaporator, enable rapid concentration of larger volumes at the speed of a rotary evaporator. Such systems operate without the solvent bumping and consequent need for continual monitoring associated with rotary evaporators.

Blow-Down Evaporation



In these evaporator systems, an inert gas such as nitrogen is blown down through needles onto samples in tubes, vials or microplates to create a flow over the liquid surface. This alters the equilibrium between the vapour and liquid phases to favour the vapour phase. Heat is normally applied to the samples to hasten evaporation and pre-warmed gases can also be used. The technique enables open access equipment use, and is comparatively inexpensive ranging from self-assembled apparatus to simple commercial systems. Although blow down evaporation is relatively fast for volatile solvents, it can be slow for

solvents with high boiling points or those that are difficult to evaporate such as water. Using blow down evaporation samples are prone to becoming hot during the process, being at the temperature of the heating block or bath during evaporation, and consequently the technique offers poor recovery of volatile analytes. As a manual process – blow down evaporation requires continuous monitoring by the user to detect the end point of the drying process. The technique typically achieves poor final dryness and splashing may occur, particularly if the gas flow rate is too high, leading to sample cross-contamination. A common use of blow down is for concentrating large volumes to just a few millilitres for subsequent processing by other techniques. Specialised teat-ended tubes are available for this procedure and some commercial systems have basic automatic stop mechanisms based on liquid level detection.

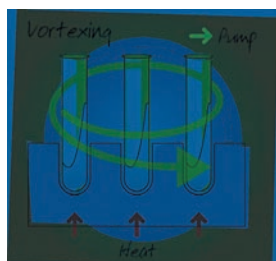
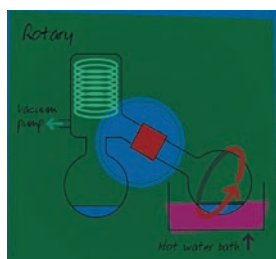
Vortex Evaporation

These systems boil batches of samples under vacuum, to keep the samples cold throughout vaporisation, while swirling the sample tubes to create a vortex.

A rotary evaporator is essentially similar, but is for a single sample contained in a flask. The vortex created generates a large sample surface area for evaporation, making the process relatively fast.

However, the resultant dried product is spread across the vessel walls, which can make sample recovery more difficult. Moreover, in contrast to centrifugal concentrators, the swirling movement generates insufficient g force to prevent solvent bumping and hence vortex evaporators are prone to sample loss and cross contamination.

In some vortex systems, evaporation is further accelerated by use of heating lamps directed into the sample tubes, but these systems are prone to overheating all or part of a sample as it becomes dry.



Factors Affecting the Speed of Evaporation

Three factors affect the speed of concentration: heat energy supply, vapour removal and solvent surface area. For boiling solvents, the faster heat energy is supplied the faster solvents boil. Similarly for evaporative systems, more heat equals faster evaporation, although samples are at the set system temperature rather than boiling where the boiling point of the solvent controls the sample temperature.

Heat is supplied by lamps, heated blocks/baths or, in the latest generation of centrifugal systems, by low-temperature low-pressure steam. In these latest systems, water takes heat from the chamber wall, turns to vapour, then condenses on sample vessels (which are cold due to solvent boiling within) to transfer the heat to the samples.

The heating vapour reaches all areas, minimum heat is wasted and the time to reach the required temperature is very rapid, thereby improving the efficiency of concentration.

In addition to the effects of heating rate, the faster vapours can be removed the faster solvents will boil. As a wet, boiling sample is at the boiling temperature of the solvent, then the better a system is at getting heat into the sample, the faster will concentration occur, and the more efficient a system will be at removing vapours by condensation in a cold trap. However, although speed of concentration can increase with higher vacuum level, this remains true only to a certain point. At the highest vacuum levels achievable by modern evaporator systems, a volatile solvent will boil at extremely low temperatures that are so cold that a cold trap cannot effectively catch the solvent and is hence rendered useless.

For optimal system performance, a balance is therefore essential between the heat input to the sample and the heat removal at the condenser. If a system is not balanced, either the cold trap does not capture solvent such that solvent goes to the pump, the pressure in the system rises and solvent recovery is lost – or the cold trap effectively controls the pressure such that the system operates at a slower rate than optimal. In a freeze dryer, vapour flow rate is the controlling factor, such that the faster vapour is removed, the faster samples dry. This is most affected by cold trap power and temperature. Finally, concentration or evaporation can be accelerated through generating a greater solvent surface area, and blow down systems speed up evaporation through a shift in equilibrium between phases.

Latest Developments in System Technology

An understanding of the evaporative process and factors affecting it, involving careful selection of a vacuum pump, cold trap or condenser and consideration of pressure control, is therefore key to obtaining a balanced system that ensures optimum performance in solvent removal and solvent recovery.

Vacuum Pumps

Vacuum evaporative systems, including freeze dryers and centrifugal concentrators, require a vacuum source. This may be a tap aspiration pump, traditional vacuum pump or new-generation dry pump.

The use of tap aspiration pumps is declining due to their negative environmental impact – there is both high water usage and solvent vapour condenses in and contaminates the water going to waste.

Traditional vacuum pumps can provide good performance, attaining pressures below 0.02 mbar with a high flow rate. However, the mineral oil used to lubricate the pump vanes is “messy” and over time can be degraded by solvent vapours leading to loss of pump performance or pump seizure.

The most modern diaphragm vacuum pumps are now very reliable and also solvent resistant, however their effective ultimate vacuum level is 1 mbar or 2 mbar which means they are ideal for working with volatile solvents, but not suitable for drying high boiling point solvents or freeze drying as their vacuum is insufficient.

Newer, dry scroll pumps do not require any pumping fluid or lubricant and are suitable for use with evaporating and concentrating systems. Dry scroll pumps (Figure 2) also provide high performance, down to pressures of 0.07 mbar at high flow rates, but with very low maintenance and minimal environmental impact compared with traditional vacuum pumps or tap aspiration.



Figure 2. Scroll Pump

Cold Traps and Condensers

A cold trap or condenser is used to capture the solvent removed by concentration or evaporation systems. The cold trap should not impede vapour flow, be easy to clean, and prevent solvent reaching and attacking the vacuum pump.

Additionally, an efficient and well-designed cold trap offers the advantages of good solvent recovery and an accelerated evaporation process through its contribution to the vacuum generated. When solvents vaporise there is huge volume expansion, of the order of 20,000 times. As vapours condense back to liquid at the cold trap, the volume reduction helps pull a vacuum.

Traditionally, a cold trap has comprised a stainless steel vessel with cooling coils around the outside that is positioned in the vapour path between the concentrator and the vacuum pump and chilled to below 0°C by a gas compressor system. Although effective, these cold traps can be awkward and time-consuming to empty. To remove condensed solvent, particularly water that freezes to ice, these cold traps must be defrosted before emptying, adding to the downtime of the system.

To overcome this obstacle, some systems use interchangeable glass flasks that are placed within the stainless steel vessel. At the end of the concentration process, the condensate-containing flask can be removed and substituted with a fresh flask. However, successful chilling of the glass flask relies on a silicon-based thermal transfer fluid between the stainless steel vessel and the flask that causes the flask exterior to become slippery and potentially hazardous to handle, and hence this method has not proved popular.

SPOTLIGHT

feature

Sample Preparation & Processing

These problems of defrosting and interchanging slippery flasks are avoided in simple evaporator systems. Rotary evaporators, for example, collect removed solvent as a liquid in a glass flask, using a glass condenser chilled with cooling water or dry ice. The principle of the simple Graham condenser has recently been applied to cold trap technology. These new generation gas compressor cold traps, such as the miVac Speed Trap™ (Figure 3), have cold coils suspended in the vapour path; solvents condense on the coils and are collected directly as liquids into an insulated glass vessel on the front of the trap. These cold traps deliver up to 50% more condensing power than earlier designs, thereby providing higher solvent recovery, and require no cooling water or dry ice to operate. Moreover, the glass flask is easily removed for rapid transfer of solvent to waste, and can be replaced immediately without having to wait for the system to defrost.

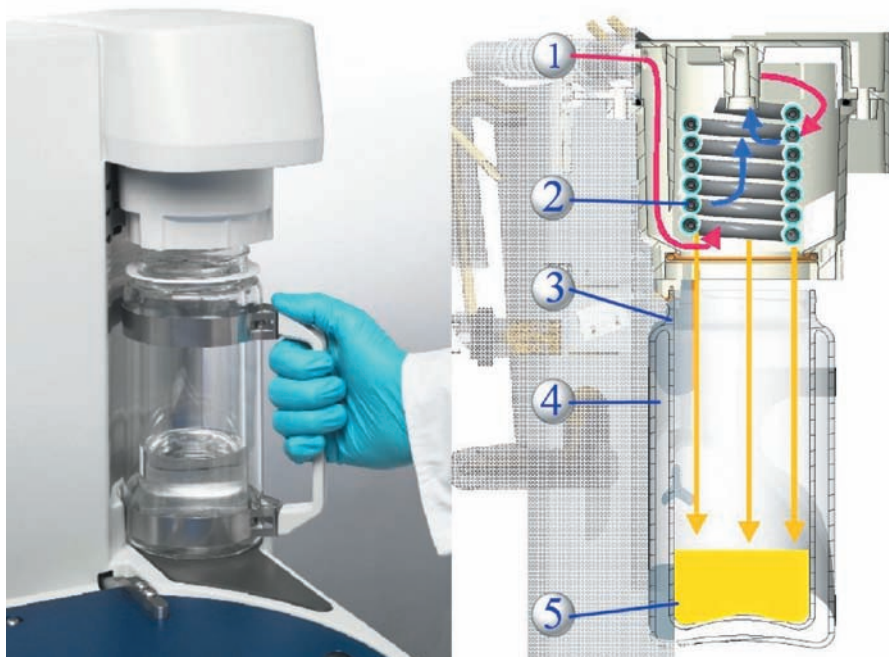


Figure 3. miVac SpeedTrap Design - 1. Hot vapours enter. 2. Condensing coils with ice shell. 3. Glass collecting flask. 4. Vacuum insulation. 5. Solvent collects as a liquid.

Condensing Power

For an efficient cold trap, condensing power is more important than low trap operating temperature. Traps running at very low temperatures (e.g., -80°C to -100°C) often consume almost all of the available power to attain these extreme temperatures rather than to condense solvent vapour. Such systems may be adequate for freeze drying which is a relatively slow process, however they are inefficient as cold traps for high speed concentration due to their limited condensing power. Gas compressors provide cold traps with maximum condensing power down to approximately -20°C (Figure 4); beyond this, condensing power declines rapidly. Optimal performance from a cold trap is therefore best attained by controlling the boiling point of solvents to -20°C or higher to ensure that the gas compressor system of the trap operates with full condensing power. Thus, to optimise the solvent recovery process in a vacuum system, it is critical to consider vacuum pump and cold trap function and to have a pressure controller and knowledge of the solvents used.

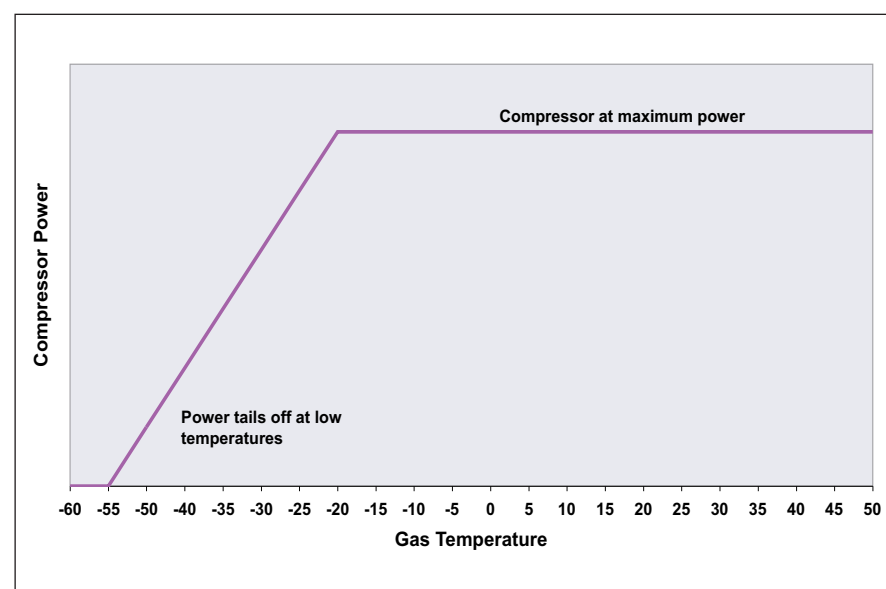


Figure 4. Compressor Power v Gas Temperature

The Importance of Pressure Control

Pressure control in a vacuum evaporation system is critical for (1) ensuring optimum trapping of evolved vapours; (2) speeding up the evaporation of complex mixtures; and (3) preventing sample loss by sublimation.

Control of solvent boiling point is achieved by controlling the pressure and manipulating the pressure to attain a boiling point of -20°C (see above). A gas compressor cold trap operates at maximum condensing power and highest efficiency for trapping evolved vapours, and samples remain in a typically preferred cold state. Operation at higher temperatures is feasible although likely to be slower due to reduced heat input to samples.

Sample freezing is undesirable in concentrator systems as this slows down evaporation, therefore, pressure should be kept higher to attain an appropriate boiling point. For instance - water will freeze if evaporated below 6mbar, the optimum pressure for water concentration is 8 mbar, at this pressure water boils at $+4^{\circ}\text{C}$.

In the case of complex mixtures (e.g. high performance liquid chromatography (HPLC) fractions) where often both water and an organic solvent are present, the organic solvent must be removed without freezing the water, or evaporation is very slow. This can be achieved with correct pressure control. Detailed technical guidance for optimising these specific types of applications is generally available from the leading evaporator / concentration system manufacturers.

Most samples can become volatile under the right conditions. Generally, the smaller the size of a molecule the easier it is to volatilise, and this is especially true for organic molecules.

However, when a sample is of low molecular weight (less than 300) and/or has high volatility – for example, a straight-chain organic molecule with few side groups – then some sample may also be lost through sublimation during the evaporation process. Good pressure control can prevent this sublimation and it is important to stop the evaporation process as soon as the samples are dry. Control measures to achieve this have been developed for some systems.

Summary

Today a wide range of evaporation and concentration systems are available to accommodate the diversity of applications and samples requiring solvent removal. The correct choice of vacuum pump and cold trap is critical to ensuring optimum evaporation and concentration performance. Pumps with appropriate vacuum level and having high flow rates are recommended. Highly efficient cold traps are now available that not only speed concentration and drying rates, but also recover solvents in liquid form thereby reducing environmental impact and eliminating time lost to defrosting procedures.