



## Freeze Drying Adviser Basics and Applications

This freeze drying guide includes background information, practical tips and application examples providing the necessary understanding for successful freeze drying in your laboratory. With the Lyovapor™, BUCHI offers the first laboratory freeze dryer with unlimited capacity and maximum user convenience by Infinite-Technology™ and Infinite-Control™. Learn more about freeze drying and benefit from the market leader in laboratory evaporation.

Freeze drying is the most gentle process to dry various types of perishable materials. The principle of freeze drying is based on the direct transition of a substance from the solid to the gaseous state, called sublimation. Initially, the product is frozen and then dried by sublimation in an environment of reduced pressure. The low pressure enables direct changeover of the frozen solvent into vapor. In most freeze drying applications, the solvent to be removed from the product is water. However, other solvents can be used to a certain extent, such as ethanol.

Freeze drying is mainly used for preservation of delicate materials that are subject to degradation or decomposition. Product stability is massively increased by reducing its water content due to the direct link between water presence and biological and chemical activity which are mainly responsible for product degradation. Compared to other dehydration methods, freeze drying causes less product damage and avoids shrinkage or agglomeration of the material (Figure 1). Common preservation methods use high temperatures that affect the shape and colour of a product, as well as its flavour, smell and nutritional content. Freeze drying is a very gentle process and particularly suitable for heat-sensitive and precious products. Freeze drying is applied in many different areas with various objectives, such as preservation of product characteristics (e.g. pharmaceuticals),

maintaining the initial shape (archaeology, flowers) or product conditioning for further use (e.g. fruits in yogurt).

The initial freezing process creates ice crystals within and on the surface of the product. By turning into ice, the individual water molecules lock up into a well defined grid (Figure 2). As the water molecules sublime from the product, they leave little pores and gaps within the product and thus maintain its shape and structure. Rehydration of the product is therefore quick and simple, a particularly important feature in pharmaceutical applications. Freeze dried products can last many years at room temperature if they are well sealed and protected against moisture and oxygen.

### Sublimation: Solid to gaseous

Sublimation describes the direct transition of a substance from the solid phase to the gaseous phase while bypassing the liquid phase completely. Sublimation can only occur at low pressure and low temperature. The ideal freeze drying conditions strongly depend on the sample to be freeze dried as well as on the solvent to be removed from the product. The main solvent being water, the three states are ice, water and water vapor. Freeze drying is fundamentally based on the phase diagram (Figure 3). The phase diagram illustrates the three physical states of water as a function of pressure and temperature.

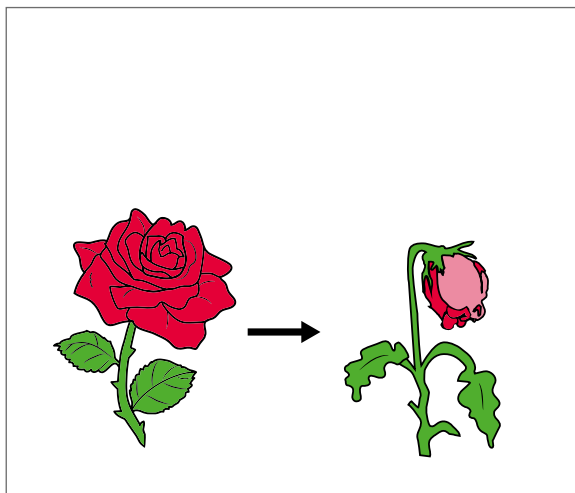


Figure 1: Common preservation methods apply heat to the product leading to structural changes, loss of colour, taste and smell as well as nutritional constituents (e.g. vitamins).

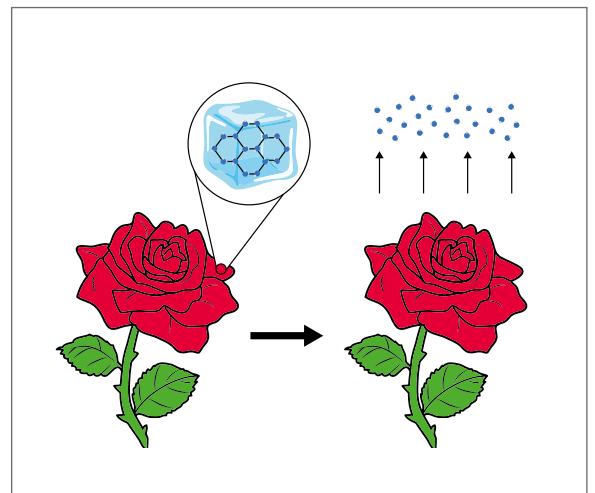


Figure 2: In freeze drying, ice directly transitions into water vapor by sublimation allowing to maintain product structure and characteristic. Freeze drying is a very gentle process.

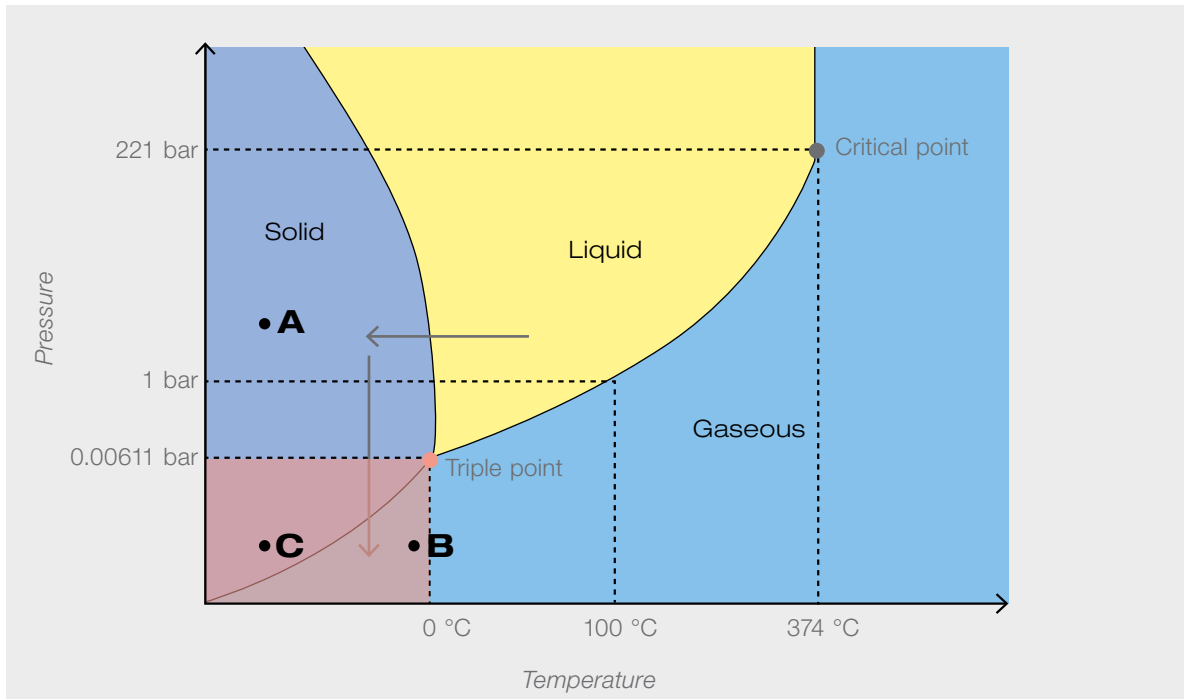


Figure 3: Phase diagram of water as a function of pressure and temperature. All states coexist at the triple point. Freeze drying occurs below the triple point and requires low pressures as well as temperatures.

Phase transitions occur at the lines separating the different states, the so-called phase boundaries. As shown in Figure 3, water goes through all physical states (solid, liquid and gaseous) as long as the prevalent pressure is above 6.11 mbar. At exactly 6.11 mbar, the three states coexist (triple point), and below 6.11 mbar water occurs only as ice or water vapor. The product to be freeze dried is initially often a liquid. Considering the phase diagram, the freeze drying process leads via two phase changes, first to the solid and further to the gaseous phase (red arrow). For sublimation to take place once the product is completely frozen, the pressure must be below the triple point, as shown in Figure 3.

### Pressure and temperature

The crucial parameters in freeze drying are pressure and temperature. Typically, the freeze drying process involves three stages; freezing, primary drying and secondary drying. Each process step has distinctive requirements in terms of pressure and temperature. Initially, the product is frozen at a temperature low enough to guarantee it is completely frozen (Figure 3, point A). The freezing phase is discussed in more detail

in the following section. For the primary drying stage, conditions for efficient sublimation need to be established. At the same time, it is essential to maintain product characteristics by ensuring the product's temperature remains below a distinct temperature, the so-called critical temperature. Above this temperature the product structure collapses leading to shrinkages or cracks. Ideally, freeze drying is performed at temperatures just below the critical temperature. The drying chamber pressure is decreased to activate the drying process. The prevailing pressure and temperature readings are now both below the triple point (Figure 3, point B).

Sublimation creates water vapor in the drying chamber. If not removed from the system, the water vapor equilibrates and no further ice particles sublimate. The vapor particles are removed by means of the ice condenser, a cooling device running at temperatures far below the critical product temperature (Figure 3, point C). The vapor pressure resulting from the low temperature of the ice condenser is therefore lower than the vapor pressure of the product and naturally forces the water molecules to travel towards the ice

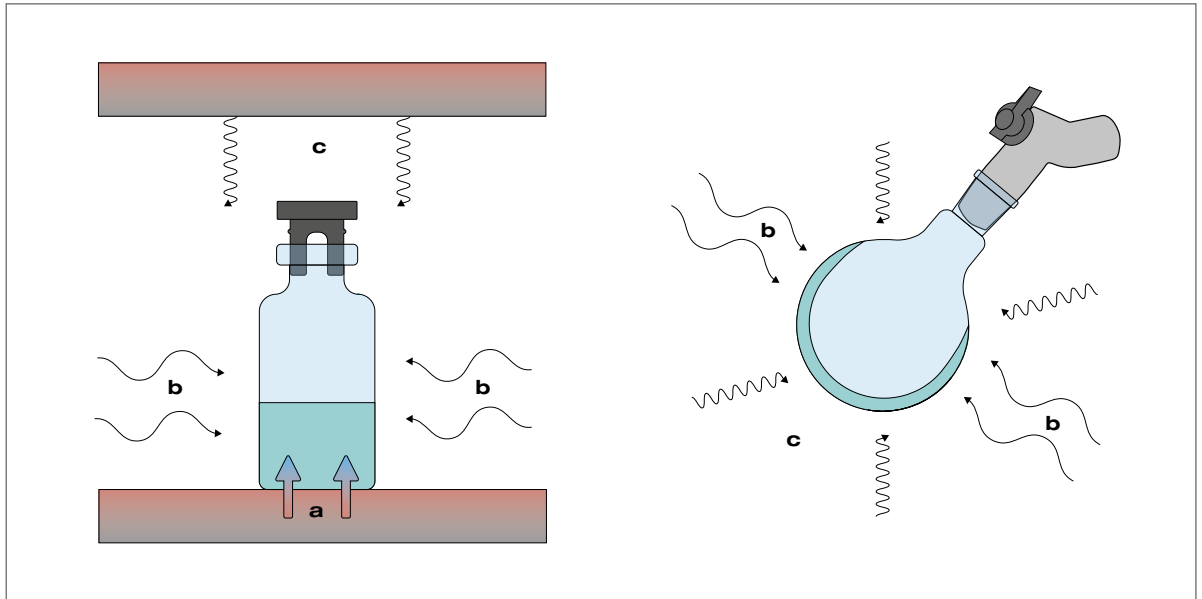


Figure 4: Different ways of heat transfer exist; conduction (a), convection (b) and radiation (c). Using heated shelves, heat is directly carried over through the surface by conduction, whereas ambient heat is transferred by convection and radiation additionally. Manifold application apply heat from the environment only.

condenser. Consequently, the water vapor, as well as other condensable gases, freezes on the ice collector forming ice.

The rate of sublimation is basically defined by the difference in vapor pressures; the vapor pressure over the product on one hand, and the vapor pressure over the ice condenser on the other hand. Generally, the larger the difference, the faster the sublimation. At the same time, the closer the product temperature is to the triple point, the larger is the pressure difference. As example, Figure 5 illustrates that combination A (product at  $-5\text{ }^{\circ}\text{C}$ , ice condenser at  $-25\text{ }^{\circ}\text{C}$ ) results in a larger

difference than combination B (product at  $-25\text{ }^{\circ}\text{C}$ , ice condenser at  $-45\text{ }^{\circ}\text{C}$ ). It is therefore beneficial to work as close to the triple point as possible.

### Driving force heat

Sublimation is an endothermic process requiring energy in the form of heat. The product experiencing sublimation therefore gives off heat, and would eventually cool down if the required heat is not supplied otherwise. As learned above, higher product temperatures lead to larger pressure differences and hence to a more efficient sublimation process. The additional input of heat boosts the sublimation and represents the real driver of freeze drying.

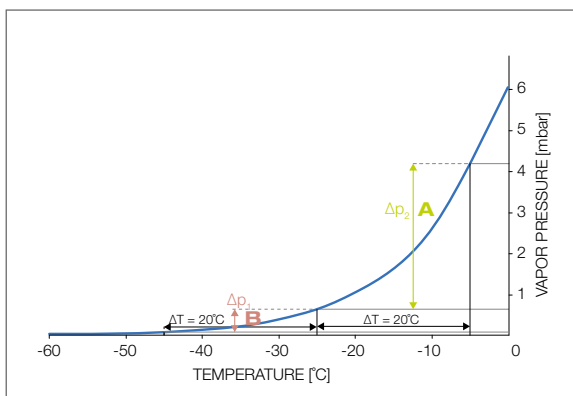


Figure 5: Example of variations in sublimation speed.

Heat transfer methods include conduction, convection and radiation (Figure 4). Direct heat conduction is applied in shelf drying with shelf heating ability. Convection and radiation are important in terms of indirect heat input from the surrounding environment. In manifold drying, heat is entirely provided by ambient heat in the form of convection and radiation. In shelf drying, the dominant transfer method is thermal conduction, however, effects from convection and radiation are noticeable and should be considered, particularly for samples close to the drying chamber boundary.

## Main components of a freeze dryer



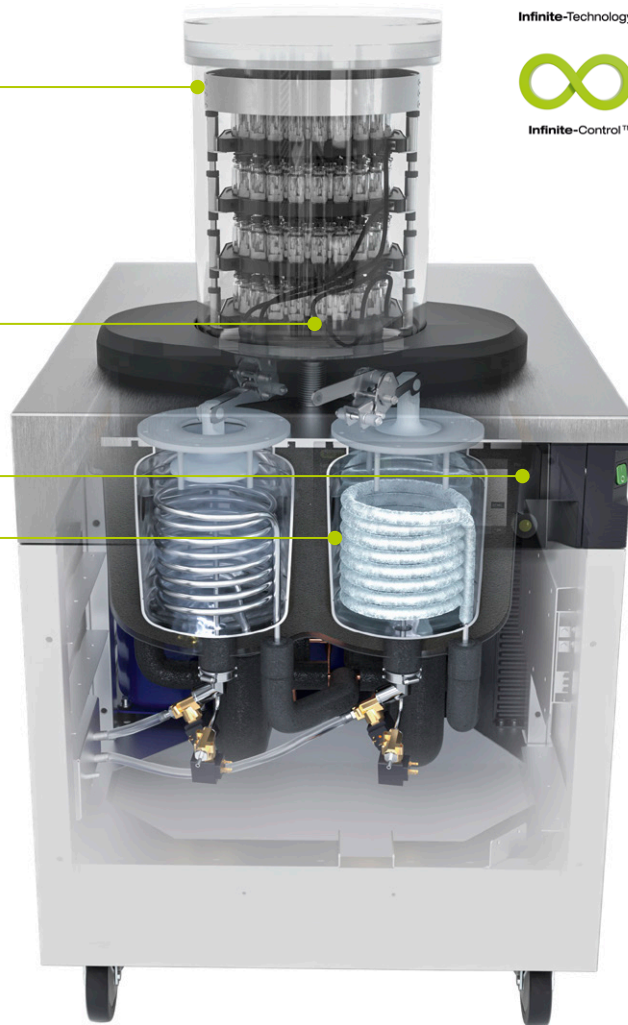
**Drying chamber**  
For sample placement  
(e.g. in vials or trays)

**Vapor path**  
Water vapor migrates towards  
lower vapor pressure

**Control unit**  
Regulation of pressure  
and temperature

**Ice condenser**  
Water vapor forms ice and  
aggregates at condenser

**Vacuum pump**  
Maintain very low  
system pressure



The amount of heat added to the system requires vigilant control; the product temperature may increase but must not exceed the critical temperature. The amount of heat required depends upon the product volume and arrangement as well as on the vessel used. Heated shelves allow for precise control of the heat input with a defined gradient and set temperature. Manifold drying on the other hand offers little control in terms of heat input.

### Freeze dryer setup

The main components of a freeze dryer are the drying chamber or specific drying attachments, the vacuum pump and the ice condenser. The

sample to be freeze dried is either placed in a system of shelves inside the drying chamber or it is filled into single flasks that are attached to the manifold. The vacuum pump is connected to the drying chamber via ice condenser and is responsible for evacuation of the drying chamber. Additionally, the vacuum pump acts as remover of all non-condensable gases that did not aggregate at the ice condenser.

The main task of the ice condenser is the collection of the water vapor and all other condensable gases. Water molecules travel naturally towards the ice condenser encouraged by the difference in vapor pressure. The ice condenser temperature must be significantly lower than the frozen product

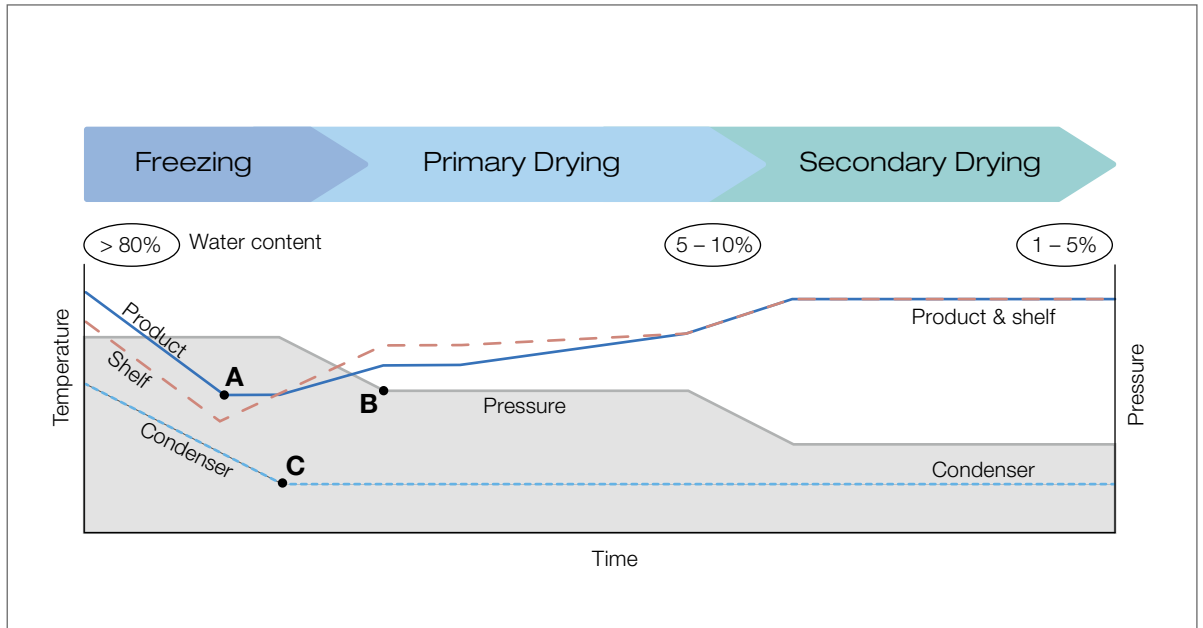


Figure 6: The freeze drying process includes three main stages: freezing, primary drying and secondary drying. The initial water content is usually reduced to more than 95%.

temperature, i.e. minimum 15 °C colder. The operating temperature of the Lyovapor™ L-200 of -55 °C is therefore well suited for most applications. Using organic solvents instead of water demands even colder temperatures to guarantee removal of the sublimated particles. For this reason, the Lyovapor™ L-300 is capable of cooling to -105 °C. Additionally, an adequate control system supports the pressure and heat regulation. Control systems range from basic to very advanced; BÜCHI's Infinite Control™ offers unprecedented features far beyond just pressure and temperature control.

### Infinite-Control™

This feature allows for maximum control of the Lyovapor™ at the instrument, at any connected work station, as well as on mobile devices. Real-time process monitoring, data recording and customized reports provide maximum convenience at any time. The dedicated app enables remote monitoring and instant informing by push notification.

### Infinite-Technology™

The Lyovapor™ L-300 is the first dual condenser freeze dryer with unlimited capacity in laboratory scale. Two ice condensers alternately collect ice and defrost the accumulated ice automatically. The

system automatically switches between the two condensers after a defined time. While the fresh condenser is collecting ice, automated hygienic steam cleaning is applied to the loaded condenser making it ready for further use. The alternation concept allows for continuous operation with unlimited volumes of ice avoiding down time to defrost the ice condenser.

### Process overview

The main steps in the freeze drying process are freezing, primary drying and secondary drying (Figure 6). Freezing takes place in a (deep) freezer, in liquid nitrogen or using a low temperature bath. The freezing temperature should be chosen well below the critical point (eutectic temperature or glass transition point) of the formulation to ensure the sample gets fully frozen (Figure 3 and 6, point A). At the end of external freezing, the freeze dryer is prepared for operation by initializing the condenser cooling (Figure 3 and 6, point C).

Once freezing is completed, the samples are placed in the drying chamber or the vessels containing the samples are attached to the manifold ports. The drying chamber is then evacuated and the application specific vacuum settings are applied. If heatable shelves are used,

the shelf temperature will be raised leading to a temperature increase of the product. However, the product temperature must remain below the critical temperature (Figure 3 and 6, point B). The sublimation process is now activated. In the following, each process step of freeze drying will be discussed in more detail.

## Freezing

Most liquid products, or formulations, freeze by forming ice crystals. Size and shape of the ice crystals depend on the cooling speed and define the freeze drying ability; rapid cooling results in small ice crystals while slower cooling leads to larger ice crystals. In terms of freeze drying, small ice crystals are more challenging to remove from the product than are large ones. Yet, the freezing temperature of a formulation is defined by its characteristics and composition.

Formulations can generally freeze in two different ways; eutectic mixtures contain substances that freeze at lower temperatures than the water surrounding them. When cooling an eutectic mixture, water is the first to separate from the substances and it freezes to ice. The formulation may now appear frozen but the remaining substances are actually still liquid. They form concentrated areas that freeze eventually at temperatures below the freezing point of water. The temperature where all components of the mixture are properly frozen is called eutectic temperature. This is the critical temperature of the formulation and the maximum temperature the formulation can

endure during the freeze drying process. Applying vacuum to an incompletely frozen eutectic mixture, may result in the destruction of the product as unfrozen components expand when placed under vacuum.

The other class of mixtures is amorphous and form glassy states when frozen. With decreasing temperature, the formulation becomes more and more viscous and eventually freezes to a vitreous solid at the glass transition point. For amorphous products, the critical point in terms of stability is called collapse temperature. The collapse temperature is typically slightly lower than the glass transition point. Amorphous products are generally very challenging to freeze dry.

## Primary drying

The first drying phase removes the bulk of water within the product. The temperature of the product and the heat input must now be carefully controlled. The ideal product temperature is as high as possible to maximize the vapor pressure difference, and at the same time below the product's critical temperature to preserve the frozen character. By using heated shelves, the set temperature is slowly approached at the defined heating rate. Simultaneous measurement of the actual product temperature allows for end point detection of primary drying. More information on end point detection is provided in a later section.

## Secondary drying

The vast majority of the water should be removed by the end of the primary drying phase. The residual moisture content of the product may now be 5 – 10% due to water bound to the matrix. At this stage, ice should not be present anymore. The secondary drying step removes the adsorbed water molecules by desorption. In order to achieve ideal conditions for desorption, the lowest possible pressure as well as a further increase of the shelf temperature is required. Again, product stability must be considered when choosing the shelf temperature. Secondary drying is usually performed for shorter time periods. At the end of secondary drying, the product moisture content should be in the range of 1 – 5%.

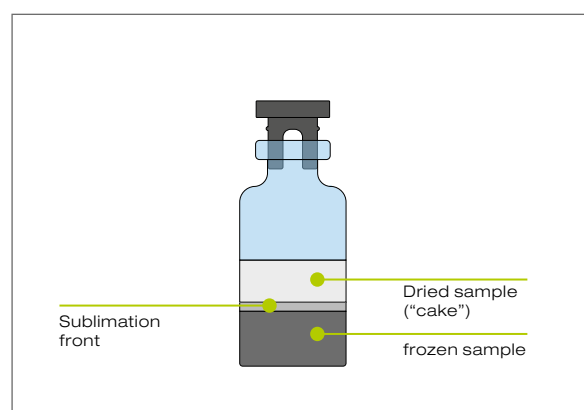


Figure 7: A well-defined sublimation front moves through the product from top to bottom.

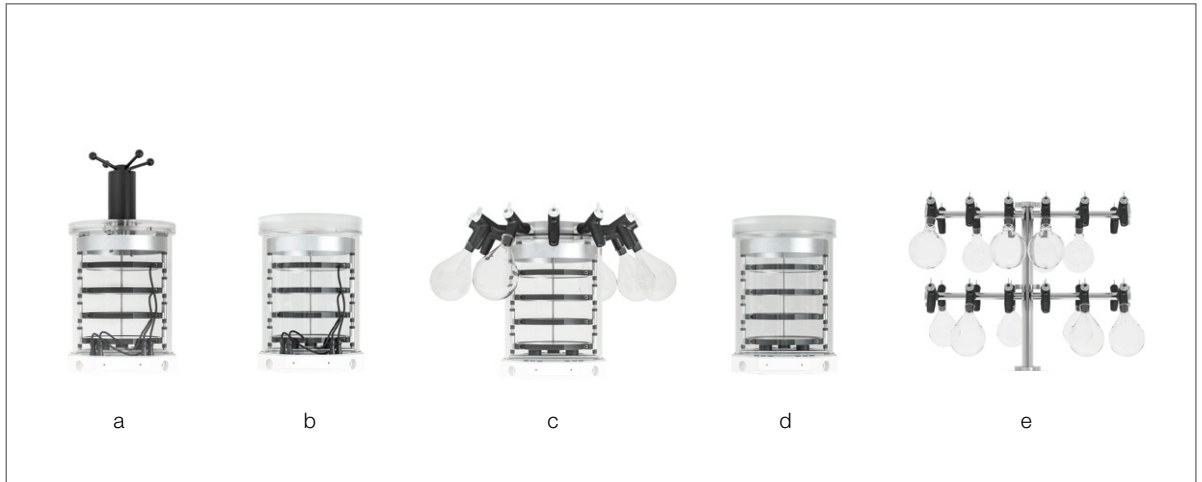


Figure 8: Range of drying attachment at BUCHI: (a) drying chamber with heatable shelves and stoppering system (b) drying chamber with heatable shelves (c) drying chamber with manifold connections and non-heatable shelves (d) drying chamber with non-heatable shelves and (e) manifold.

### End point detection

How to know when primary and secondary drying is complete? Several reliable ways exist to detect the end point of the main drying cycle. In the following, the three most commonly used methods in freeze drying are described, all available with the Lyovapor™ L-300.

#### Temperature difference test

This test is used with heated shelves where the product temperature increases gradually towards the set temperature of the shelf during primary drying. Heated by the adjacent shelf, the product temperature rises simultaneously. Yet, the product temperature is constantly lower than the shelf temperature as sublimation withdraws energy from the product. Once all water molecules are sublimated, the product temperature converges towards the shelf temperature indicating the end point of the process. The product temperature is measured by thermocouples placed in the product, whereas the shelf temperature is set in the method.

#### Pressure rise test

Water vapor is created as long as sublimation takes place. Closing off the way out of the drying chamber blocks the water vapor from migrating to the ice condenser. The pressure in the drying chamber will therefore rise in case ice still sublimates, and will remain the same if primary

drying is complete.

#### Pressure difference test

Comparative pressure measurement with two different kind of gauges is another reliable method for end point detection. Whereas the capacitive pressure gauge measures the absolute pressure regardless of gas composition, the Pirani gauge is calibrated against a specific gas, mostly  $N_2$ . Since the presence of water vapor influences the signal of the Pirani pressure gauge but not the other, the signals differ as long as water vapor is created. The end point of primary drying is reached once the two signals converge.

#### Drying efficiency

The drying rate at which a certain product is freeze dried depends on various factors with pressure and temperature being the key elements. However, the product itself also plays an important role, particularly the product volume to be freeze dried, as well as the kind of product arrangement. Generally, the greater the surface area relative to the volume, the faster the product dries. A larger surface results in more water molecules leaving the matrix. In manifold applications, it may therefore be beneficial to use shell freezing where thin product layers are created by rotating the flasks over a low temperature bath.

On the other hand, vials should be carefully filled



for freeze drying applications; the ideal filling depth is approximately 1 cm and maximum 2 cm. Considering that sublimation occurs at the surface, drying begins at the top of the product and forms a layer where drying is currently happening. During the drying process, this well-defined sublimation front travels from top to bottom of the product (Figure 7). Whereas drying is efficient at the beginning of the process, it becomes tougher as the sublimation front moves downwards. The sublimated water molecules must then pass the dried product (the “cake”) first before they can leave the matrix. Application of heat helps to overcome the aggravated circumstances.

### Drying chamber configurations

Final product requirements define the choice of the appropriate drying chamber configurations. Each configuration has specific purposes with according benefits and limitations. The commonly used approaches are described in the following.

### Manifold drying

Several types of flasks (as well as vials or ampules) are directly attached to the single manifold ports. Hence, each vessel is individually connected to the drying chamber by separate valves. This allows to hook up different flasks of various content

at different times and perform freeze drying continuously with the Lyovapor™ L-300. Manifold drying enables multiple users to work with the same instrument simultaneously. The products are frozen in the flasks by means of a freezer or a low temperature bath. Once attached to the manifold port, the vacuum is applied quickly. The energy required to drive the process is provided by ambient heat making the heat input difficult to control.

### Shelf drying

Multiple shelves in the drying chamber provide space for numerous vials filled with the product, or directly placing cut up solids onto the shelf. The products can be frozen on the shelves by means of a freezer. Once completely frozen, the shelves are placed in the drying chamber. Subsequent drying chamber evacuation initializes the freeze drying process. Heatable shelves allow for precise control of the heat input while thermocouples in the product simultaneously observe the product temperature and ensure the collapse temperature is not exceeded. In general, all vials, or the product pieces dispersed on the shelf, receive equal treatment in terms of pressure and heat input. However, small local effects may occur due to convection and radiant heat, particularly samples

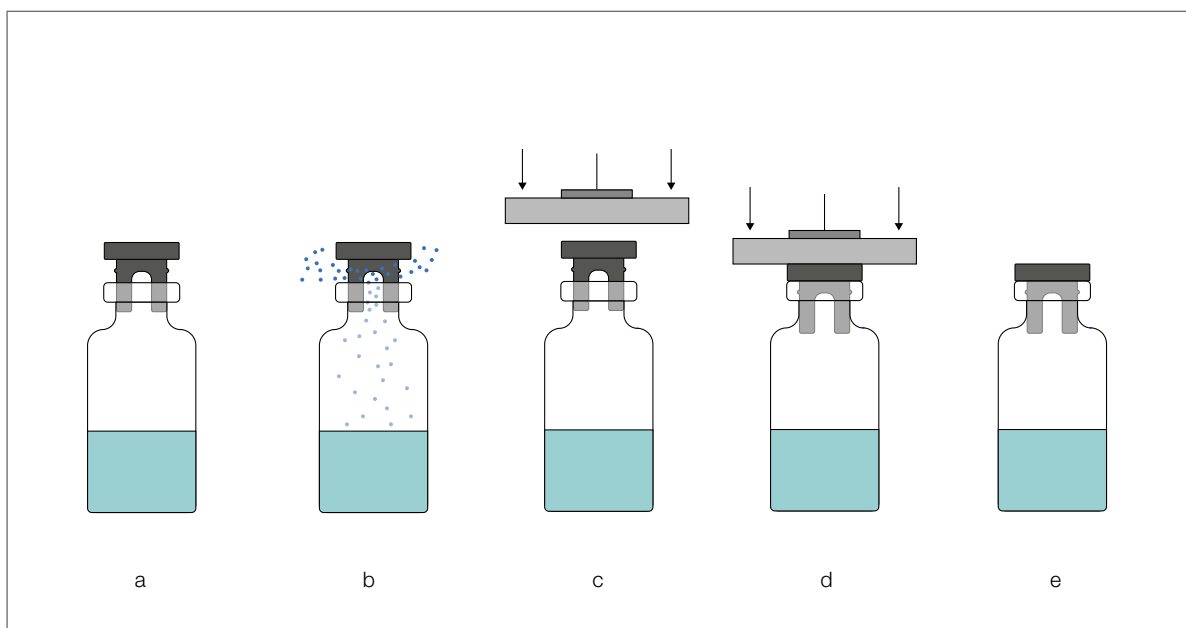


Figure 9: The stopper is loosely placed on the vial (a) allowing solvent particles to leave the vial (b). The adjacent shelf moves downwards (c) and presses the stopper into the vial (d) resulting in a sealed vial (e).

in peripheral regions from the shelf centre. Batch drying of vials further allows for internal closure under uniform environment (stopping, next section).

#### Combination of manifold and shelf drying

A combined solution with several shelves and a number of manifold ports on top are also available for users of both methods.

#### Convenient stoppering

Freeze dried products are hygroscopic and destabilize when exposed to moisture or oxygen. Appropriate isolation from the environment is therefore essential for storage of the freeze dried product. Packaging must be impermeable to moisture, as well as to air. The closure process should take place in an atmosphere free from moisture or oxygen, and therefore ideally under vacuum, as during the freeze drying process. Considering batches of vials are freeze dried in the drying chamber, this requires the vials be closed inside the drying chamber without interaction from outside.

Internal stoppering is achieved with the Lyovapor™ stoppering system and by using vials with specific stoppers for freeze drying purposes. The stoppers with notches are loosely placed on the vials prior to freeze drying (Figure 9, a). Due to the notches design, the solvent can sublime smoothly and freely leave the vial during the freeze drying process (Figure 9, b). Once the drying process is complete, the stoppering feature attached to the top of the drying chamber is being used. Turning the handles results in the shelves moving down and towards each other (Figure 9, c). By doing so, the stoppers sitting on the vials are pressed into the respective vial sealing it automatically (Figure 9, d). The shelves move upwards and reveal the airtight vials by simply reversing the process (Figure 9, e).

Vacuum conditions may not be ideal for certain freeze dried products or containers, e.g. if a syringe is used to rehydrate the material. In these cases, it is recommended to fill the empty volume with an inert gas, such as nitrogen, prior to closure. Backfilling with an appropriate, ultra-pure gas can be automatically performed with the Lyovapor™.



## **Summary of Applications** Freeze Drying

A selection of freeze drying applications are presented on the following pages. The freeze dried products include precious samples in the food industry (truffles), the generally challenging sample Mannitol as well as the lyophilisation of herbs for use in Traditional Chinese Medicine.

### Introduction

Freeze drying is a gentle form of drying and may be used to preserve foods without changing their appearance or taste.

The freeze drying process includes the freezing of the food sample and subsequent applying a fine vacuum to the frozen sample. Under these conditions, the water in the food will sublime, hence, the sample dries.

In food applications, freeze drying is commonly used to take instant coffee and to dry and conserve fruits, vegetables or herbs [1].

### Experimental

The banana was cut into pieces of approximately 5 mm width. Eleven banana slices were placed on the stainless steel tray (Figure 1) and then, frozen overnight in a deep freezer at -40 °C.

After 24 hours of deep freezing the banana slices were transferred with the tray into the Lyovapor™ L-200 for freeze drying. The shelf temperature itself was chosen such that it does not exceed 25 °C at the end of the primary and secondary drying (temperature set point). For more information about the drying sequence, please see reference [2].

After drying of the banana (see Figure 2), the residual moisture content of three banana slices were analyzed using a halogen moisture balance heated to 110°C. Therefore, the samples were ground in a mortar and transferred to the moisture analyzer within 30 seconds. The switch-off criterion refers to a change of no more than 1 mg / 140 s.

### Results and Discussion

Figure 1 and 2 show the steel tray with the banana slices before and after freeze drying process, respectively. All eleven banana slices showed a homogenous freeze dried structure and appearance. No change of their size and morphology could be observed during the drying.

To determine the drying efficiency of the Lyovapor™ L-200, the residual moisture content of three banana slices was analyzed using a halogen moisture analyzer. The results of the measured moisture contents and drying efficiencies are shown in Table 1.

The initial water content of the banana was  $76.97 \pm 1.24 \%$  (n=3). Hence, applying the described freeze drying method on the Lyovapor™ led to a water removal of  $\geq 95.92 \%$ .

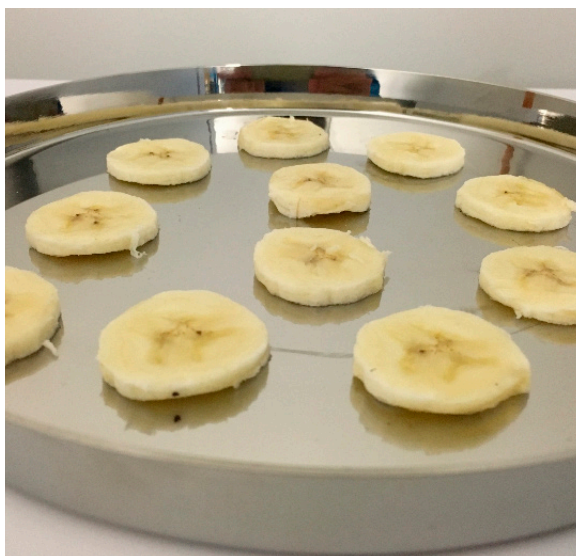


Figure 1: Tray with fresh cut banana slices.



Figure 2: Tray with banana slices after freeze drying.

Banana slice	Weight of freeze dried sample [g]	Weight of halogen dried sample [g]	Moisture content [%]
1	0.606	0.587	<b>3.14</b>
2	0.843	0.818	<b>2.97</b>
3	0.794	0.770	<b>3.02</b>

Table 1: Results of the residual moisture analysis after freeze drying with the Lyovapor™ L-200.

In general, applying the freeze drying process to foods such as banana slices, has the following advantages and disadvantages [3]:

### Advantages

- The process at low temperature and low pressure makes freeze drying an effective way to keep color, smell, flavor and heat-sensitive nutrients of food.
- Eliminates the surface hardening of the food.
- Freeze dried food is porous and easy to rehydrate and/or dissolve. It can be consumed directly or after rehydration.
- Since freeze dried food contains very low moisture, it has relatively small density and is easy to be transported. The freeze dried food can be preserved at room temperature for a long time, while the cost of transportation is much lower than that of frozen food.
- No additives are added into the food during freeze drying process.

### Disadvantages

- If exposed directly to air, freeze dried food will be rehydrated quickly, resulting in deterioration of food.
- The freeze dried products have to be vacuum- or vacuum-nitrogen packed and the packaging material must not be permeable to water vapor.
- During transportation and sale process, freeze dried food is easy to be powdered or cracked for its loose porous structure.
- Freeze drying is a time- and energy-consuming process, which leads to higher production costs.

## Conclusions

With the Lyovapor™ L-200, a high drying efficiency was achieved for the water removal of a banana. In summary, the Lyovapor™ L-200 allows freeze drying of fruits such as banana slices.

## References

- [1] G. W. Oetjen; Freeze-Drying; Ullmann's Encyclopedia of Industrial Chemistry (2004).
- [2] Application Note, 254/2016 Lyophilisation of fresh banana slices.
- [3] H. Tse-Chao Hua, L. Bao-Lin, Z. Hua; Freeze-Drying of Pharmaceutical and Food Products, (2010).

#### Introduction

Truffles are products with limited shelf life and their sensory properties are rapidly lost. Hence, the expensive fungi become less valuable within a few days. Losses of volatile compounds, oxidation and enzymatic reactions are a considerable problem during their storage. Furthermore, the aroma profile is commonly modified as a result of elevated temperature processes or enzymatic reactions.

Freeze drying the fungi avoids loss and degradation of volatile compounds due to the low temperatures applied during drying. The aromatic profile of truffles are maintained. Freeze dried truffles can either be rehydrated or directly used in dry form [1].

#### Experimental

The fresh summer truffle was cut into pieces of approximately 1 mm width (Figure 1). The slices were placed on the stainless steel tray and frozen for 24 hours in a deep freezer at -24°C.

After 24 hours of deep freezing, the truffle slices were transferred with the tray into the Lyovapor™ L-200 for freeze drying in the cube drying chamber and under ambient air atmosphere. The shelf temperature itself was chosen such that it does

not exceed 20 and 25°C at the end of the primary and secondary drying (temperature set point), respectively. For more information about the drying sequence, please see reference [2].

After drying of the truffle (see Figure 2), the residual moisture content of five truffle slices were analyzed using a halogen moisture balance heated to 110°C. Therefore, the samples were transferred to the moisture analyzer immediately after removing the dried truffle from the Lyovapor™. The switch-off criterion on the moisture analyzer refers to a change of no more than 1 mg / 140 s.

#### Results and Discussion

Figure 1 and 2 shows the tray with the truffle slices before and after freeze drying process, respectively. All truffle pieces showed a homogenous freeze dried structure and appearance. No change of their size and morphology could be observed during the drying.

To determine the drying efficiency of the Lyovapor™ L-200, the residual moisture content of five truffle slices, were analyzed using a halogen moisture analyzer. The results of the measured moisture contents are shown in Table 1.



Figure 1: Tray with fresh cut truffle slices.



Figure 2: Tray shelf with truffle slices after freeze drying.

Truffle piece	Weight of freeze dried sample [g]	Weight of halogen dried sample [g]	Moisture content [%]
1	0.187	0.179	<b>4.28</b>
2	0.119	0.115	<b>3.36</b>
3	0.165	0.161	<b>2.42</b>
4	0.211	0.204	<b>3.32</b>
5	0.153	0.147	<b>3.92</b>

Table 1: Results of the residual moisture analysis after freeze drying with the Lyovapor™ L-200.

The analyzed samples contained  $3.46 \pm 0.63$  % moisture after freeze drying process. The initial water content of the summer truffle was  $66.33 \pm 0.94$  % ( $n=3$ ). Hence, applying the described freeze drying method on the Lyovapor™ L-200 lead to an average water removal of 94.78 %. With a water removal of approx. 80 %, the freeze dried truffle has a shelf life of more than 24 months [3]. In general, applying the freeze drying process on foods such as truffle slices, have the following advantages and disadvantages [4]:

#### Advantages

- The process at low temperature and low pressure makes freeze drying an effective way to keep color, smell, flavor and heat-sensitive nutrients of food.
- Eliminates the surface hardening of the food.
- Freeze-dried food is porous and easy to rehydrate and/or dissolve. It can be consumed directly or after rehydration.
- Since freeze-dried food contains very low moisture, it has relatively small density and is easy to be transported. The freeze-dried food can be preserved at room temperature for a long time, while the cost of transportation is much lower than that of frozen food.
- No additives are added into the food during freeze drying process

#### Disadvantages

- If exposed directly to air, freeze-dried food will be rehydrated quickly, resulting in deterioration of food.
- The freeze-dried products have to be

vacuum- or vacuum-nitrogen packed and the packaging material must not be permeable to water vapor.

- During transportation and sale process, freeze-dried food is easy to be powdered or cracked for its loose porous structure.
- Freeze-drying is a time- and energy-consuming process, which leads to higher production costs.

## Conclusions

Freeze dried truffles are a promising alternative to out of season fresh truffles because flavors, smells and nutritional content remain mostly unchanged in comparison to other drying techniques [1, 3, 5].

With the Lyovapor™ L-200, a high drying efficiency was achieved for the water removal. In summary, the Lyovapor™ L-200 enables to freeze dry fungi samples such as truffle slices.

## References

- [1] I. Palacios, E. Guillamón, A. García-Lafuente, A. Villares; Effects of Freeze-Drying Treatment on the Aromatic Profile of Tuber spp. Truffles. Journal of Food Processing and Preservation, Volume 38, Issue 3, Pages 768–773, (2014).
- [2] Application Note, 269/2016 Lyophilisation of truffle.
- [3] <http://www.tartuflanghe.com/en/tartufo-bianco/dehydratedwhite-truffle>
- [4] H. Tse-Chao Hua, L. Bao-Lin, Z. Hua; Freeze-Drying of Pharmaceutical and Food Products, Woodhead Publishing Series in Food Science, Technology and Nutrition, pages 141–169 (2010).
- [5] <https://www.sialparis.com/Catalogue/Catalogue-Sial-Paris-2016/Products-list/freeze-dried-truffles-GEOOFOODSITALIAN-TRUFFLES>

#### Introduction

In this Application Note sodium chloride (NaCl) and mannitol are used for freeze drying experiments. The unambiguous crystal structure of NaCl renders this salt a model compound. In contrast, mannitol is well known to crystallize in different polymorphs [1] and it may form hydrates [2].

Nevertheless, mannitol is the most used bulking agent for freeze dried pharmaceutical formulations.

#### Experimental

Nevertheless 3 mL of 5 % aqueous mannitol or NaCl solutions (50 g/L) were transferred into the vials with a volumetric pipette (70 vials of each solution). Then, the samples were frozen overnight in a deep freezer at -40 °C on a stainless steel tray.

After 24 hours of deep freezing the vials were transferred on the steel tray to the Lyovapor™ L-200 for freeze drying. The collapse temperature was set to 30 °C. For unknown formulations, it is recommended to determine the collapse temperature by means of a freeze drying microscope. Furthermore, a safety temperature margin can be programmed to protect the sample from collapsing.

The shelf temperature itself was chosen such that it does not exceed 20 °C at the end of the primary

drying and 25 °C at the end of the secondary drying. For more information about the drying sequence, please see reference 3.

After drying, the residual moisture content of nine mannitol and NaCl samples, positioned on the shelf diameter, were analyzed using a halogen moisture balance heated to 110 °C. Therefore, the samples were ground in a mortar and transferred to the moisture analyzer within 30 seconds.

#### Results and Discussion

The vials containing pure mannitol that were located on the shelf diameter are shown in Figure 1. Generally, all vials contained a homogenous freeze dried mannitol or NaCl cake.

To determine the drying efficiency of the Lyovapor™ L-200, the residual moisture content of nine mannitol samples positioned on the tray diameter were analyzed using a halogen moisture analyzer. The results of the measured residue moisture contents are shown in Table 1.

Mannitol samples, independent of their position on the shelf, contained less than 2.0 % moisture after the freeze drying process.



Figure 1: Steel shelf with the freeze dried samples.



Figure 2: Freeze dried mannitol samples analyzed for its residual moisture.



Vial	Weight of freeze dried sample [g]	Weight of halogen dried sample [g]	Residual moisture [%]	Drying efficiency [%]
1	0.107	0.105	1.87	98.13
2	0.100	0.098	2.00	98.00
3	0.113	0.112	0.88	99.12
4	0.122	0.121	0.82	99.18
5	0.111	0.110	0.90	99.10
6	0.116	0.115	0.86	99.14
7	0.121	0.120	0.83	99.17
8	0.108	0.107	0.93	99.07
9	0.116	0.114	1.72	98.28

Table 1: Results of the residual moisture content analysis after freeze drying mannitol solutions with the Lyovapor™ L-200.

We found that the mannitol samples positioned in the middle of the shelf contained marginally less residual moisture than the samples placed on the outer radius of the shelf. For NaCl this pattern was not observed. The residual moisture contents for NaCl varied randomly between 0.79-1.59 %. This finding may indicate that during the freezing process the fraction of mannitol hydrate formed is greater on the outer than on the inner shelf area.

Furthermore, it is likely, that some water is adsorbed on the sample while preparing it for the residual moisture analysis. Hence, the drying efficiency is probably higher than reported.

Strategies to remove more moisture are i) increasing the drying time, ii) increasing the temperature during secondary drying and iii) annealing [2].

## Conclusions

With the Lyovapor™ L-200, a high drying efficiency was achieved for the water removal of a mannitol model formulation and a NaCl solution. For both compounds, the optical appearance of the dried cake was uniform, and no collapsed cakes were observed.

## References

- [1] Kim, A.I.; Akers, M.J.; Nail, S.L. *J. Pharm. Sci.* 1998, 87 (8), 931-935.
- [2] Yu, L.; Milton, N.; Groleau, E.G.; Mishra, D.S.; Vansickle, R.E. *J. Pharm. Sci.* 1998, 88 (2), 196-198.
- [3] BUCHI Application Note, 256/2017

#### Introduction

Traditional Chinese Medicines (TCM) are usually prepared by mashing and boiling in water. This process is called decoction. Thereby, health beneficial compounds are extracted from herbal and plant material. If not directly administered, these extracts can be preserved for a longer shelf life using lyophilisation.

In this Short Note we highlight the lyophilisation procedure of Tianma Gouteng Yin (TGY), as a TCM model decoction. TGY is a widely used medicine applied to treat Parkinson's disease (PD) like symptoms. Recently, it has been reported that this TCM decoction exerts neuroprotective effects in animal and cell models of PD [1].

#### Experimental

##### Equipment:

- BUCHI Lyovapor™ L-200 Pro
- BUCHI Lyovapor™ Software
- Deep Freezer -24 °C, Bauknecht
- Stainless steel tray
- BUCHI Rotavapor® R-300, System Rotavapor® Dynamic

##### Sample:

- The TGY mixture was prepared by St. Peter Apotheke Zurich, Switzerland.

A human dose of TGY1 contains *Gastrodiae Rhizoma (Tianma)* 9 g, *Uncaria Ramulus Cum Uncis (Gouteng)* 12 g, *Haliotidis Concha (Shijueming)* 18 g, *Gardeniae Fructus (Zhizi)* 9 g, *Scutellariae Radix (Huangqin)* 9 g, *Cyathulae Radix (Chuanniuxi)* 12 g, *Eucommiae Cortex (Duzhong)* 9 g, *Leonuri Herba (Yimucao)* 9 g, *Taxilli Herba (Sangjisheng)* 9 g, *Polygoni Multiflori Caulis (Shouwuteng)* 9 g, *Poria (Fuling)* 9 g.

#### Results

The TGY sample was extracted, concentrated and freeze-dried. In the upper part of Figure 1, the sample is shown during the lyophilisation process. In the lower part of Figure 1, the freeze dried product, in a stainless steel tray, is shown.

It is well visible that the product has a highly porous structure. Thanks to this structure as prepared, TCM doses are easily rehydrated and instantly dissolved. It can be consumed directly or after rehydration. The health beneficial compounds are preserved during lyophilisation [1].

Furthermore, as prepared TCM decoctions have a long shelf life in a vacuum- or vacuum-nitrogen charged packaging.



Figure 1: Steel shelf with TGY extract being dried in the Lyovapor™ L-200 Pro.



Figure 2: Dried TGY sample exhibiting a porous structure.

## Conclusion

Freeze-drying is a suitable method to prepare TCM decoctions for a long shelf life and for simple use by rehydration. Here, we successfully dried a human dose of a Tinma Gouteng Yin sample that is reported to have neuroprotective effects in in vitro and in vivo models of Parkinson's disease.

Thanks to the gentle preparation using the combination of extraction, concentration, and lyophilisation, health beneficial compounds are preserved in the medicine. With the here presented process TCM does are made durable and readily available.

## References

[1] Liu L.-F. et al. Nature Scientific Reports, 5, 16862, 2015.

