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Review article

Freeze-drying of bioproducts: putting principles into practice

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Abstract

The product and process parameters that determine successful freeze-drying are described and their interrelationships are explored. It is shown that the thermochemical and thermomechanical properties of water-soluble, amorphous materials form the basis of effective formulation design and that coordinated approaches to formulation and process development achieve optimum results with a minimum of trial-and-error experimentation. © 1998 Elsevier Science B.V.

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1. Introduction

Freeze-drying as an industrial process dates from the time during World War II when the demand for human blood plasma reached critical proportions. Before that, drying by freezing and sublimation of ice had been a laboratory curiosity, although the concentration effects of freezing had been well appreciated very much earlier. Thus, the Uighurs of Turfan prepared 'frozen-out wine' as early as the seventh century.

Even in present times, however, it is not universally appreciated that freezing is actually synonymous with drying, because solvent water is removed from a solution or a dispersion in the form of a pure solid (ice) phase. The fact that the ice is subsequently sublimed, to be recondensed elsewhere, does not affect the residual solution phase. There are other misconceptions about freezing and drying, among them the notion that '…the transformation of water into ice brings to an end all chemical reactions' [1]. Industrial conventional wisdom also still has it that freeze-drying processes can only be developed empirically by trial and error, i.e. by testing the finished product, or that '…anything can be freeze-dried in 24 hours'. It is the purpose of this review to convince the reader that the various stages of lyophilisation are based on very sound physical, chemical and engineering principles and can be controlled to the extent that the outcome of a given process performed on a given product can often be estimated to within fairly close tolerances, without the need for trial-and-error experimentation. Even more important, stable products can frequently be designed by matching an optimum product formulation with its associated optimum drying process cycle.

2. Why freeze-dry?

Despite its technical complexity, freeze-drying, as a unit operation, is not mentioned in standard chemical engineering texts. It is also given short shrift in the biochemical literature, usually being disposed of in one sentence. It is therefore not immediately obvious how a process as capital and energy intensive, as lacking in a fundamental engineering analysis and as time consuming as freeze-drying should nevertheless have established itself as the drying method of choice by the pharmaceutical and bioindustries.

The prime reason is probably a conception that drying enhances chemical stability. Although from a practical

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standpoint ambient-temperature stable liquid state formulations would in most cases be preferable, the chances of success must be considered as negligible. The substances of most concern, peptides, proteins and complex synthetic organic molecules, are subject to a variety of chemical reactions in aqueous solutions, many of which are quite unacceptable in terms of product performance or safety. Included among such reactions which tend to reduce specific biological activity are hydrolysis, cross-linking, oxidation, aggregation and disulfide rearrangements.

Experience has taught that in the dry state such reaction can be substantially retarded. That still leaves the question why freezing, followed by ice sublimation, should have become the preferred route of water removal. The answer is probably to be found in an assumption that a process, performed at low temperatures, is less likely to be injurious to labile bioproducts than drying at ambient or higher temperatures. Whatever may be the validity of the underlying assumptions, the fact is that freeze-drying is accepted by the regulatory authorities as a suitable unit operation in the manufacture of therapeutic products.

3. Basic principles, process and quality parameters

At its most basic, the process consists of the separation of liquid water from a wet solid product or from a solution or dispersion of given concentration in the form of a solid phase, ice, and its subsequent removal by vacuum sublimation, leaving the solutes or substrates in their anhydrous, or almost anhydrous states. This bald statement hides a multitude of complex and interacting problems, relating to the chemical composition of the wet product, chemical and physical changes that may accompany freezing, heat and mass transfer within the equipment used, and the performance of the resulting dried product.

Freeze-drying was at one time popular in the food industry [2] but has largely been superseded by more economical drying processes. The quality criteria for food products are governed largely by consumer acceptance. They are crude compared to those applied to pharmaceutical and other bioproducts, especially when administered by injection, where acceptability and profitability are governed by complex biochemical criteria, e.g. units of biological activity per milligram of product or the presence of low amounts of contaminant, introduced during the process or thereafter, during storage.

The freeze-drying of biologicals is only now becoming a subject considered as worthy of study by the relevant sectors of industry [3]. While not claiming that this review provides all the answers, the author hopes to demonstrate that freeze-drying can be analysed rationally by the application of well-established physical, chemical and engineering principles, and that formulations and process cycles can be established in a predictive manner that will result in acceptable products, with a minimum of trial and error experimentation.

4. What can be controlled?

As a first approximation, the nature of the preparation to be freeze-dried and the dimensions of the containing vessel determine the optimum process conditions that must be applied. For the sake of clarity in presentation the various parameters that govern freeze-drying are treated separately at this stage. They are summarised below.

Product:	composition/formulation/concentration, solid content, fill volume
Container:	type (vial, ampoule, syringe), geometry, stoppers
Equipment:	freeze-drier model, loading (shelf, trays), probes (number, position, type)
Process:	shelf temperature, chamber pressure, time (cooling, annealing, primary/secondary drying cycles)

In most practical situations a compromise must be achieved between various competing demands relating to formulation, processing conditions, cost and other considerations. Thus, the quality and appearance of the dried product depend on the composition, concentration and volume of the solution to be dried, the geometry of the containing vessel and several equipment and process parameters which govern heat and mass transfer but are beyond the scope of this discussion.

5. Formulation

The composition profile of a bioproduct to be freeze-dried will be governed by several factors. Frequently the biologically active component is present at a very low concentration, so that bulking agent (excipient) is added to create physical stability. This provides for ‘body’, mechanical strength and an attractive appearance. Alternatively, the excipient may also perform the function of chemical stabiliser for the bioproduct during the process of freeze concentration. In addition, the solution may require pH buffers and may also contain other salts, either carried over from downstream processing or added to give the final product the correct tonicity upon reconstitution. A solution formulation (X) containing a biologically active substance might have the following composition [4]: bioactive agent, 20 mg; 0.1 M buffer solution, 0.6 ml; stabiliser/excipient, 50 mg; NaCl, 3 mg; water for injection, 1 ml.

The bioactive agent might be a conventional organic molecule, a peptide, a high molecular weight protein or a supermolecular structure, e.g. a virus. We shall refer to the processing characteristics of formulation X and its dry state behaviour subsequently. The composition profile of X is not altogether typical, because in many pharmaceutical preparations the bioactive product content amounts to no more than a few per cent. The freeze-drying characteristics of such a

preparation are then governed mainly by the physical and thermomechanical behaviour of the excipient mixture.

6. Freezing and associated changes

Freezing to completion requires the removal of more than 99% water from an initially dilute solution. The total solute concentration increases rapidly and is a function of the temperature only; it is thus independent of the initial solution concentration. For example, an isotonic saline solution (0.15 M) increases in concentration to 3 M when frozen, under equilibrium conditions, to −10°C [5]; i.e. a 20-fold concentration increase! All components in a mixture will suffer similar concentration increases. It is such dramatic salt concentration increases, much more than the much discussed 'ice formation', which damage labile bioproducts during freezing. A secondary consequence of freeze-concentration relates to its effect on chemical reaction rates in the residual solution phase. Contrary to the prediction of Arrhenius kinetics, relating reaction rate to temperature, rates actually accelerate, usually by orders of magnitude, during the freezing of an aqueous solution [6]. The phenomenon, mistakenly referred to as freeze denaturation in the case of proteins, should more correctly be termed concentration denaturation.

According to textbook chemistry, all solutes in a mixture precipitate in the crystalline state at various stages (eutectic phase separation) during the freeze concentration process, when their respective saturation solubilities are reached. Such differential precipitation of buffer components can be quite damaging, because it will cause major pH shifts that cannot be detected; because by the time the dried product is reconstituted in water, the buffers will have redissolved. Contrary to the predictions of textbook chemistry, clean eutectic phase separation during freeze concentration of multicomponent mixtures is, however, rare. The worst possible situation is the uncontrolled partial precipitation of some component(s) of a mixture during freezing and/or drying.

Such unpredictable and/or undesirable precipitation processes can be prevented by the use of suitable excipients that do not readily crystallise from a frozen solution. If present at high enough concentrations, they will also inhibit the precipitation of salts and cause the freeze concentration process to proceed well beyond the limit of saturation solubility. Freezing then continues but slows down, because with decreasing temperature and an increasing degree of supersaturation, the solution viscosity rises, increasingly steeply, until it reaches a point at which ice growth comes to a stop, at least in real time; the 'solution' phase might still contain up to 50% of unfrozen water. The mixture is then said to have undergone a glass transition, characterised by a glass temperature T_g' and a water content w_g'; the solution now has the mechanical properties of an amorphous solid, but the molecular structure of a liquid, i.e. it is devoid of a long-range molecular order. The product, as a whole, now consists of a mass of ice crystals embedded and dispersed in the vitreous, freeze-concentrated solution, with the whole occupying the original solution volume. At this stage the appearance of the product at the microscopic level depends on the characteristic size distribution of the ice crystals which, in turn, depends largely on the initial cooling rate but also on some other factors associated with details of the heat transfer within the solution during freezing. Although in principle the ice crystal size distribution is a function of the cooling rate, in practice the necessary cooling rates to significantly modify this distribution require heat transfer rates far higher than can be achieved in a commercial freeze drier, where vials are standing on refrigerated shelves. The ice will eventually be removed by sublimation, so that the texture, porosity and total surface area of the dried residue should closely resemble that of the frozen solution, with large spaces which had previously been occupied by ice crystals.

Carbohydrates are favoured as excipients, because they are chemically innocuous and can be easily vitrified during freezing. T_g' values of disaccharides and higher oligomeric sugars lie above −30°C [5,7–9], rendering these substances attractive as freeze-drying excipients. Caution must nevertheless be exercised, because some carbohydrates, notably mannitol and lactose, can also separate from a frozen solution in the form of crystalline phases, depending on the processing conditions employed. They can even crystallise within dried products. The electron micrographs in Figs. 1 and 2 show the very distinct appearances of mannitol and sucrose, respectively, both freeze-dried from 10% w/w solutions. Mannitol has crystallised during freezing and/or drying, whereas sucrose remains in the amorphous state right through the drying process. This results in the marked morphological differences of the two products. A secondary factor is probably the mechanism of ice nucleation and propagation, arising from the high viscosity of the sucrose solution, as compared to mannitol at the same mass concentration.

Other chemical compounds that can serve as excipients include amino acids, water soluble polymers and some salts, especially those of organic acids, e.g. citrates and lactates [11]. As discussed above for the carbohydrates, the main factors determining the choice of excipient(s) are chemical compatibility with the bioactive material to be dried and the phase behaviour of the freeze-concentrate, i.e. phase separation or vitrification of the excipient(s).

Volatile compounds, whether salts or non-electrolytes, form a special class of excipients. Salts, such a ammonium formate, acetate or bicarbonate, are readily removed during the ice sublimation stage and will therefore not remain in the dried product. In formulations containing amorphous excipients, e.g. sucrose, they also enhance the rate of ice sublimation [12]. A similar ability has been ascribed to tert.-butanol, although here the effect is said to be due to a modification in the ice crystal habit, producing needle-shaped ice crystals with a larger surface area for sublimation [13].

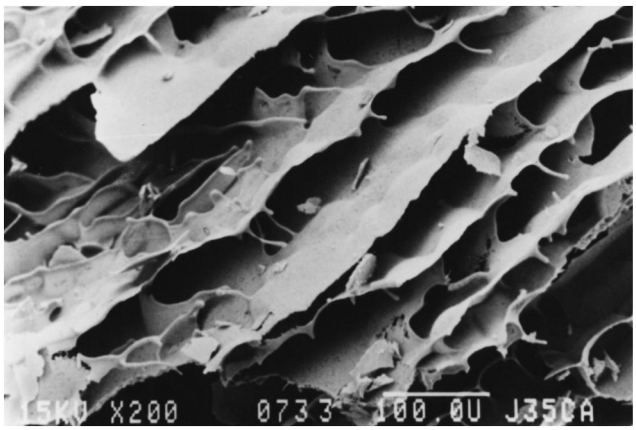

Fig. 1. Scanning electron micrograph of a 10% freeze-dried mannitol solution illustrating the typical directional appearance of a substance that crystallised from solution during freezing and/or drying. Scale bar = 100 μm.

The physico-chemical and mechanical properties of water-sensitive glasses have been the subject of several recent reviews [7,8]; although of fundamental importance, they will not be described in detail here. The most important features from the point of view of freeze-drying and the stabilisation of labile bioproducts are the effects of temperature and moisture content on T_g. Fig. 3 is a solid–liquid state representation of the binary system water–sucrose [9,10], showing how the phase composition of an initially dilute solution (A) changes with temperature during freezing and subsequent heating/drying. The important point to note is that, despite the predictions of classical phase equilibria, sucrose does not precipitate as a crystal phase when its solution is cooled to the eutectic point T_e but becomes subject to progressive supersaturation. In this connection, it is important to realise that the supersaturated solution is thermodynamically unstable and relies for its apparent stability on the extremely high viscosity. Critical for the drying process is the realisation that once the two-phase mixture of

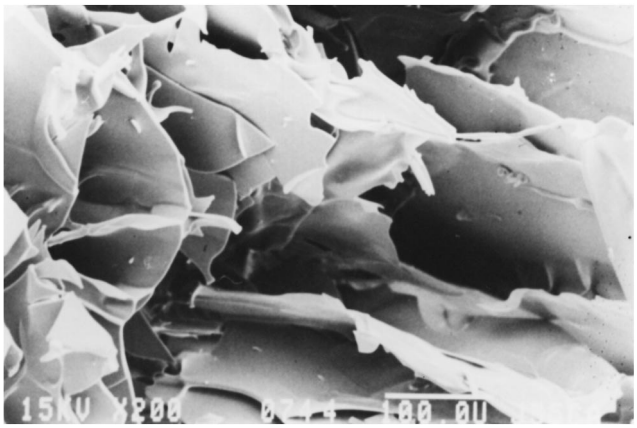

Fig. 2. Scanning electron micrograph of a 10% freeze-dried sucrose solution. Freeze-drying was performed according to 'best practice', i.e. the temperature of the sample was not allowed to exceed T_g by a significant amount or for a significant period. The appearance is typical of an amorphous solid. Scale bar = 100 μm.

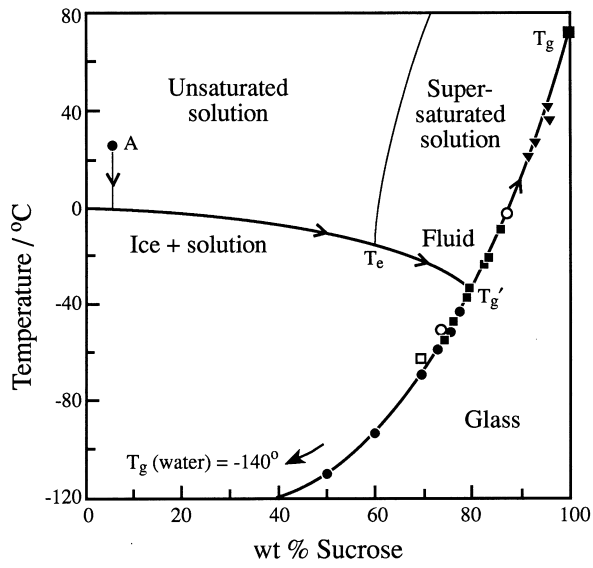

Fig. 3. Solid–liquid state diagram for the sucrose–water system and the freeze-drying path of a solution (A) with an initial solids content of 5%; for explanations, see text. Symbols refer to experimental results from different sources; details in ref. [10].

ice + supersaturated solution has reached its limiting composition, ice sublimation can be started, but it must be performed at, or below T_g'. If the temperature is allowed to rise, ice will melt back into the solution, causing a dramatic drop in its viscosity, with damaging and irreversible consequences.

The point of intersection of the freezing and glass curves, T_g', depends on the solution composition, but not on its initial concentration. The vapour pressure of ice which provides the driving force for water removal increases logarithmically with increasing temperature. From a process economic standpoint, therefore, the ice sublimation stage profits from as high a temperature as possible. The setting of the correct sublimation temperature depends on the formulation details. In practice, T_g' values above –40°C should be aimed at by judicious formulation; for instance, composition X has a T_g' of –38°C. For most products, T_g' will be governed by the nature and proportions of excipients and salts in the product.

7. The container

Materials to be freeze-dried essentially fall into two classes: solids with a high water content, e.g. food products, or homogeneous solutions, e.g. of peptides or conventional drugs. Intermediate states comprise dispersions, e.g. liposomes or single cells (microorganisms, yeasts). Solids are generally placed on trays within the freeze-drier, whereas liquids are either processed in bulk on trays or in small volumes in a variety of containers, e.g. vials, ampoules, syringes, bottles, microtitre plates. The nature and shape of the containing vessel and the stopper impact on the processing conditions in several ways [14].

(1) The sublimation rate of ice is a function of the area over which sublimation takes place and the thickness and porosity of the dried plug that develops. Thus, for a given fill volume, maximum sublimation rates are achieved with thin plugs with a large surface area.

(2) Heat transfer from the freeze-drier to the solution relies on three contributing mechanisms: radiation from the walls of the chamber, conduction through the sides and bottom of the container, and conduction by collisions of gas molecules with each other and with the walls of the container. The area of contact between container and shelf, as well as the container shape and any constriction caused by the way moisture is allowed to leave, i.e. the nature of the stoppers, will therefore affect the drying rate.

8. Equipment

The model of freeze-drier to be employed is usually beyond the control of the operator. It will, however, affect the quality and uniformity of the dried product. Of particular importance is the maintenance of a uniform shelf temperature across each individual shelf and between shelves. Where vials are used, then consideration must be given to their contact with the shelf. This depends on the flatness of the vial base and whether the vials are introduced into the drier on trays or whether they are placed directly on the shelves.

Where vials are loaded on trays, a perfect contact of the tray with the shelf is of critical importance. If a tray is even slightly warped and does not make perfect contact with the shelf, this can give rise to temperature gradients of several degrees, resulting in a marked reduction of the sublimation rate in vials standing on this particular area of the plate.

Temperature and pressure monitoring devices and their positioning also affect process control. Electrical resistance is a favoured method for monitoring the progress of freezing and drying in commercial equipment. In conducting solutions, this may be appropriate, because order of magnitude changes in the resistance accompany phase transitions. In nonelectrolyte mixtures, however, where glass transitions, rather than phase changes, are of crucial importance, changes in electrical resistance, if they are observed at all, cannot reliably be correlated with such transitions. It is in any case preferable to employ a more direct temperature measurement, such as thermocouples. Some commercial equipment employs resistance thermometers, but their use is to be discouraged, because of their large thermal capacity, leading to local heating and quite unrepresentative results. Thin wire or thin foil thermocouples are to be preferred, although even then, the temperature/time output from the particular vial carrying the thermocouple may not be representative of the drying process in other vials. A completely noninvasive measurement would be preferred for monitoring the progress of drying; the use of an anemometer, placed in the manifold between chamber and condenser has been reported [15], but such equipment is not available commercially.

9. The process cycle

The driving force for freeze-drying is provided by the partial pressure difference of water at the subliming ice surface (p_i) and at the condenser (p_c), in the form of $\log(p_c/p_i)$. Since vapour pressure is a function of temperature, the driving force can also be expressed in terms of the temperatures at the subliming surface and the heat sink (condenser). It is important to note that the employment of a vacuum only affects the rate of drying but not the driving force, or the extent to which ice can be sublimed.

The cycle consists of four distinct stages that may, however, partly overlap:

1. The solution, containing the product to be dried, is frozen on refrigerated shelves.
2. The shelves are heated and the ice is sublimed, usually, but not necessarily under reduced pressure (primary drying); the water vapour is condensed.
3. Unfrozen water remaining in the product (w_g) is removed by a judicious raising of the temperature of the shelves (secondary drying).
4. The condenser is heated to melt and remove the collected ice.

Only three process parameters can be directly controlled: condenser and shelf temperatures, chamber pressure and time. The parameter that is of greatest importance is, however, the product temperature which cannot be directly controlled. Its change with time can be measured (see above) only in a very limited number of positions within the drier, and with an uncertain degree of accuracy. Such measurements are not necessarily representative of samples in other positions within the drier.

With modern equipment, a cycle can be programmed in advance and made subject to in-process control. Once product formulation, container type and fill volume have been set, then the setting of the correct process parameters requires a knowledge of:

1. The correct temperature to which the solution must be cooled, i.e. slightly below T_g', and the time required for maximum freeze concentration in all containers. Thus, a reliable measurement of T_g' is of overriding importance.
2. The time required for ice sublimation to reach completion under given process conditions.
3. The correct (maximum) heating rate to effect secondary drying to a predetermined residual moisture content, but avoiding excessive product softening and eventual collapse.

Several attempts are on record for the design of complete freeze-drying cycles, based on laboratory experimental data

and/or computer simulation [16], or of specific stages, e.g. primary drying [17,18].

The freezing stage can be quantified, provided that T_g' is known. It must be noted, however, that the onset of freezing cannot be controlled [5]. In an array of many vials placed on a cold shelf, different vials become subject to different degrees of undercooling before ice is nucleated [19]. In practice, a random spread of freezing temperatures, amounting typically to ca. ±3 degrees about the mean, introduces a degree of variability in product appearance and porosity that cannot be prevented but will affect subsequent drying rates.

The optimised ice sublimation stage basically depends on a balancing of coupled heat and mass transfer within the product, given by

$$K_v(T_s - T_i) = \Delta H(dm/dt) \quad (1)$$

where K_v is a heat transfer coefficient that depends on the net mechanism of heat flow from the shelf to the product; it will typically contain contributions due to radiation, direct conduction from the shelf to the product and conduction arising from the kinetic energy transfer by collisions of gas molecules during their passage from the subliming surface to the condenser. T_s and T_i are the temperatures of the shelf and the product, respectively, (dm/dt) is the rate of mass transfer, i.e. the mass of ice, m, subliming in time t, and ΔH is the latent heat of sublimation of ice per unit mass at the appropriate temperature T_i. It is implied in Eq. (1) that T_i can be regarded as a so-called product temperature, i.e. no temperature gradient exists within the product. Barring submillimetre fill depths, this constitutes a gross oversimplification (see below); allowance must be made for such temperature gradients.

The major practical control problem lies in the balancing of Eq. (1). The energy absorbed during the sublimation process must be compensated by a supply of energy, at the correct rate, from the heated shelf to the product. Failing this, the product temperature will drop and sublimation will slow down; this also reduces the driving force (see above). If, on the other hand, the shelf temperature is raised excessively, causing the product temperature to rise to above T_g', then ice will melt back into the freeze concentrate, causing structural collapse and possible chemical deterioration.

A secondary problem concerns the effect of the receding ice front on mass transfer. As sublimation proceeds, so the water molecules to be removed encounter an increasing resistance to diffusion by the dried portion of the porous plug. The sublimation rate then decreases and, with a constant shelf temperature, there is again a danger of overheating the product. The above discussion illustrates how the total composition and the solids content of the solution, as well as the fill volume and the fill depth impact on processing conditions. Best results are obtained with solid contents of the order of 10% w/w and fill depths not exceeding 20 mm.

The other control variable that affects the sublimation rate is the chamber pressure. Its influence arises mainly from the contribution to energy transfer by collisions between water molecules. Surprisingly, this contribution is in many cases larger than that due to direct heat conduction from the shelf to the subliming ice front. It therefore follows that an increase of the chamber pressure, e.g. by the supply of a dry non-condensable gas, such as argon, increases the rate of ice sublimation. The effect of chamber pressure on the sublimation rate can be quantified in a simplified manner with the aid of the kinetic theory of gases which relates pressure and temperature with molecular collisions, according to Eq. (2):

$$dm/dt = fp_i/(2\pi MkT_i)^{1/2} \quad (2)$$

where p_i is the saturation vapour pressure of ice, M is the mass of a gas molecule, k is the Boltzmann gas constant, and f, the 'drying factor', being the probability that a water molecule is removed from the ice surface before it can recondense. For a maximum sublimation rate, therefore, $f = 1$. Eq. (2) is based on the assumption that other mechanisms of heat transfer are negligible compared to conduction by molecular collisions. The simplified form of the equation needs to be modified where the ice crystals are partially or wholly covered by a 'product skin', as they invariably are in practical freeze drying situations.

Any increase in mass transfer produced by a rise in the chamber pressure will inevitably result in a cooling of the product, unless additional heat is supplied via the shelf. Fig. 4 illustrates the coupled effects of shelf temperature and chamber pressure on the ice sublimation rate at different product temperatures. It must be stressed that the actual numbers shown can refer only to a particular formulation and fill condition [20]. The trends can, however, provide a generalised picture of the interplay between the various parameters. The overriding consideration must always be to maintain the product temperature just below T_g', i.e. $T_i < T_g'$.

Consider a formulation with an assumed T_g' of −18°C. Allowing for safety margins, sublimation might be carried out close to −20°C. The drying conditions could then be set, according to point A in Fig. 4. At a constant shelf temperature, the sublimation rate could be decreased (B) or increased (C) by altering the chamber pressure. However, raising the pressure to 55 mPa would produce an unacceptable rise in the product temperature. A safe increase in the sublimation rate might be achieved by a reduction of the pressure to 25 mPa and a simultaneous increase in the shelf temperature to 40°C, thus maintaining a constant product temperature (D). In practice, however, especially with larger fill depths, it would not be good practice to operate with a shelf temperature 60 degrees in excess of T_g'. Bearing in mind temperature gradients between the shelf and the subliming ice surface, the bottom of the product might well become subject to overheating and collapse.

The above example demonstrates that the maintenance of a constant product temperature T_i throughout the duration of the process by adjustments of the shelf temperature and the

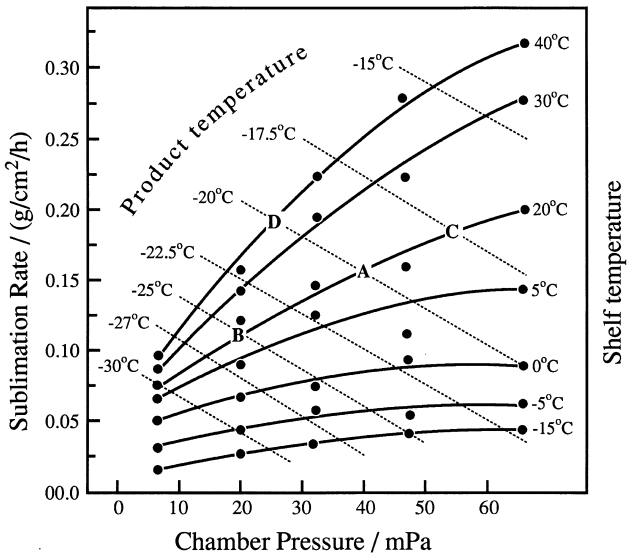

Fig. 4. Interrelated effects of shelf temperature and chamber pressure on product temperature [17] for a recombinant human interleukin preparation (1 ml) formulated in 2% w/v glycine, 1% w/v sucrose and 10 mM sodium citrate buffer (pH 6.5) in 3-ml vials; modified and redrawn from ref. [17].

chamber pressure can present problems. For instance, temperature gradients between the shelf and the subliming ice surface can amount to 3°C/mm, depending on the fill depth as well as on the thickness and material of any metal trays used. Familiarity with the particular equipment to be used is thus essential. Overheating during either or both drying stages is a common cause of product failure.

Secondary drying, i.e. the removal of dissolved (unfrozen) water from the product, is not easily quantifiable. The rate is governed by diffusion of water from the product filaments and its subsequent desorption and condensation. The diffusion process is not subject to simple kinetics [21] but, as might be expected, drying is accelerated by an increase in the temperature. The temperature dependence of water removal from a vitreous carbohydrate film, as determined by thermogravimetry, is illustrated in Fig. 5. The differential mass loss Δw at each temperature is normalised, by means of a factor F, to the initial water content, w_o, i.e. $F = \Delta w/w_o$. The drying rate is diffusion-limited and tends to a plateau at each temperature. Analysis of the mass loss curves demonstrates that drying does not follow simple diffusion kinetics but depends on w_o. Of significance for freeze-drying is that, here again, care must be taken not to overheat the product, because after the removal of ice, the residual product has lost most of its mechanical strength and has become extremely fragile, vulnerable to structural collapse. The onset of such collapse is shown in Fig. 6, an electron micrograph of a freeze-dried Ficoll solution that had, during the secondary drying stage, been exposed for a short period to a temperature slightly in excess of T_g. Collapse on the microscopic scale first occurs at the tips of the product filaments but, on continued exposure at $T > T_g$, the well-preserved porous structure is completely destroyed.

In contrast to primary drying, the chamber pressure does not appear to affect the secondary drying rate to any marked extent [14]. Ideally, therefore, secondary drying conditions should be such as to track the glass transition profile, as displayed in Fig. 3, starting from T_g', up to the desired storage temperature and water content.

Fig. 7 shows specimen recorder output traces from a freeze-drier, taken from a manufacturer's brochure. The cycle may be adequate, but it hardly corresponds to the optimum process conditions, whatever the T_g' value of the formulation. The product vials were apparently frozen to –50°C and kept at this temperature for a further 18 h. The shelves were then heated to –20°C and the vacuum applied; the pressure was reduced to approx. 1 mmHg (0.3 mPa), probably corresponding to full vacuum. These two adjustments produced an immediate product temperature rise to –30°C. Three product temperature probes show different responses to drying, indicating substantially different sublimation rates. Eventually, after 2.5 days, the product temperatures approached the temperature of the shelf, indicating the completion of sublimation. Secondary drying was performed in an acceptable manner, by ramping the shelf temperature, and allowing the product temperatures to rise in response, as water was removed by diffusion. The whole process lasted approx. 5 days, exclusive of loading of the freeze drier.

Since the product details are not known, only a limited amount of information can be extracted from Fig. 7. It is reasonable to conclude, however, that the process cycle could have been shortened by taking into account the factors discussed above. Assuming also that T_g' of the product prob-

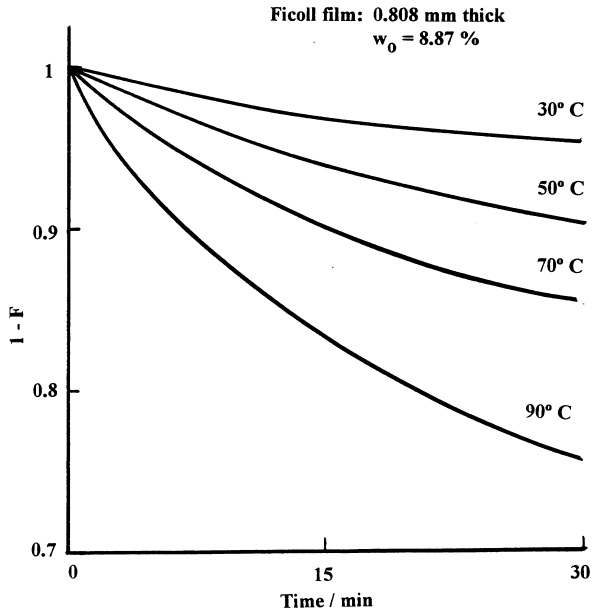

Fig. 5. Removal of water by diffusion from Ficoll films in the glassy state at several temperatures; recalculated and redrawn from data in ref. [18]. Ficoll® (ex Pharmacia) is a three-dimensionally cross-linked polymer of sucrose, with the cross-links effected by reaction with ethylene chlorhydrin.

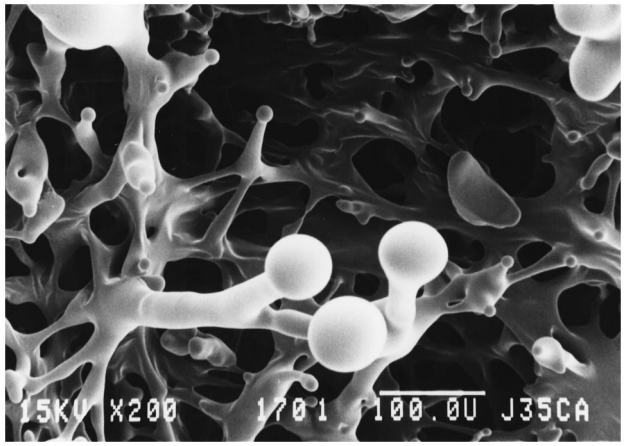

Fig. 6. Scanning electron micrograph of a freeze-dried 10% Ficoll solution, illustrating incipient collapse. Scale bar = 1 μm.

ably lay in the neighbourhood of −30°C, this temperature might, in principle, have been raised by a change in the formulation, making possible a shortening of the sublimation process. It is also not immediately obvious why the product was frozen to −50°C and maintained at this temperature for a protracted period, since it was subsequently warmed to −30°C and dried at that temperature.

10. Rational co-development of formulation and process

In the above discussions, stress was placed on the interdependence of formulation and processing. Working within external constraints that would also affect the choice of a particular formulation, e.g. regulations, pharmacokinetic or marketing considerations, best results will be achieved by co-ordinated product and process development. An example of this type of strategy provides the basis of product X, earlier referred to [4]. Three buffer systems were considered: lactate, citrate and acetate. Lactate was discarded for pharmacokinetic reasons, and acetate was also excluded because, being volatile, acetic acid is removed during the sublimation of ice, causing a pH shift in the reconstituted solution. Sodium citrate, on the other hand, tends to vitrify during freezing, with an acceptable T_g' value. Two excipients were considered: mannitol and sucrose. Mannitol enjoys a great popularity in the pharmaceutical industry. Of all the simple carbohydrates, it is however the one most prone to inadvertent crystallisation, during freeze-drying or thereafter, during storage. A factor in its favour is the attractive appearance of a freeze-dried, crystalline mannitol plug. Set against that must be the incidence of vial cracking reported for formulations based on mannitol [22]. Vial breakage was also encountered during the development work on product X. For these reasons, sucrose was chosen as excipient, despite the need for a longer freeze-drying cycle. Marketing factors also required that, on reconstitution with water, the solution should be isotonic. It was thus necessary to add enough NaCl to the formulation to provide for an isotonic product for injection. From a processing point of view, the addition of salts is to be avoided, because of their ability to depress T_g'. Experiments with solutions of constant tonicity, but a varying sucrose/salt ratio, yielded formulation X as the best compromise. An acceptable freeze-drying process could be calculated (and subsequently performed) for this formulation.

11. Assessment of stability

Two aspects of stability need to be considered: the product yield immediately after the completion of freeze-drying and long-term storage stability under specified conditions. A bioproduct that is to be freeze-dried must first of all be able to withstand a freeze–thaw cycle without unacceptable losses in activity. Provided that it passes this test, then it should be possible to devise a suitable drying protocol. Long-term storage stability is more difficult to assess without real-time testing. Accelerated storage testing by product stressing is subject to pitfalls which can lead an unwary or unskilled operator to false conclusions of the true shelf-life under ambient conditions [23]. This is particularly true for testing performed at elevated temperatures, where a glass transition may occur somewhere within the temperature range used. In such cases, extrapolations based on Arrhenius kinetics and/or Q_{10} factors provide erroneous results, because these kinetic equations are inoperative at temperatures in the neighbourhood of T_g. Appropriate kinetic rate equations for chemical and physical processes taking place in amorphous materials in the temperature range immediately above T_g have been discussed in the literature [7,8].

12. Conclusions

The parameters that govern successful freeze-drying of bioproducts from their dilute aqueous solutions are many and they are subject to complex interrelationships, some of

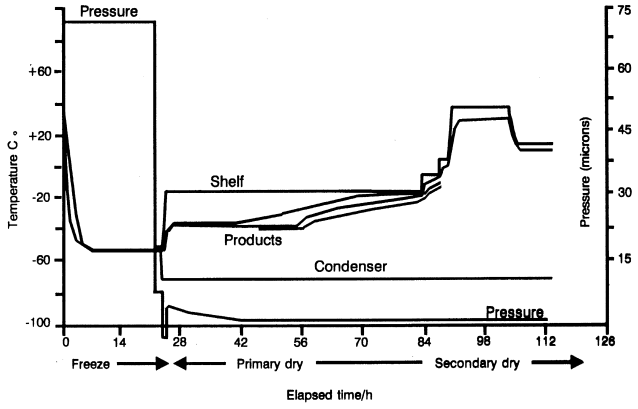

Fig. 7. Diagrammatic representation of a typical freeze-drier output, showing shelf, product and condenser temperatures, and chamber pressure.

which have been discussed in this review. Product formulation, containing vessel geometry and available equipment, all play a part in the design of a suitable process cycle. Experience teaches that best results are obtained where an integrated approach, combining both product and process development activities, can be adopted.

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References

[1] L. Rey, Freezing and freeze-drying, Proc. R. Soc. Lond. B 191 (1975) 9–19.
[2] J.McN. Dalgleish, Freeze-drying for the Food Industries, Elsevier Applied Science, London, 1990.
[3] J.C. May, F. Brown (Eds.), Biological Product Freeze-drying and Formulation, Dev. Biol. Standardization, Vol. 74, 1992.
[4] R.H.M. Hatley, F. Franks, S. Brown, G. Sandhu, M. Gray, Stabilisation of a pharmaceutical drug substance by freeze-drying. A case study, Drug Stability 1 (1996) 73–85.
[5] F. Franks, Freeze-drying: from empiricism to predictability, Cryo-Lett. 11 (1990) 93–110.
[6] R.H.M. Hatley, F. Franks, H. Day, The effect on the oxidation of ascorbic acid of freeze concentration and undercooling, Biophys. Chem. 24 (1986) 187–192.
[7] H. Levine, L. Slade, Water as a plasticizer: physico-chemical aspects of low-moisture polymeric systems, Water Sci. Rev. 3 (1988) 79–185.
[8] J.M.V. Blanshard, P.J. Lillford (Eds.), The Glassy State in Foods, Nottingham University Press, Nottingham, 1993.
[9] F. Franks, Freeze-drying: a combination of physics, chemistry, engineering and economics, Jap. J. Freezing Drying 38 (1992) 5–16.
[10] A.P. MacKenzie, Non-equilibrium freezing behaviour of aqueous systems, Proc. R. Soc. Lond. B 278 (1977) 167–189.
[11] B.S. Chang, C.S. Randall, Use of subambient thermal analysis to optimize protein lyophilization, Cryobiology 29 (1992) 632–656.
[12] J. Oesterle, F. Franks, A.D. Auffret, Volatile excipients as aids to pharmaceutical freeze-drying, Pharm. Dev. Technol. 3 (1998) in press.
[13] K. Kasraian, P.P. DeLuca, The effect of tertiary butyl alcohol on the resistance of the dry product layer during primary drying, Pharm. Res. 12 (1995) 491–495.
[14] M.J. Pikal, M.L. Roy, S. Shah, Mass and heat transfer in vial freeze-drying of pharmaceuticals: role of the vial, J. Pharm. Sci. 73 (1984) 1224–1237.
[15] E.Yu. Shalaev, Ph.D. Thesis, Koltsovo, 1991.
[16] M.J. Pikal, Use of laboratory data in freeze-drying process design: Heat and mass transfer coefficients and computer simulation of freeze drying, J. Parenter. Sci. Technol. 39 (1985) 115–138.
[17] M. Kochs, Ch. Körber, I. Heschel, B. Nunner, The influence of the freezing process on vapour transport during sublimation in vacuum-freeze-drying of macroscopic samples, Int. J. Heat Mass Transfer 36 (1993) 1727–1738.
[18] M.P. Schoen, B.K. Braxton, L.A. Gatlin, R.P. Jeffries, A simulation model for the primary drying phase of the freeze-drying cycle, Int. J. Pharm. 114 (1995) 159–170.
[19] R.W. Michelmore, F. Franks, Nucleation rates of ice in undercooled water and aqueous solutions of polyethylene glycol, Cryobiology 19 (1982) 163–171.
[20] B.S. Chang, N.L. Fischer, Development of an efficient single-step freeze-drying cycle for protein formulations, Pharm. Res. 12 (1995) 831–837.
[21] B.J. Aldous, A.L. Greer, F. Franks, Diffusion of water within amorphous carbohydrates, J. Mater. Sci. 32 (1997) 301–308.
[22] N.A. Williams, T. Dean, Vial breakage by frozen mannitol solutions: correlation with thermal characteristics and effect of stereoisomerism, additives and vial configuration, J. Parenter. Sci. Technol. 45 (1991) 94–100.
[23] F. Franks, Accelerated stability testing of bioproducts: attractions and pitfalls, Trends Biotechnol. 12 (1994) 114–117.