1

Introduction to Freeze-drying and Ice Templating

1.1 Introduction

Freeze-drying is a widely used drying technique in pharmaceutics, biologics, and food industries. It is a preferred drying technique when dealing with temperature-sensitive chemicals/substances, as it can produce dry powders or cakes for easy storage and transport. From the perspective of pharmaceutical applications, the freeze-dried products should also have a reasonable shelf life and be readily reconstituted with the anticipated drug activity [1–4].

A freeze-drying process, especially for pharmaceutics and biologics, consists of three steps, i.e. freezing on a cold shelf in a freeze-dryer chamber, primary drying, and secondary drying [1, 4]. The freeze-drying process is usually applied to aqueous solutions or suspensions. When freezing aqueous solutions or suspensions (usually in small vials), the majority of the water in the sample is either free water or gets frozen. However, a small percentage of the water bound to or having strong interaction with pharmaceutics/excipients remains as liquid. The amount of liquid water present in the frozen sample depends on the freezing temperature as well as the components in the solution. The frozen ice crystals are sublimed in the primary drying process, i.e. a change from solid state to vapour state to remove the ice crystals. In the secondary drying step, the bound water needs to diffuse and transport outside of the porous matrix to be removed. Therefore, the rate of primary drying may be controlled or enhanced by careful control of the freezing temperature, the shelf temperature, and the vacuum, whilst the nature and the porosity of the matrix is more important for the secondary drying stage.

In chemistry, biological and materials research, freeze-drying is more commonly known as a *drying approach*. The cool samples may be either directly placed on a shelf in a freeze-dryer or frozen in a freezer or in liquid nitrogen before being placed into a freeze-dryer. The main purpose is to keep the samples at low temperature (to maintain the component activity) and to prevent dense aggregation or considerable shrinkage during the drying process. This is in contrast to a conventional drying process of aqueous solution or wet samples, e.g. drying in open air, N_2 flow drying or vacuum drying. Because of water's high surface tension, removing liquid water can generate significant surface force that brings small particles together (hence aggregation of particles) or the collapse of porous structure. For this reason, freeze-drying has been frequently used

Ice Templating and Freeze-drying for Porous Materials and their Applications, First Edition. Haifei Zhang.

in materials science in order to prepare materials with highly interconnected porosity and high surface areas.

During a freeze-drying process, either in a pharmaceutical industry environment or in a research lab practice, the process needs to be conducted so that the samples remain frozen, avoiding partial melting and annealing, following which porous cakes or structures without dense skin and shrinkage are prepared. This is, of course, not just intended to produce a material with an 'aesthetic look' [4]. Such materials can have desirable properties for subsequent applications. After the removal of ice crystals, the voids left behind are pores within the materials, hence the porous materials. In the pharmaceutical industry and other relevant research areas, good cakes with homogeneous structures are desirable for their subsequent applications. The porous structures of such cakes are examined with the intention of relating the quality of the freeze-dried materials with subsequent application performance, rather than as a technique to prepare porous materials with controllable porosity and morphology.

Porous materials are used in a wide range of applications and have been intensively investigated [5, 6]. According to the IUPAC definition, the pores can be categorized as macropores (>50 nm), mesopores (2–50 nm), and micropores (<2 nm) [7]. Templating is probably the most common approach used for the preparation of porous materials. The templates used may be hard templates (e.g. colloids, particles, sacrificial pre-formed porous structures) or soft templates (e.g. assembly of surfactants, polymers, droplets, emulsions) [5]. In recent years, ice templating has been developed and intensively investigated as an effective approach to the production of a variety of porous materials [8–11]. This templating method exhibits some unique characteristics that the other techniques do not have. For instance, a directional freezing process can be employed to prepare aligned porous materials. By careful control of the freezing conditions, layered porous materials can be formed and can be subsequently utilized to produce tough composite materials mimicking natural structures. Furthermore, the ice templating method is not restricted to water-based solutions or suspensions. It can be used for organic solutions/suspensions, compressed CO_2 solutions, and emulsions [8].

Sometimes, there is a misconception that freeze-drying and ice templating may be the same, as seen in some published articles. However, they are different but are related with each other. In brief, freeze-drying is a drying method (in pharmaceutical industry the freezing process is usually completed on the freeze-dryer shelf). Figure 1.1 shows the schematic representation of a freeze-drying process. Ice templating is a templating method where ice crystals are used as templates in order to produce the desired templated structures. A controlled freezing process is usually required in order to control the orientation, size, and morphology of the ice crystals. As in any other templating methods for the preparation of porous materials, the ice templates need to be removed. The most common method to remove ice crystals is by freeze-drying. However, freeze-drying is not the only method that can be used. Solvent exchange may be employed in a cold miscible solvent (and it is an insoluble solvent to the solute in the frozen solvent) where the temperature is below the melting point of the frozen sample. After the frozen solvent is completely removed, the materials may be dried by usual drying methods

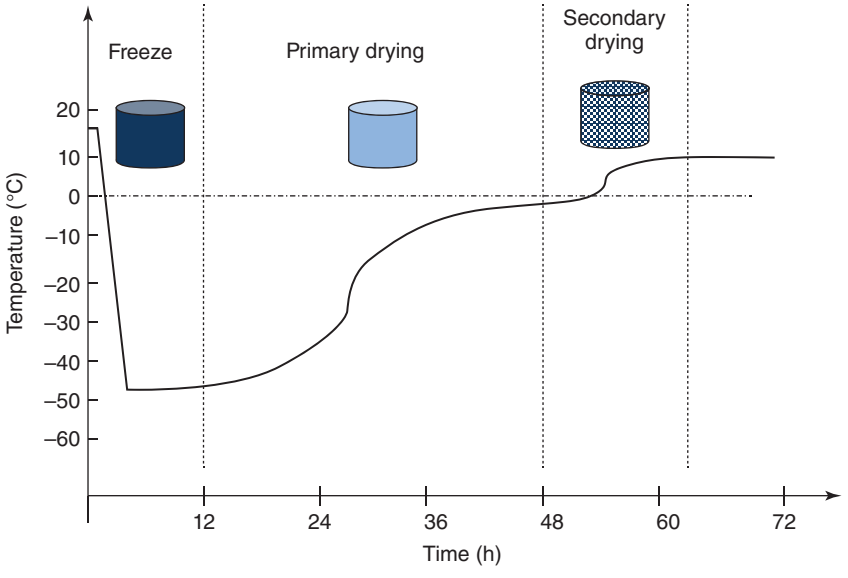

Figure 1.1 Schematic representation of a freeze-drying process. The temperature and time scales are approximate and for indication only.

such as air drying or vacuum drying. There is another option when monomers and crosslinkers or reactive reagents are included in the frozen samples. A frozen polymerization/crosslinking process can be applied, which will lock in the frozen structure [12]. After polymerization, the frozen samples may be warmed up to room temperature and then dried in air or by vacuum drying.

Because of the close link between freeze-drying and ice templating, in this chapter, we will first introduce the basics and key aspects/parameters of a freeze-drying process. The same controls applied in freeze-drying may be applied to the ice templating approach as well when ice crystals are removed by a freeze-drying process. However, it must be pointed out that there is very limited research on the effects of the freeze-drying process on porous materials or nanostructures. Secondly, the key aspects, important parameters and progress of ice-templating method will be covered. The practice and experience of the Zhang group in ice templating and freeze-drying for porous materials will be described last, hopefully to provide useful and practical information for researchers who are new to this research area.

1.2 The Freeze-drying Process

Water is essential for life. However, the presence of water tends to make materials or products degrade fast. To preserve samples, to make storage longer and transport easier, suitable drying methods are required. This is particularly important for biological and pharmaceutical samples. Indeed, freezing itself is a drying technique. During the freezing process, water turns into ice crystals, which can

exclude any impurities, including polymers, particles, and dissolving molecules. The freezing front rejects the solutions from forming ice crystals; this concentrates the solution and makes the dissolved substances 'dry'. However, the frozen 'dry' samples have to be kept at low temperature (below the melting point) and are normally stored in a freezer. This incurs storage costs and difficulty in transporting. Potential freezer break down or power cut can melt and damage the samples. Thus, a freeze-drying process is a preferred drying process.

The use of freeze-drying has a long history, tracing back to 1250 BCE for preserving materials by dehydrating, which involves the freezing stage and drying at low temperature under vacuum. Freeze-drying is also widely known as '*lyophilization*', a term mostly attributed to Rey LR's work in 1976. Because the process produces a dry porous structure, its high porosity and exposed surface can rapidly resorb the solvent, e.g. the water, vapour or moisture in the surrounding environment. The freeze-dried materials exhibit such 'lyophil' characteristics towards the solvent (mostly water), hence the term '*lyophilization*' process [4].

But it is not all positive for a freeze-drying process. The disadvantages associated with freeze-drying processes are low scale-up potential, longer drying time and energy-intensive processing. This results in high operational costs. A freeze-drying process is therefore only viable for high-value products, such as pharmaceutics and biologics. There is continuing effort in developing new procedures and/or optimizing the freezing stage and drying stage in order to reduce the operation costs. It is critically important for biological and pharmaceutical samples not to be damaged or deactivated during the freezing stage as the growth of ice crystals can be harmful.

1.2.1 Additives in the Solution

1.2.1.1 Additives to Maintain Integrity/Activity of Biological Samples

Freeze-drying can be used for cryopreservation, i.e. long-term storage of biological materials such as in biomedical applications and agriculture. The use of additives can help stabilize biomolecules in the freeze-dried samples, probably more importantly to reduce the damaging impact of growing ice crystals on biological samples.

During a freezing process, extremely low temperature (e.g. −196 °C of liquid nitrogen) may be applied. The growing ice crystals can pierce (from outside) or tear apart the cells (from inside) by mechanical force. Further, due to the freezing concentration effect, the composition in the liquid phase may change, for example, leading to high ion concentrations or dramatic pH change. This can be detrimental to the cells. Furthermore, because of the freezing dehydrating and concentration effect, this can also cause the change of osmotic pressure across cell membranes and dehydrate the cells [13].

To mitigate these adverse effects, additives acting as cryoprotectants are added to the solution. These additives should be non-toxic or low toxic, can help balance the surrounding environment of the cells, and reduce the size and amount of ice crystals during the freezing stage. Alcohols, glycerols, and polymers (e.g. polyvinyl alcohol (PVA)) have been often used. Antifreeze proteins, as observed

in some arctic fish, may be also utilized [14]. It can help lower the ice nucleation temperature and inhibit ice nucleation and crystal growth so to produce small ice crystals without damaging cell activity.

1.2.1.2 Lyoprotectants to Prevent Denaturation of Biopharmaceutics or Enhance Reconstitution of Pharmaceutics

In order to be effective, it is suggested that the lyoprotectants must be retained in the amorphous state. Crystallization of lyoprotectants during the freeze-drying process or subsequent storage may potentially damage the stability of proteins. For this purpose, non-reducing sugars, especially sucrose and trehalose have been widely used. It should be mentioned that the amorphous state in the freeze-dried product does not necessarily mean that no crystallization occurs during the freeze stage and drying stage. Indeed, one study found that trehalose could crystallize during annealing and form amorphous materials after freeze-drying [15].

As it is widely known, polymer excipients are processed with active pharmaceutical ingredients (API) as tablets or in other formulations. Similar excipients, e.g. hydroxypropyl methyl cellulose (HPMC), may be included in the solution, which can help to stabilize and re-constitute the freeze-dried formulations [16].

1.2.1.3 Stabilizer/Binders for Particulate Suspensions

Suspensions are frequently freeze-dried to produce porous structures. To ensure a high-quality freeze-dried product, a homogeneous and well-dispersed suspension is required [10, 17]. In order to achieve this, polymeric stabilizers such as PVA, polyvinylpyrrolidone, and industry-grade commercial stabilizers may be used. In order to produce samples with good mechanical stability, binders are usually required in the suspensions. It is possible for the same polymer to act as both a stabilizer and a binder. This part is particularly important for the preparation of porous ceramics by freeze casting and more details may be found in Chapter 5.

1.2.2 Optimizing the Freezing Stage

It is recognized that freeze-drying is a time-consuming and energy-intensive process. For an industrial freeze-drying process, freezing usually takes a few hours whilst the drying process requires days with the primary drying taking much longer than that of the secondary drying. In the primary drying stage, the driving force for the removal of ice crystals is the difference of vapour pressure between the sample chamber and the condenser chamber. It is known that vapour pressure is proportional to temperature. It is more efficient to raise the temperature of the frozen sample rather than to reduce the temperature in the condenser chamber. Therefore, designing a freezing stage that allows nucleation and ice growth at higher temperature can considerably benefit the primary drying process [18, 19].

Freezing is a very complicated process. Water may look like a simple molecule but it has over 10 crystalline phases. The presence of hydrogen bonds between water molecules and the interaction with solutes result in complex phenomena.

The nucleation temperature determines the size, number, and morphology of ice crystals. A good process should lead to homogenously dispersed small ice crystals, which can in turn contribute to a faster drying process and a high-quality freeze-dried cake.

Typically, the process of freezing water or water solutions consists of four steps: (i) cooling the solution below the equilibrium freezing temperature; (ii) formation of ice nucleus that acts as primary nucleation; (iii) Secondary nucleation that allows the growth to ice crystals; and (iv) complete freezing to form frozen solids [20]. However, formation of ice nucleus does not occur just below the equilibrium freezing temperature. Pure water can be supercooled to −48 °C to initiate ice nucleation whilst impurities/solutes in a solution may act as nuclei and allow growth of ice crystals under less supercooled conditions (often seen as −15 to −20 °C) [21, 22]. A higher freezing temperature that allows the spontaneous formation of ice crystals would in principle lead to a faster primary drying process. Among different polymorphic forms of ice, cubic shape and more often hexagonal shape crystals are observed under typical freezing conditions. For the hexagonal ice crystals where each oxygen atom is tetrahedrally surrounded by four other oxygen atoms, it can form dense structures with crystal growth thus excluding the solutes from the ice front. Controlling the freezing condition is also important in tuning the size of crystals, where the spontaneous formation of a large number of nuclei and higher temperature gradient may contribute to small ice crystals. There is a link between the drying rate and ice crystal size. Larger ice crystals, usually generated involving an annealing process, increase sublimation rates. This is due to the larger pores left behind by the removal of large ice crystals, which facilitate the transport of water vapours [19]. Controlling the freezing process may also lead to the formation of more homogeneous ice crystals in the frozen samples, which may result in freeze-dried pharmaceutical cakes of better quality.

In order to maintain and improve freeze-dried product quality whilst reducing freeze-drying time, different freezing methods have been developed. Some of the common ones are described below:

(i) *Ice fog technique*. This technique was first reported by Rowe [23]. After the vials are cooled to the desired nucleation temperature, ice nuclei are introduced to facilitate ice crystal growth by a flow of cold N_2. N_2 gas is transported into the chamber through a metal coil immersed in liquid nitrogen. Water vapour already present in the chamber can be cooled by the cold N_2 and forms a vapour suspension of ice crystals (a 'fog'). The N_2 gas enters the chamber at minimum overpressure and passes the ice nuclei into pre-cooled vials to induce crystallization. Different types of ice fog techniques have been described in the review paper by Geidobler and Winter [19].

(ii) *Electrofreezing*. In this method, a high voltage pulse is applied to generate ice nuclei on an electrode, which induces ice crystallization [24]. For this method to be successful, suitable electrodes have to be developed and fixed into a freeze-dryer. Each vial requires one electrode, which makes this method impractical and difficult to scale-up.

(iii) *Ultrasound-induced freezing*. Applying ultrasonic waves to liquids produces acoustic cavitation, the formation of gas-filled bubbles with short-lived high temperature and pressure [25]. The collapse of the cavitating bubbles results in the increase of equilibrium freezing temperature due to very high pressures. This increases the supercooling level and is the driving force for ice nucleation. The method can be easily realized by attaching an ultrasound generator to an aluminium plate combined with the freezing shelf. Therefore, there is no direct contact with the freezing solution, thus avoiding a potential contaminating issue. This technique was demonstrated by freeze-drying of pharmaceutical proteins. It was found that larger and directional ice crystals were formed at high temperatures whilst smaller and heterogeneous ice crystals were produced at lower temperatures [26].

(iv) *Directional freezing*. In this case, ice nucleation is induced at the bottom of the vial by contact with a cold stage. Ice propagation is orientated in the vertical direction and either lamellar or vertical dendrite ice crystals are formed [27]. The directional freezing process may also be realized by applying a gradient freezing stage which may allow the advance of freezing front at a constant velocity, facilitating homogenous cooling of the samples. This technique may be very important for cryopreservation of cells. When ice crystals grow outside the cell, causing water to osmotically move out of the cells, as a result of which the cells shrink and eventually get damaged and die. The directional freezing process contributes to uniform cooling of the samples, producing lamella ice crystals between which the cells are trapped [13]. To achieve uniform cooling, the dissipation of heat of fusions during water freezing is very important, which can be done in two ways: the metal block closely surrounding the sample and from the ice front into the unfrozen part.

Other freezing methods may also be used. For example, shelf-ramped freezing, pre-cooled shelf freezing, and an annealing process (to make ice crystals more uniform) can be readily performed by using different shelf temperature programs on a modern freeze-dryer. Quench freezing is basically freezing a solution in a cool liquid such as liquid nitrogen or dry ice bath with a chosen organic solvent (e.g. ethanol, acetone). The frozen samples are then placed in a freeze-dryer. This is the typical method that materials researchers use to produce freeze-dried porous materials. By varying the vacuum in the chamber (evaporation of water to reduce local temperature), pressurizing/depressurizing (using the principle of the freezing temperature of water lowered at high pressure and vice versa) the chamber, ice nucleation can be induced. But these methods are not practical or difficult to scale up [18, 19].

1.2.3 Primary Drying in Freeze-drying

During the freezing process, the solute is excluded from the freezing front and the solute phase becomes concentrated, which is termed as *freeze concentrate*. By the end of the freezing process, the majority of the water in the solutions turns into ice crystals [28]. The primary drying stage is to remove the ice crystals by sublimation.

During primary drying, the chamber is under vacuum and the pressure is used as the partial pressure of the water vapour. In the chamber, the temperature of the sample is lower than the shelf temperature. The driving force for the primary drying is the difference between the water vapour pressure at the sample temperature and partial pressure in the chamber. Therefore, in order to speed the primary drying process, the sample temperature should be as high as possible without the collapse of the sample structure and a high level of vacuum is maintained in the chamber. It should be mentioned that raising the sample temperature is more efficient for a faster primary drying process. A very high vacuum is not only difficult to maintain but also may hinder the drying process. This is because when ice crystals are sublimed, heat input into the frozen sample is required (so to maintain the sample temperature). The heat transfer is usually achieved from the shelf (higher temperature) to the sample (lower temperature) and by radiation from the surrounding. At very high vacuum, a Thermos flask effect may be developed in the chamber, which inhibits heat transfer from the shelf. The heat transfer by gas/vapour is reduced as well at high vacuum. This leaves the heat for sublimation provided predominantly by the inefficient radiation. It has been reported that moderate chamber pressure (100–150 mTorr, 100 mTorr is 13.3 Pa) could give optimal heat transfer in a set of vials [29].

Primary drying can take days depending on the freeze-dryer set-up, much longer than the freezing stage and secondary drying (both taking hours). To reduce the freeze-drying cycle time without an impact on product quality, the most important thing is to optimize primary drying parameters. The fine tuning of the sample temperature and the shelf temperature is the key to a fast primary drying process. It is known that each 1 °C increase in sample temperature can reduce the primary drying time by about 13% [30]. Therefore, the sample temperature should be as high as possible but below the temperature of sample structure collapse (usually several degrees). The safety margin between the sample temperature and the collapse temperature may be selected by considering the benefit of fast primary drying or higher risk of sample collapse [28]. The shelf temperature is typically 5–40 °C higher than the sample temperature. At constant chamber pressure and shelf temperature, the sample temperature increases 1–3 °C from beginning to the end of primary drying [29]. The bigger the difference between sample temperature and shelf temperature, the faster the primary drying process, but the higher the risk of the sample collapse (Figure 1.2) [28].

By the end of primary drying, all the ice crystals have been removed by sublimation. The sample temperature increases to the shelf temperature because there is no more ice sublimation and there is no heat removal by sublimation. Therefore, it signals the end of the primary drying stage when a steep rise of sample temperature is observed. There are different ways to measure sample temperatures [28], for example, thermocouples or RTD (resistance temperature detector) temperature sensors in the sample vials (invasive, measuring the bottom temperature), and manometric temperature measurement (MMT, measuring the temperature at the sublimation interface, more relevant to the drying process). The temperature discrepancy between the measured sample and the rest of the samples in the chamber should be noticed as a result of the invasive impact of thermocouple and the layout/locations of the samples in the chamber. The sample temperature

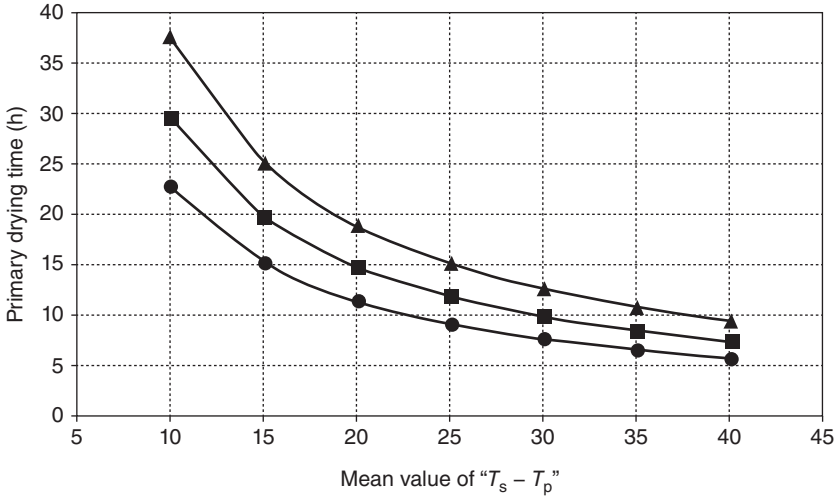

Figure 1.2 The diagram shows the relation of primary drying time with the temperature difference between the shelf temperature (T_s) and the product sample temperature (T_p). Solid circles, low dry layer resistance (solids ≤ 1%); solid square, medium dry layer resistance (10% > solids > 1%); solid triangles, high dry layer resistance (solids ≥ 10%). *Source:* Tang and Pikal 2004 [28]. Reprinted with permission from Springer.

is usually higher at the front and side but lower in the interior due to additional radiation heat transfer from the door and chamber walls.

1.2.4 Secondary Drying in Freeze-drying

The secondary drying is to remove the unfrozen water by desorption from the highly concentrated solute phase. The water transports from the desorption site across the interconnected porosity in the dry matrix and then vaporizes and is removed under vacuum. At the start of secondary drying, there is still a relatively large amount of residual water (5–20% on dry solid) present. With the increase of sample temperature, there is a great risk of sample collapse. At the end of the secondary drying, the residual moisture content is usually less than 1%, which ensures a good stability and long storage capability.

Although there is an increase of sample temperature, the shelf temperature is still higher because the heat of transfer is required for the vaporization of liquid water phase. Corresponding to the increase of sample temperature, the shelf temperature should increase slowly because the fast increase can cause the collapse of the not completely dry sample. Owing to the high residual moisture content in the amorphous sample early in secondary drying and thereby low glass transition temperature, the possibility of structural collapse is quite high. It is usually regarded as a safe procedure if the shelf temperature increases at a rate of 0.1–0.15 °C min^{-1} for amorphous solids. For crystalline solids, they do not have potential collapse during secondary drying and, therefore, a higher ramp rate (0.3–0.4 °C min^{-1}) is suggested [28].

The shelf temperature is directly related to secondary drying time. It is better to run a high shelf temperature for a short time rather than low temperature for a long period, provided that the sample quality can be maintained. Longer drying period at this region of temperature may be detrimental to the stability of biomolecules such as proteins. The drying time may vary depending on the solution concentration. A higher solute concentration can lead to a solid with smaller surface areas, less porosity, and enhanced barrier for water transport, and thereby a longer drying time. Usually, secondary drying times of 3–6 h is probably best with the terminal temperature varying in the region of 40–50 °C, even for protein formulations [31].

1.3 Ice Templating for Porous Structures

Ice templating has been used widely for the preparation of different types of porous materials. This section describes the key but general parameters that have been investigated. Detailed discussion and specific information are given in the following chapters. Unlike freeze-drying for pharmaceutics and foods, the ice-templating method is mainly used in research for the fabrication of novel porous materials and their potential applications. There is either no mass production or rare mass production of materials reported by the ice-templating approach, to the best of the author's knowledge. As such, the preparation time and cost of ice-templated materials have not been evaluated as a priority, as with freeze-drying in industries.

1.3.1 Solutes or Particles

(i) *Solutions widely used for ice-templating processes*. Small molecules, polymers or biomacromolecules are dissolved in suitable solvents. The solutes are excluded from the freezing ice front during freezing. To form a porous material, it is difficult to work with only small molecules because powders with low porosity are usually formed. It seems like a pre-requisite to have polymer or large biomolecules in the solution to form porous structures with tuneable morphology. The larger polymer molecules can entangle or interconnect together to give stability and porosity.

(ii) *Particles suspended in liquid medium*. These particles can include nanoparticles, nanofibres, platelets, and microparticles. To form a homogenous structure, a stable suspension is required before freezing. Therefore, surfactants or stabilizers are always present in the suspensions. For nanoparticles or nanofibres, because of their small sizes, it is generally easy to form stable dispersions. For larger nanoparticles or even microparticles, a higher concentration of stabilizer (usually polymeric, also to increase the viscosity of the suspension) is normally required. This has been observed frequently when using ceramic powders to prepare porous ceramic materials by ice templating.

(iii) *Emulsions where liquid droplets dispersed in another liquid phase*. An emulsion can be formed, usually by stirring or homogenization, to disperse liquid droplets in another immiscible continuous liquid phase [32]. A surfactant (either ionic or non-ionic polymeric surfactant) is added to stabilize the droplets by preventing them from coalescing. When monomers or polymers are dissolved in the continuous phase, freezing the emulsion followed by freeze-drying can produce porous polymers, combining ice templating and emulsion templating. Small molecules or polymers can be also dissolved in the droplet phase, which produces complex materials after freeze-drying the emulsion [33, 34].

1.3.2 Solvents

(i) *Water*. There is no doubt that water is the mostly used solvent. In the freeze-drying formulations for pharmaceuticals and biologics, water is the only solvent used although some polar organic solvents may be added as additives. When employing ice templating for porous materials, as the name 'ice' suggests, water-based solutions or suspensions have been mostly used.

(ii) *Organic solvents*. Organic solvents can also be frozen and the resulting frozen solvent or ice crystals ('ice' here means the general frozen solvent) are used as templates to generate porous materials. This is particularly important when preparing hydrophobic porous materials or when the precursors are insoluble in water. Despite the potential toxicity, flammability, and environmental concerns, similarly to the use of organic solvents in chemical plants, it is sometimes necessary to utilize these organic solvents. When choosing suitable organic solvents, their melting points and vapour pressures are important parameters to be considered. Owing to the limit in shelf temperature or the temperature of condensing chamber, only organic solvents that have relatively high melting points should be selected, e.g. melting points higher than −60 °C, depending on vapour pressure, type of solutes, freezing volume, and exposed surface area of the frozen sample. Table 1.1 shows the organic solvents that have

Table 1.1 Properties of common organic solvents used in the ice-templating approach.

Solvent	Density (ml g^{-1} at 25 °C)	Melting point (°C)	Vapour pressure (mmHg)
Dichloroethane	1.256	−35	87 at 25 °C
Chloroform	1.48	−63	160 at 20 °C
O-xylene	0.879 at 20 °C	−26 to −23	7 at 20 °C
Dimethyl sulfoxide	1.10	16–19	0.42 at 20 °C
1,4-Dioxane	1.034	10–12	27 at 20 °C; 40 at 25 °C
Cyclohexane	0.779	4–7	77 at 20 °C
tert-Butanol	0.775	23–26	31 at 20 °C
Camphene	0.85	48–52	2.5 at 25 °C

been used in literature. An important application is the use of organic solvents including dichloroethane, *o*-xylene, dioxane as templates for the preparation of hydrophobic biodegradable poly(ε-caprolactone) and poly(lactide-*co*-glycolide) materials. Dimethyl sulfoxide (DMSO) is often used because of its high melting point and the ability to dissolve polar polymers. Tertiary-butanol has been employed quite often for the preparation of silica and porous ceramics. Another unique solvent is camphene, which is solid at room temperature with high vapour pressure. Camphene melts at a moderate temperature of around 60 °C so it is still convenient to form solutions or suspensions. The subsequent freeze-drying process can be operated at room temperature compared to the usual freeze-drying process, thus reducing the operation costs.

(iii) *Compressed CO_2*. Supercritical CO_2 or compressed CO_2 has been regarded as a green and sustainable solvent. CO_2 is not toxic, inflammable, and cheaper and has low critical points. The solvent properties can be readily tuned by changing temperature and pressure. Below the critical temperature, compressed CO_2 acts like an organic solvent and can be used as a solvent for reactions or separation [35]. The very attractive point is that compressed CO_2 can be easily removed by depressurization. Zhang et al. reported the freezing of compressed CO_2 solution for the preparation of a porous organic material. The process used no organic solvent, and there was no need for freeze-drying because the frozen CO_2 could be just sublimed when the reactor valve was open and the frozen sample warmed up gradually [36].

(iv) *Mixing solvents*. Two miscible solvents may be mixed together. The point is that each solvent may exhibit different sizes or morphology of ice crystals upon freezing. The mixing solvents may be used accordingly to tune the pore morphology and pore sizes.

(v) *Emulsions*. This is an extended use of organic solvents. Oil-in-water emulsions have been mostly investigated. In addition to the preparation of porous materials with systematically tuneable porosity, the emulsion-freeze-drying method has been explored to form poorly water-soluble drug nanoparticles [33, 37]. Cyclohexane (high melting point, high vapour pressure, low toxic) and chloroform (good solvent for many poorly water-soluble drugs and relatively high melting point) have been mostly used for this purpose [33, 38].

1.3.3 Controlled Freezing

1.3.3.1 Control Orientation of Ice Crystal Growth

(i) *Random freezing*. This usually means freezing without control; for example, placing a solution in a freezer, in a cold bath of dry ice/acetone, or even just immersing in liquid nitrogen. There is no long-range order for the orientation of ice crystals. A schematic diagram is shown in Figure 1.3a. This, however, does not exclude locally ordered arrangement of ice crystals. As long as the temperature gradient exists, the local order of ice crystals will

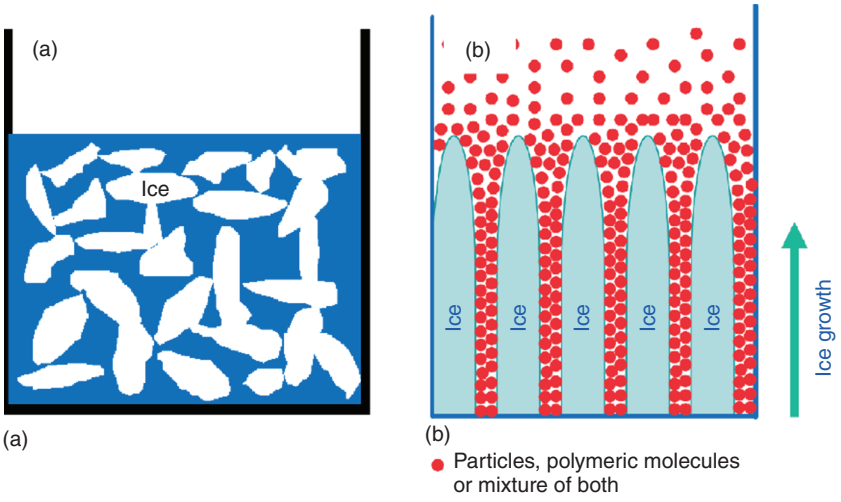

Figure 1.3 Schematic representation of controlled freezing: random freezing (a) versus directional freezing (b). *Source:* Qian and Zhang 2011 [8]. Reprinted with permission from John Wiley & Sons.

be present. This is also reflected in the pore structure of the freeze-dried materials.

(ii) *Directional freezing*. In contrast to random freezing, directional freezing indicates the orientated growth of ice crystals [22]. It has also been called *unidirectional freezing* by other researchers [9, 10]. This is usually realized by applying a constant temperature gradient to the liquid sample. The greater the temperature gradient, the faster the freezing and the smaller the ice crystals (and hence narrower spacings). A schematic representation of directional freezing process is given in Figure 1.3b. A directional freezing process can be performed by a simple and manual way, for example, by dipping a test tube containing liquid phase into liquid nitrogen slowly. In a more controlled way, one can use a motor to lower the sample vial into liquid nitrogen or keep the sample vial still but use the motor to push the liquid nitrogen dewar up. A dip coater may be used to dip a glass slide with the spread of liquid phase into liquid nitrogen, thus forming a thin film on the glass slide. A computer-controlled freezing stage can be employed to produce 2-dimensional surface patterns. A detailed description for this process is given in Section 1.4.

1.3.3.2 Ways of Freezing Samples

(i) *Freezing bulk samples*. The solutions or suspensions contained in a vial, beaker, or mould can be frozen and then freeze-dried to produce monoliths of different shapes (conforming to the shape of the container). The sample volume may vary from 1 to 100 ml, or even bigger volumes. The key point is that the fill depth or the thickness of the frozen sample should be normally less than 2 cm. Otherwise there is a risk of pore structure collapse or a

very long drying time may be required. Indeed, in the freeze-drying of pharmaceutics, it is suggested that the fill depth in the vial should be no greater than 2 cm. Otherwise, the quality of the freeze-dried cakes may be compromised.

(ii) *Injection into liquid nitrogen*. Instead of freezing the whole sample, the solution or suspension can be injected using a syringe pump and dropped into liquid nitrogen. The drops allow fast freezing and the resulting frozen beads contribute to fast freeze-drying, usually facilitating the formation of nice porous structures. For these freeze-dried beads, it is very easy to transfer them into different vessels or weigh out the required amount. These beads are usually quite uniform and can pack together nicely, e.g. for better separation. The down side is that it takes longer time to inject the solutions compared to just freezing the whole solution.

(iii) *Spray freezing*. This is quite often used for the production of protein or pharmaceutical powders. Basically, a solution is sprayed into liquid nitrogen using a nozzle. The nozzle can be either above the surface of liquid nitrogen [8, 33] or beneath it [39]. In the latter case, the nozzle is usually pressurized to avoid freeze-blocking or other measures are taken to prevent nozzle blocking. The sprayed droplets are fast frozen, which may help maintain the amorphous stage of pharmaceutics and avoid/reduce denaturation of proteins. The frozen particles are usually in the micron range. The larger surface area and short transport path allow a fast and uniform drying.

1.3.4 Removal of Ice Crystal Templates

(i) *Freeze-drying of frozen solutions or suspension*. The common feature for this type of samples is that upon warming up, the frozen samples melt and turn back into the liquid phase. Pharmaceutical formulations, polymer solutions and colloidal suspensions are normally in this category. The freeze-drying process is performed to allow the complete sublimation of ice crystals and the subsequent secondary drying to remove the unfrozen bound water (or other solvent). If for any reason the freeze-drying process goes wrong, the frozen sample may completely collapse, partially melt or shrink significantly. As a result, the ice-templated porous structure may be lost. Another general characteristic is that these freeze-dried materials can be readily dissolved into the original solvent (and other soluble solvents) and produce the solutions or suspensions again [1, 4, 8]. This is what is called as *reconstitution* in freeze-dried pharmaceutics. This feature is also employed to produce aqueous drug nanoparticle dispersions by the emulsion-freeze-drying approach [33, 37].

(ii) *Freeze-drying of gels or crosslinked samples*. For this type of samples, precursors or monomers are dissolved in a suitable solvent. After freezing the solutions, instead of immediate freeze-drying, a gelation process or crosslinked polymerization is allowed to occur while the samples remain frozen to maintain the ice-templated structures. Reported examples include

the silica gelation in the frozen state [40], UV-initiated frozen polymerization (a UV initiator and a crosslinker present with the monomers) [12], and the crosslinking polymerization in the freeze-concentrated monomer-rich phase in a frozen sample [41]. After the gelation or polymerization, a freeze-drying process is applied to remove the ice templates with the main purpose of generating highly interconnected porous structures with minimal shrinkage. Usually, if the materials are highly crosslinked, it is possible to dry them using the traditional vacuum oven instead of freeze-drying. To avoid shrinkage or pore-structure collapse by drying from water (due to the high surface tension of water), an immiscible organic solvent may be used to replace water and then removed by a common drying method. This can usually reduce the degree of shrinkage considerably.

There are instances where freeze-drying is used as a technique to produce porous materials with higher porosity and minimal shrinkage. Examples include the freeze-drying of metal–organic framework [42], gels [43], resorcinol–formaldehyde resins [44]. Under these circumstances, the materials are chemically crosslinked, physically assembled or stable frameworks containing solvents. The materials are not so flexible and hence a freezing process is not thought to change the original material structures. Freeze-drying is only functional as a better drying technique but there is no ice templating involved that leads to ice-templated materials.

(iii) *Solvent exchange with frozen samples*. It is possible to prepare ice-templated porous materials without performing a freeze-drying process. Apart from the frozen polymerization/reaction and subsequent conventional drying process mentioned in the earlier section, a solvent exchange process may be employed. For this method to work for a frozen sample, a second solvent that is miscible with the frozen solvent but which does not dissolve the solute should be selected. The melting point of this second solvent should be lower than the glass transition temperature (T_g) of the frozen solvent. The frozen sample is soaked in the second solvent at the temperature where the second solvent is still liquid and the frozen sample remains frozen. After replacing with fresh solvent at regular intervals, the frozen solvent is dissolved and completely removed. The materials can then be dried by vacuum drying to produce ice-templated porous materials. This method has been demonstrated by processing frozen DMSO-polymer samples with icy water [45]. It is expected that this approach can be extended to other systems.

1.3.5 Ice-templated Materials

1.3.5.1 Shape and Form

When making bulky or monolithic samples, the shapes of the materials can be controlled by choosing different vessels or moulds. This can be very important for engineering materials where the materials may be too hard, brittle or soft to be cut into the desirable shapes. Powered samples can be fabricated by a spray freezing process or by ice-induced self-assembly of colloids. Beads with diameters

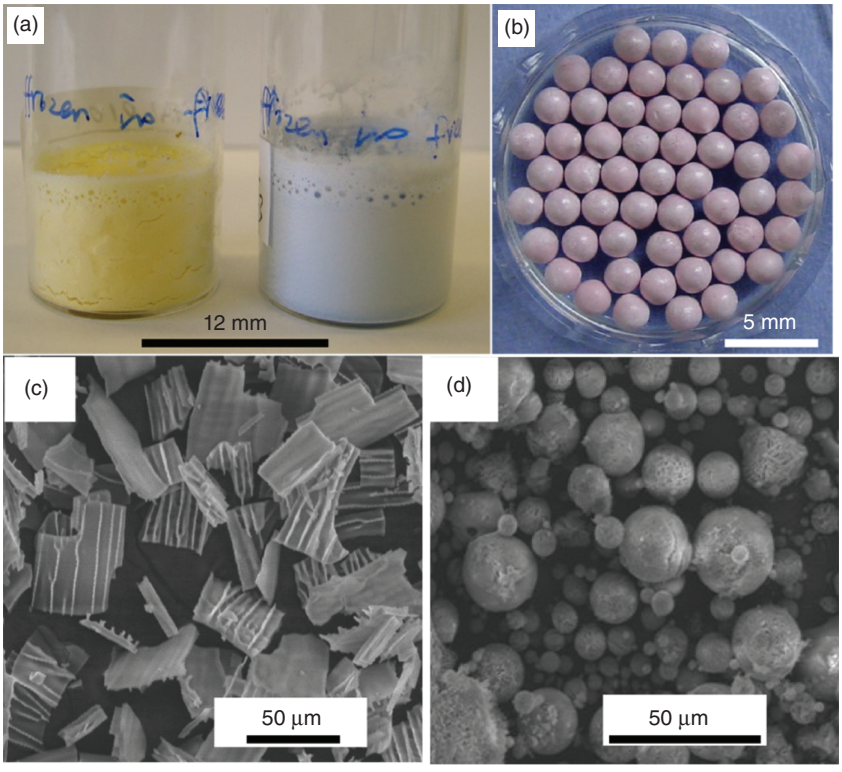

Figure 1.4 Examples showing different shapes and forms of freeze-dried materials. (a) Monoliths in glass vials; (b) polymer beads containing gold nanoparticles; (c) powders of silica microplates; and (d) powder of porous microspheres.

in millimetres can be prepared by injection into liquid nitrogen. Figure 1.4 shows examples of the freeze-dried materials with different forms.

1.3.5.2 Pore Structure and Morphology

Freeze-drying of a solution or suspension produces porous materials. By controlling the freezing process, it is possible to generate porous materials with random pores, aligned microchannels or layered porous structures (Figure 1.5a–c). It has been found that when a very diluted solution or suspension (e.g. 0.1 wt%) is processed, one-dimensional nanostructures can be generated. For example, polymer nanofibres can be formed from dilute aqueous polymer solutions (Figure 1.5d) [46]. In the case of nanoparticle suspensions, microwires/nanowires consisting of assembled nanoparticles can be constructed (Figure 1.5e,f) [47]. When combining emulsion templating and ice templating, porous materials with tuneable porosity exhibit both ice-templated and emulsion-templated pore morphologies (Figure 1.5g). Moreover, when a second polymer is dissolved in the droplet phase, a composite of aligned porous microspheres embedded in an aligned porous matrix can be formed (Figure 1.5h). Simply dissolving the porous matrix can generate the ice-templated microspheres (Figure 1.5i).

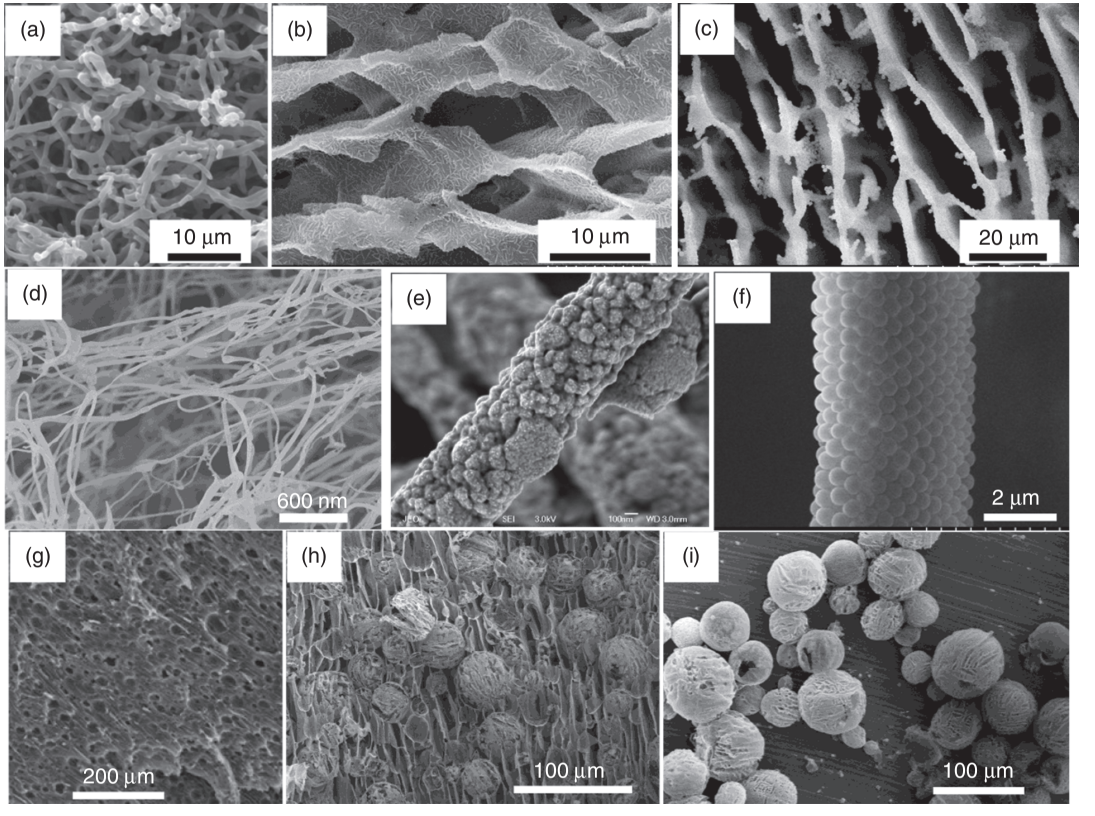

Figure 1.5 Illustration of various morphologies of freeze-dried materials produced in our laboratory. (a) Porous poly(lactide-*co*-gylcolide); (b) porous poly(vinyl alcohol) with inorganic salt; (c) silica colloids with poly(vinyl alcohol); (d) sodium carboxymethyl cellulose. *Source:* Reprinted with permission from Ref. [46]. (e) Nanowires consisting of gold nanoparticles. *Source:* Reprinted with permission from Ref. [47]. (f) Microwire consisting of polystyrene colloids; (g) emulsion (with 50% oil phase)-templated porous poly(vinyl alcohol) with sodium dodecyl sulfate as surfactant; (h) porous poly(ε-caprolactone) microspheres in aligned porous poly(vinyl alcohol); and (i) aligned porous poly(ε-caprolactone) microspheres.

1.3.5.3 Type of Materials

As the ice-templating method can be used for solutions, suspensions or emulsions, and water or organic solvents, a wide range of materials can be prepared. Some of the examples are illustrated in Figure 1.5. Hydrophilic polymeric materials can be prepared from aqueous polymer solutions whilst hydrophobic organic materials are formed from organic solutions. Porous silica and metal oxides can be constructed either directly from the precursor or by using the pre-formed polymer structures as templates [48]. For porous metals, usually porous metal oxides can be prepared first and followed by reduction, e.g. in H_2 atmosphere [49]. It is also possible to start with metal nanoparticles and fuse the nanoparticles into metal materials by heating in an inert atmospheres. Porous carbon materials can be generated as well. This may be achieved by freeze-drying of carbon nanotubes or graphene suspensions [50]. Or a porous carbon-rich polymer may be prepared first followed by carbonization in N_2 or argon [51]. A large number of activities have been carried out on the fabrication of porous ceramics, which normally starts with the suspensions of ceramic particles either in aqueous medium or in a suitable organic medium [10, 17].

1.4 The Practice in Our Laboratory

These are the procedures and set-ups we have been using in our laboratory. It works well from a research point of view but is not necessarily the optimized procedures or can work on a large scale. The purpose is to give new researchers in this area or relevant research areas basic practices and some ideas to start with.

1.4.1 Controlled Freezing

The controlled freezing process usually refers to directional freezing, in order to allow orientated growth of ice crystals. This is in contrast to random freezing where we usually place the solutions/suspensions in a freezer or a fridge depending on the freezing temperature of the solvent or solution. The directional freezing may be simply carried out by using tweezers or a tong to hold the sample vial and slowly dip into liquid nitrogen. It is still the practice we are using when a new student needs to be familiar with the directional freezing procedure (Figure 1.6a). An alternative approach is to fix the sample vial and to raise the level of liquid nitrogen (Figure 1.6b). It would be best to use a motor to raise the platform that supports the liquid nitrogen dewar. It is also fine to use a jack and raise the level of the jack manually. This works quite well for our experiments.

To better control the directional freezing process, we have also used a syringe pump to lower a sample vial into liquid nitrogen at a defined rate (Figure 1.6c). Of course, a motor may be also used similarly. More precisely, a computer-controlled dip coater can be used. A sample vial (or glass slide with spreading liquid) can be lowered into liquid nitrogen at the controlled rates. But trial experiments need to be conducted before establishing a suitable procedure. This is because when setting the sample up, the liquid nitrogen vapour may just

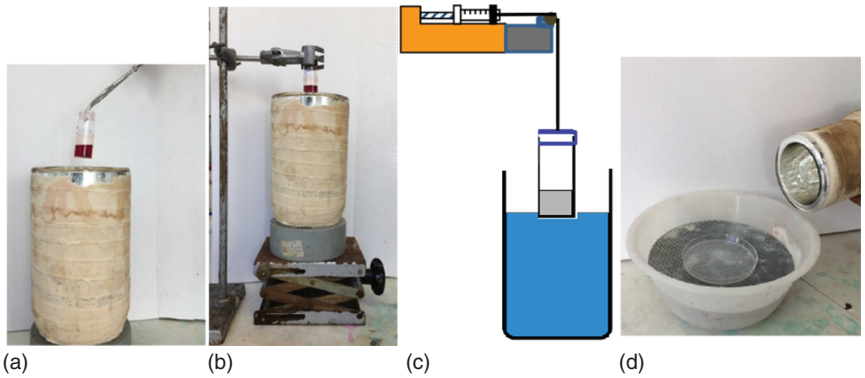

Figure 1.6 Photographs or diagrams show the set-ups that have been used. (a) Dip the glass vial into liquid nitrogen manually; (b) fix the glass vial into the level of liquid nitrogen dewar on a jack manually; (c) dip the glass vial via a syringe pump; and (d) a petri dish on a metal mesh which is stacked in a bowl. Liquid nitrogen is added from the side. (Note: This photograph is for illustration only with the plastic bowl. For the experiments, an open-mouthed dewar is always used.)

freeze the sample particularly when the volume is small. A suitable starting point and the lowering rate are key parameters.

Directional freezing has been used to produce aligned porous materials. By contacting the vial bottom with liquid nitrogen and then lowering down into liquid nitrogen gradually, nucleation starts at the bottom and ice crystals grow upwards in a parallel way. To produce a uniformly aligned porous structure, it is important to allow a continuous upward growth of ice crystals and reduce disturbance from sample moving and from the sidewall. It is believed it is better to have a sample vial with a good thermal conductive bottom (e.g. metal) and a poor thermal conductive wall (e.g. plastic, PTFE). Another way is to use a vial with a large diameter to reduce the sidewall effect to the majority of the samples. For example, a large petri dish can be filled with solution or suspension at shallow depth and placed on a metal mesh that is just located at the level of liquid nitrogen. Once nucleation and ice crystal growth starts (that can be observed quite easily), more liquid nitrogen can be poured from the side (Figure 1.6d). The raising level of liquid nitrogen contributes to the continuous freezing and growth of ice crystals until completion.

The above procedures are used to produce relatively bulky or monolithic samples. Similar procedures may be used to prepare thin films and two-dimensional surface patterns, especially with the dip coater approach. In our lab, we have used computer-aided two temperature-controlled freezing stages to prepare aligned patterns on flat substrates. This instrument is largely used to observe the freezing process (see discussion in section 1.4.2). The difference is that after the slide is completely frozen, it is moved to liquid nitrogen quickly (to keep being frozen) and then transferred into a freeze-dryer.

In order to produce ice-templated beads, a solution or an emulsion can be simply injected into liquid nitrogen using a syringe pump at a controlled slow rate (Figure 1.7a). It takes some time to fully freeze the drops. Indeed, once the drops

move off from the needle and hit the surface of liquid nitrogen, the heat of fusion from freezing water leads to the rapid evaporation of liquid nitrogen, which drives the drops moving around quickly on the surface of liquid nitrogen. The drop will then fully freeze and sink into liquid nitrogen. The time taken to fully freeze the drop varies depending on solvent type (or emulsion), solute, and concentration. In order to prepare discreet beads, the injection rate should be slow enough to allow one drop to be fully frozen and sinking before another drop hits on the surface of liquid nitrogen. When there are two drops rapidly moving around the surface, it is highly likely they will collide and form fused particles. Owing to this slow injection rate, the needle should be well above the surface of liquid nitrogen to prevent the cold liquid nitrogen vapour to freeze the liquid in the needle and block it. Even from a laboratory point of view, the production of beads by injection with one syringe is very slow. To improve the productivity, a peristaltic pump can be used, where a number of silicone tubes may be used to inject a solution (or emulsion) into multiple dewars containing liquid nitrogen. Figure 1.7b shows the set-up we have used in our laboratory. Before using this set-up, a suitable injection rate must have been established for the liquid system in question.

To produce ice-templated microspheres, we have used a simple aerosol sprayer (bought from Fisher Scientific) to spray either aqueous solutions or emulsions into liquid nitrogen. The spraying nozzle is above the surface of liquid nitrogen. Once the fine droplets are sprayed onto the liquid nitrogen, they will rapidly freeze and settle into the liquid nitrogen. The frozen powders can then be collected after decanting the liquid nitrogen and subjecting to normal freeze-drying.

1.4.2 Observation of Freezing and Freeze-drying

Observation of the freezing process and more specifically the growth of ice crystals have been investigated by various researchers, with the aim to understand the process and provide fundamental evidence for freezing theories. The detailed discussion on this topic can be found in Chapter 2.

Figure 1.7 (a) The diagram shows a solution/emulsion/suspension injected into liquid nitrogen. (b) The photograph shows parallel injection into liquid nitrogen using a peristaltic pump. In this case, a milky oil-in-water emulsion is injected into liquid nitrogen contained in a few dewars. (c) Illustration of a solution/suspension/emulsion being sprayed into liquid nitrogen using a simple aerosol sprayer. (Note: This photograph is for illustration only with the plastic bowl. For the experiments, an open-mouthed dewar is always used.)

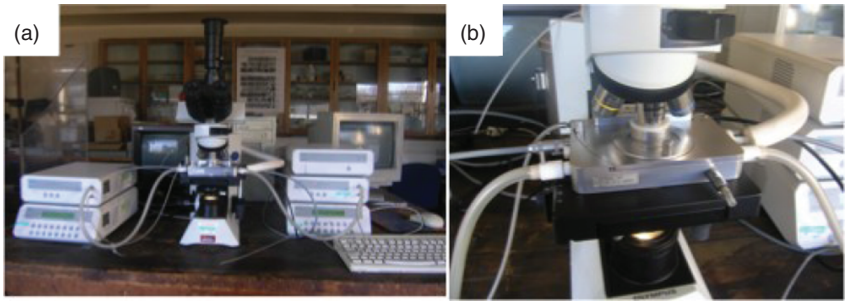

Figure 1.8 Photographs showing the set-up used to observe the freezing process – an optical microscope equipped with a computer-controlled freezing stage. (a) The whole set-up and (b) close view of freeze stage connections just under the optical lens.

We have used an Olympus CX41 microscope equipped with a digital camera and a computer-controlled freezing stage (Link-am software, shown in Figure 1.8, borrowed from Dr Michael Butler of Unilever). There are two separately temperature-controlled metal plates (a gap of 2 mm between them) achieved by pumping liquid nitrogen at controlled rates. A sample holder connected to a micromotor (which is controlled by the computer) can move a glass slide at different rates (usually 10–200 $\mu m\ s^{-1}$). Thin metal rings (different heights) are placed on a glass cover slide and a defined volume of solution is deposited in the metal ring (to control the thickness of the liquid film) and then covered by another glass cover slide (to prevent solvent evaporation for longer observation). This set-up has been used to observe the directional growth of ice crystals for aqueous PVA solution, aqueous PVA/silica colloidal suspension, and aqueous gold nanoparticle suspensions. The study with the controlled freezing stage has provided direct observation for orientated ice crystal growth and demonstrated the viability to control the width of the aligned channels by varying the freezing rate.

In extended studies, the freezing stage can be tightly sealed and connected to a vacuum pump. After the freezing process is completed (no top cover glass slide is used), the vacuum pump is switched on so that the freeze-drying process can be directly observed using the microscope. We did not observe the gradual sublimation of ice crystals (as we had hoped). It was difficult to get clear optical images during the freeze-drying process. After some time, suddenly, it seemed that all the ice crystals have been moved and clear optical images could be obtained (Figure 1.9). For easy observation, an oil-in-water emulsion (cyclohexane emulsified into aqueous PVA solution containing sodium dodecyl sulfate as surfactant) is investigated. The emulsion is spread on a glass slide (no cover glass on the top used) and then subjected to freezing and freeze-drying. As can be seen from Figure 1.9, there is no visible difference between the emulsion prepared and the emulsion frozen. However, after freeze-drying, some cellular pores are really bright because that allows complete light through them while the PVA part looks a bit dark.

Because of the fine control on the freezing process using a computer-controlled freezing stage, this set-up has been used to produce aligned polymer (and with

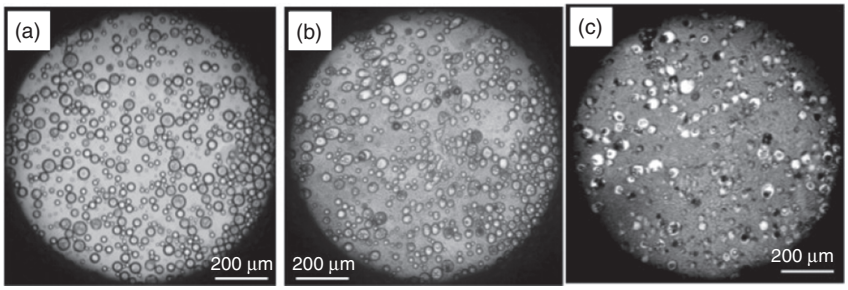

Figure 1.9 Observation of freeze-drying an oil-in-water emulsion (cyclohexane emulsified into aqueous PVA solution) using an optical microscope. (a) The emulsion as prepared, spread on a glass slide; (b) this emulsion is frozen in the freezing stage; and (c) the same sample after freeze-drying by subjecting the freezing stage compartment to a vacuum pump.

silica or metal oxide nanoparticles) patterns and grid structures [52]. These patterned substrates can be used to guide stem cell growth.

1.4.3 Freeze-drying Procedure

We have rarely freeze-dried the samples with a total volume of >200 ml. In principle, provided that the freeze-drying capacity is sufficient, the frozen samples with large exposed surface area and a thickness <2 cm (we usually freeze-dry samples with a fill depth of 1 cm or smaller in the lab) can be freeze-dried to produce ice-templated porous materials with minimal shrinkage. When the solvents other than water are used, the removal of cyclohexane or DMSO can be equally efficient. For the solvents with lower melting point, a smaller volume and higher exposed surface area should be used to ensure the production of freeze-dried porous materials.

It is known that small molecules, particularly inorganic solvents, may considerably reduce the freezing point (not the supercooled temperature) when dissolved in water. However, this has not impacted the preparation of polymer materials in our laboratory. Indeed, the use of polymers either as main solutes or as stabilizers is essential for the preparation of freeze-dried porous materials, both in aqueous and organic solutions. For a mixture solution of polymer and inorganic salts, the melting point can be still much lower than frozen water or polymer solution. This should be taken into account and a trial and error process may be required for such types of solutions. The freeze-drying process works well with nanoparticle suspensions (usually > 15 nm) although stabilizers are still required.

Once the freezing process is completed, the frozen samples may be stored in liquid nitrogen for a short while before being placed in a freeze-dryer. One may choose to leave the frozen samples in a freezer overnight (depending on the solvents used) for freeze-drying next day. The ice-templated macroporous structures seem stable but we have not investigated the possible annealing or other effects on the pore structures.

Below is the procedure adopted in our laboratory. We have used bench-top freeze-dryers. The current freeze-dryer is CoolSafe 90.

(1) Before switching on, check that any liquid in the condenser chamber has been completely drained.
(2) Switch on the freeze-dryer and keep running at least 15 min. Ensure that the vacuum level and the required condenser temperature are reached.
   - Close the draining valve.
   - Ensure that the acrylic chamber is properly positioned
   - Switch on the freeze-dryer.
   - Switch on the pump, ensure that the valve between the pump and freeze-dryer is open.
   - Ensure that the freeze-dryer is running stably when the yellow light (on the panel) is not flashing and the condenser temperature is down to about −100 °C.
(3) Place the frozen samples into the freeze-dryer.
   - Ensure you have all the frozen samples ready (not one by one) beside the freeze-dryer. The frozen samples should be stored in liquid nitrogen (it may be alright for water samples to be stored in a freezer, but liquid nitrogen is recommended to avoid partial melting during transferring because of the small volumes involved).
   - Close the valve between the pump and the freeze-dryer.
   - Open the draining valve to release the vacuum.
   - Open the acrylic chamber, immediately place the frozen samples (it helps to have some liquid nitrogen with the frozen samples) on the stack. Do not place the frozen samples directly on the acrylic base because the low temperature may stiffen the plastics.
   - Once the samples are placed, immediately re-position the acrylic chamber.
   - Close the draining valve and open the valve to the pump.
(4) Leave the samples to be freeze-dried normally for 48 h. Check the status of the frozen samples in the first 30 min. If for any reason the freeze-drying is not running properly, for example, the acrylic chamber is misplaced, or is leaking somewhere, one will notice the partial melting of the frozen samples. If this happens, stop the freeze-drying process and sort out the problems.
(5) When the freeze-drying is finished, it is alright to repeat procedure 3 and freeze-drying another batch of frozen samples. Because we only freeze-dry small volumes of frozen samples, this should not exceed the cold chamber capacity or impact the freeze-drying process obviously. But this should be examined carefully if large volumes of frozen samples are freeze-dried.
(6) Switch off the freeze-dryer when no further samples are to be freeze-dried
   - Switch off the freeze-dryer.
   - Open the draining valve to release the vacuum.
   - Take out the freeze-dried samples and store in a desiccator.
   - Switch off the pump.
   - Allow the condenser to warm up, melted solvent is drained, and flush the condenser chamber using water (only do this at room temperature) if it does not look clean.

Other types of freeze-dryers can also be used. For example, we have also used a VirTis AdVantage freeze-dryer where the shelf temperature can be programmed

to facilitate the freeze-drying process. The important thing is to know the melting points of the frozen sample, vapour pressure of the frozen solvent (or approximately judge based on the volatility), the sample depth, and the volume. Different labs may have different freeze-drying practices but the overall target is the same–to produce ice-templated materials with desirable pore structures and pore volumes.

References

1 Franks, F. and Auffret, T. ed. (2007). *Freeze-Drying of Pharmaceuticals and Biopharmaceuticals: Principles and Practice*. RSC Publishing.
2 Rey, L. and May, J.C. ed. (2010). *Freeze-Drying/Lyophilization of Pharmaceutical and Biological Products*. CRC Press.
3 Hua, T.-C., Liu, B.-L., and Zhang, H. ed. (2010). *Freeze-Drying of Pharmaceutical and Food Products*, Food Science, Technology and Nutrition. Woodhead Publishing Limited.
4 Varshney, D. and Singh, M. ed. (2015). *Lyophilized Biologics and Vaccines: Modality-Based Approaches*. Springer.
5 Roberts, A.D., Li, X., and Zhang, H. (2014). Porous carbon spheres and monoliths: morphology control, pore size tuning and their applications as Li-ion battery anode materials. *Chem. Soc. Rev.* 43: 4341–4356.
6 Silverstein, M.S., Cameron, N.R., and Hillmyer, M.A. ed. (2011). *Porous Polymers*. Hoboken: Wiley.
7 Sing, K.S.W., Everett, D.H., Haul, R.A.W. et al. (1985). Reporting physisorption data for gas/solid systems with special reference to the determination of surface area and porosity. *Pure Appl. Chem.* 57: 603–619.
8 Qian, L. and Zhang, H. (2011). Controlled freezing and freeze drying: a versatile route for porous and micro-/nano-structured materials. *J. Chem. Technol. Biotechnol.* 86: 172–184.
9 Gutierrez, M.C., Ferrer, M.L., and del Monte, F. (2008). Ice-templated materials: sophisticated structures exhibiting enhanced functionalities obtained after unidirectional freezing and ice-segregation-induced self-assembly. *Chem. Mater.* 20: 634–648.
10 Deville, S. (2010). Freeze-casting of porous biomaterials: structure, properties and opportunities. *Materials* 3: 1913–1927.
11 Pawelec, K.M., Husmann, A., Best, S.M., and Cameron, R.E. (2014). Ice-templated structures for biomedical tissue repair: from physics to final scaffolds. *Appl. Phys. Rev.* 1: 021301.
12 Barrow, M., Eltmimi, A., Ahmed, A. et al. (2012). Frozen polymerization for aligned porous structures with enhanced mechanical stability, conductivity, and as stationary phase for HPLC. *J. Mater. Chem.* 22: 11615–11620.
13 Wolkers, W.F. and Oldenhof, H. ed. (2015). *Cryopreservation and Freeze-Drying Protocols*. New York: Human Press, Springer.
14 Deville, S. (2013). Ice-templating, freeze casting: beyond materials processing. *J. Mater. Res.* 28: 2202–2219.

15 Sundaramurthi, P. and Suryanarayanan, R. (2010). Trehalose crystallization during freeze-drying: implications on lyoprotection. *J. Phys. Chem. Lett.* 1: 510–514.

16 Levina, M. and Rajabi-Siahboomi, A.R. (2004). The influence of excipients on drug release from hydroxypropyl methylcellulose matrices. *J. Pharm. Sci.* 93: 2746–2754.

17 Li, W.L., Lu, K., and Walz, J.Y. (2012). Freeze casting of porous materials: review of critical factors in microstructure evolution. *Int. Mater. Rev.* 57: 37–60.

18 Kasper, J.C. and Friess, W. (2011). The freezing step in lyophilisation: physio-chemical fundamentals, freezing methods and consequences on process performance and quality attributes of biopharmaceuticals. *Eur. J. Pharm. Biopharm.* 78: 248–263.

19 Geidobler, R. and Winter, G. (2013). Controlled ice nucleation in the field of freeze-drying: fundamentals and technology review. *Eur. J. Pharm. Biopharm.* 85: 214–222.

20 Searles, J.A., Carpenter, J.F., and Randolph, T.W. (2001). The ice nucleation temperature determines the primary drying rate of lyophilization for samples frozen on a temperature-controlled shelf. *J. Pharm. Sci.* 90: 860–871.

21 Moore, E.B. and Molinero, V. (2011). Structural transformation in supercooled water controls the crystallization rate of ice. *Nature* 479: 506–508.

22 Zhang, H., Hussain, I., Brust, M. et al. (2005). Aligned two- and three-dimensional structures by directional freezing of polymers and nanoparticles. *Nat. Mater.* 4: 787–793.

23 Rowe, T.D. (1990). A technique for the nucleation of ice. Proceedings of International Symposium on Biological Product Freeze-Drying and Formulation, Basel, New York: Karger, c1992. ISBN 3805554664.

24 Petersen, A., Rau, G., and Glasmacher, B. (2006). Reduction of primary freeze-drying time by electric field induced ice nucleus formation. *Heat Mass Transfer* 42: 929–938.

25 Bang, J.H. and Suslick, K.S. (2010). Applications of ultrasound to the synthesis of nanostructured materials. *Adv. Mater.* 22: 1039–1059.

26 Nakagawa, K., Hottot, A., Vessot, S., and Andrieu, J. (2006). Influence of controlled nucleation by ultrasounds on ice morphology of frozen formulations for pharmaceutical proteins freeze-drying. *Chem. Eng. Process.* 45: 783–791.

27 Patapoff, T.W. and Overcashier, D.E. (2002). The importance of freezing on lyophilisation cycle development. *BioPharm* 15: 16–21.

28 Tang, X. and Pikal, M.J. (2004). Design of freeze-drying processes for pharmaceuticals: practical advice. *Pharm. Res.* 21: 191–200.

29 Pikal, M.J., Roy, M.L., and Shah, S. (1984). Mass and heat transfer in vial freeze-drying of pharmaceuticals: role of the vial. *J. Pharm. Sci.* 73: 1224–1237.

30 Pikal, M.J. (1990). Freeze-drying of proteins. Part I: Process design. *BioPharm* 3: 18–28.

31 Pikal, M.J., Shah, S., Roy, M.L., and Putman, R. (1990). The secondary drying stage of freeze drying: drying kinetics as a function of temperature and chamber pressure. *Int. J. Pharm.* 60: 203–217.

32 Zhang, H. and Cooper, A.I. (2005). Synthesis and applications of emulsion-templated porous materials. *Soft Matter* 1: 107–113.

33 Zhang, H., Wang, D., Butler, R. et al. (2008). Formation and enhanced biocidal activity of water-dispersible organic nanoparticles. *Nat. Nanotechnol.* 3: 506–511.

34 Zhang, H., Edgar, D., Murray, P. et al. (2008). Synthesis of porous microparticles with aligned porosity. *Adv. Funct. Mater.* 18: 222–228.

35 Cooper, A.I. (2000). Polymer synthesis and processing using supercritical carbon dioxide. *J. Mater. Chem.* 10: 207–234.

36 Zhang, H., Long, J., and Cooper, A.I. (2005). Aligned porous materials by directional freezing of solution in liquid CO_2. *J. Am. Chem. Soc.* 127: 13482–13483.

37 Grant, N. and Zhang, H. (2011). Poorly water-soluble drug nanoparticles via an emulsion-freeze-drying approach. *J. Colloid Interface Sci.* 356: 573–578.

38 McDonald, T.O., Martin, P., Patterson, J.P. et al. (2012). Multicomponent organic nanoparticles for fluorescence studies in biological systems. *Adv. Funct. Mater.* 22: 2469–2478.

39 Yu, Z., Garcia, A.S., Johnston, K.P., and Williams, R.O. (2004). Spray freezing into liquid nitrogen for highly stable protein nanostructured microparticles. *Eur. J. Pharm. Biopharm.* 58: 529–537.

40 Mukai, S.R., Nishihara, H., and Tamon, H. (2004). Formation of monolithic silica gel microhoneycombs (SMHs) using pseudosteady state growth of microstructural ice crystals. *Chem. Commun.* 874–875.

41 Gun'ko, V.M., Savina, I.N., and Mikhalovsky, S.V. (2013). Cryogels: morphological, structural and adsorption characterisation. *Adv. Colloid Interface Sci.* 187–188: 1–46.

42 Ma, L., Jin, A., Xi, Z., and Lin, W. (2009). Freeze drying significantly increases permanent porosity and hydrogen uptake in 4,4-connected metal–organic frameworks. *Angew. Chem. Int. Ed.* 48: 9905–9908.

43 Sai, H., Xing, L., Xiang, J. et al. (2013). Flexible aerogels based on an interpenetrating network of bacterial cellulose and silica by a non-supercritical drying process. *J. Mater. Chem. A* 1: 7963–7970.

44 Tamon, H., Ishizaka, H., Yamamoto, T., and Suzuki, T. (2000). Influence of freeze-drying conditions on the mesoporosity of organic gels as carbon precursors. *Carbon* 38: 1099–1105.

45 Roberts, A.D., Lee, J.-S.M., Wong, S.Y. et al. (2017). Nitrogen-rich activated carbon monoliths via ice templating with high CO_2 and H_2 adsorption capacities. *J. Mater. Chem. A* 5: 2811–2820.

46 Qian, L., Willneff, E., and Zhang, H. (2009). A novel route to polymeric sub-micron fibers and their use as templates for inorganic structures. *Chem. Commun.* 3946–3948.

47 Zhang, H., Lee, J.-Y., Ahmed, A. et al. (2008). Freeze-align and heat-fuse: microwires and networks from nanoparticle suspensions. *Angew. Chem. Int. Ed.* 47: 4573–4576.

48 Qian, L., Ahmed, A., Foster, A. et al. (2009). Systematic tuning of pore morphologies and pore volumes in macroporous materials by freezing. *J. Mater. Chem.* 19: 5212–5219.
49 Ran, H., Feng, P., Liu, Z. et al. (2015). Complex-shaped porous Cu bodies fabricated by freeze-casting and vacuum sintering. *Metal* 5: 1821–1828.
50 Nardecchia, S., Carriazo, D., Ferrer, M.L. et al. (2013). Three dimensional macroporous architectures and aerogels built of carbon nanotubes and/or graphene: synthesis and applications. *Chem. Soc. Rev.* 42: 794–830.
51 Roberts, A.D., Li, X., and Zhang, H. (2015). Hierarchically porous sulfur-containing activated carbon monoliths via ice-templating and one-step pyrolysis. *Carbon* 95: 268–278.
52 Qian, L., Ahmed, A., Glennon-Alty, L. et al. (2015). Patterned substrates fabricated by a controlled freezing approach and biocompatibility evaluation by stem cells. *Mater. Sci. Eng. C* 49: 390–399.