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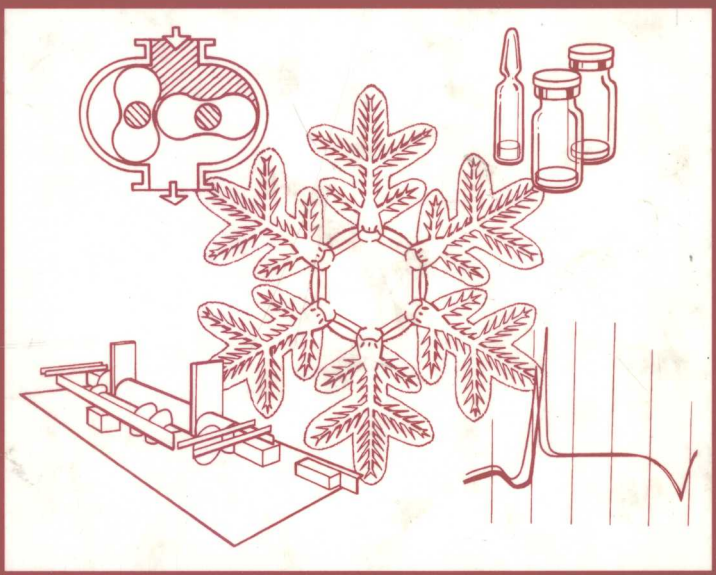
Freeze-Drying/Lyophilization of Pharmaceutical and Biological Products



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Freeze-Drying/Lyophilization of Pharmaceutical and Biological Products

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Preface

Since its discovery by Altman at the turn of the century and its subsequent development by Gersh, freeze-drying has been used for the preservation of delicate biologicals and biochemicals that present a high instability at room temperature because of their important water content.

More or less considered a laboratory curiosity until the end of the 1930s, drying from the frozen state underwent striking development during the last world war. Under the impetus of Earl Flosdorf in the United States, Ronald Greaves in the United Kingdom, and François Henaff in France, it became a routine technology for the preparation of blood plasma and plasma fractions, which were of massive use on most battlefields and saved millions of lives throughout the world. The process was then named *lyophilization* since it helped to prepare dry products with a great affinity for their hydrated solvants. Later, the late Sir Ernst Boris Chain, Nobel Laureate, applied freeze-drying for the first time for the preservation of the newly discovered penicillin, and less than a decade later it became commonly used for the stabilization of vaccines, thanks to Charles Merieux and his associates.

At the same time, on a purely engineering basis, extensive work was conducted in all major developed countries to design and build large-scale reliable freeze-drying plants that were able to handle batches of several tens—sometimes hundreds—of thousands of vials or ampoules in well-controlled sterile conditions. In parallel, an important amount of basic research was conducted in several laboratories to understand and monitor the freeze-drying process. Our own work in the field started in 1954 and has been pursued ever since.

During that time, the scope of application of freeze-drying was opened to the food and chemical industries, and new semicontinuous and even purely continuous processes developed for the operations of large multitons/day plants.

However, as biological research evolved into the more sophisticated approach of physiological biochemistry, and with the rocketing advance of genetical engineering, freeze-drying appeared in most cases as a unique technique for the long-term perservation of very small amounts of highly refined biological products. This, in turn, compelled most researchers to increase their knowledge on the role and performances of bulking agents, additives, cryoprotectants, antioxidants, and free radical scavengers, which were discovered to play a leading role in the protection of the integrity of the active substances, during both freezing and drying as well as in the course of storage.

In that field, a better understanding of the behavior of complex molecules, such as proteins, during lyophilization was acquired, as well as more and more information gathered on the leading role of water in the whole process. The basic properties and the different "states" of this critical component of all living matter were studied with cutting-edge technologies such as X-ray and neutron diffraction, nuclear paramagnetic resonance, or Debye dipolar absorption in addition to the already classical tools of conventional physical chemistry. Concomitantly, the structure and ultrastructure of freeze-dried products were investigated with direct or indirect technologies ranging from classical to electron scanning microscopy, to more advanced methods such as thermoluminescence.

The purpose of *Freeze-Drying/Lyophilization of Pharmaceutical and Biological Products* is *not* to present an exhaustive view of the process, but essentially to shed light on some focal areas of the field in which pioneering research has been achieved and assess its impact on current manufacturing practices. This book also provides a critical review of such wide issues as the design and construction of equipment to identify the main trends and sometimes locate the specific sectors where our technological know-how is still incomplete.

To the end, the editors have selected a group of knowledgeable experts who share a long experience in the field and have a large understanding of the adjacent and complementary disciplines. The contributors range from pure theoreticians to confirmed technicians with a wide in-depth field practice. They agreed to present their views in a clear and comprehensive way, avoiding unnecessary semantics and specialized mathematical treatments, in order to remain readily accessible to the diversified group of potential users of freeze-drying in the pharmaceutical industry. Special emphasis has been given to some grass-roots approaches to the process, which are of basic importance for operators and can also incline theoreticians to take a closer look into the actual unfolding of lyophilization.

The editors hope that this somewhat unconventional approach to freeze-drying will be of interest and help not only for those who plan to enter this area, but also to the great majority of the experienced operators who wish to better understand the odds and ends of a challenging process.

Louis Rey

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Contents

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Glimpses into the Realm of Freeze-Drying: Classical Issues and New Ventures

Louis Rey
*Cabinet d'Études, Lausanne, Switzerland**

In their 1906 paper to the Académie des Sciences in Paris (almost a century ago), Bordas and d'Arsonval [1] demonstrated for the first time that it was possible to dry a delicate product from the frozen state under moderate vacuum. In that state it would be stable at room temperature for a long time and the authors described, in a set of successive notes, that this technique could be applied to the preservation of sera and vaccines. Freeze-drying was then officially borne, despite its having been in use centuries ago by the Inca who dried their frozen meat in the radiant heat of the sun in the rarified atmosphere of the Altiplano.

However, we had to wait until 1935 to witness a major development in the field when Earl W. Flosdorf and his coworkers [2] published some very important research on what they called, at the time, lyophilization (this name was derived from the term *lyophile* coming from the Greek λυος and φιλειν, which means "likes the solvent," describing the great ability of the dry product to rehydrate again). Freeze-drying had then received a new name that has been in current use since then, together with cryodesiccation.

Many authors, in different comprehensive books dedicated to freeze-drying, have already described in full detail the scientific history of this method [3], and we will not attempt to do it again. Moreover, in the last 60 years, much research and substantial development have been devoted to freeze-drying, and it would be of little use to list papers that are well known and available to all of the specialists concerned.

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This, indeed, is the very reason why the present book has been designed to present to the readers essentially new experimental methods and data, as well as recent developments on our own basic understanding of the physical and chemical mechanisms involved in cryodesiccation.

Nevertheless, it would not be fair to skip the names of some of the great pioneers in the field. Earl Flosdorf, Ronald Greaves, and François Henaff fought the difficult battle of the mass production of freeze-dried human plasma, which was used extensively during World War II. To that end, they engineered the appropriate large-scale equipment. Sir Ernst Boris Chain, the Nobel Laureate for penicillin, introduced freeze-drying for the preparation of antibiotics and sensitive biochemicals. Isidore Gersh and, later on, Tokio Nei and Fritjof Sjøstrand produced remarkable photographs of biological structures prepared by freeze-drying for electron microscopy. Charles Merieux, on his side, opened wide new areas for the industrial production of sera and vaccines. In parallel, he developed a bone bank, a first move in a field where the U.S. Navy Medical Corps invested heavily a few years later under Captain Georges Hyatt.

At the same time, cryobiology was getting its credentials with many devoted and gifted scientists such as Basil Luyet, Alan Parkes, Audrey Smith, Harry Meryman, Christopher Polge, Peter Mazur, and others. We had the privilege of living this exciting period together with all of these people since 1954 and most of them were present in Lyon in 1958 when Charles Merieux and I opened the first International Course on Lyophilization, with the sad exception of Earl Flosdorf who had agreed to deliver the opening address but died tragically a few weeks before the conference.

Today, 42 years later, we are pleased to see that freeze-drying still holds a remarkable place in our multiple panel of advanced technologies and more particularly in the pharmaceutical field. It was thus a wise and sound decision of our publisher to propose that a collective book be devoted to that topic.

I. BASIC FREEZE-DRYING

Lyophilization is a multistage operation in which, quite obviously, each step is critical. The main actors of this scenario are all well known and should be under strict control to achieve a successful operation.

The product, i.e., the "active" substance which needs to keep its prime properties.

The surrounding "medium" and its complex cohort of bulking agents, stabilizers, emulsifiers, antioxidants, cryoprotectors, moisture-buffering agents.

The equipment, which needs to be flexible, fully reliable, and geared to the ultimate goal (mass production of sterile / nonsterile drugs or ingredients, experimental research, technical development).

The process, which has to be adapted to individual cases according to the specific requirements and low-temperature behavior of the different products under treatment.

The final conditioning and storage parameters of the finished product, which will vary not only from one substance to another one but in relationship with its "expected therapeutic life" and marketing conditions (i.e., vaccines for remote tropical countries, international biological standards, etc.). In other words, a freeze-dryer is not a conventional balance; it does not perform in the same way with different products. *There is no universal recipe for a successful freeze-drying operation* and the repetitive claim that "this material cannot be freeze-dried" has no meaning until each successive step of the process has been duly challenged with the product in a systematic and professional way and not by the all-too-common "trial-and-error" game.

The freeze-drying cycle. It is now well established that a freeze-drying operation includes:

The ad hoc *preparation of the material* (solid, liquid, paste, emulsion) to be processed taking great care not to impede its fundamental properties.

The freezing step during which the material is hardened by low temperatures. During this very critical period, all fluids present become solid bodies, either crystalline, amorphous, or glass. Most often, water gives rise to a complex ice network but it might also be imbedded in glassy structures or remain more or less firmly bound within the interstitial structures. Solutes do concentrate and might finally crystallize out. At the same time, the volumetric expansion of the system might induce powerful mechanical stresses that combine with the osmotic shock given by the increasing concentration of interstitial fluids.

The *sublimation phase or primary drying* will follow when the frozen material, placed under vacuum, is progressively heated to deliver enough energy for the ice to sublimate. During this very critical period a correct balance has to be adjusted between heat input (heat transfer) and water sublimation (mass transfer) so that drying can proceed without inducing adverse reactions in the frozen material such as back melting, puffing, or collapse. A continuous and precise adjustment of the operating pressure is then compulsory in order to link the heat input to the "evaporative possibilities" of the frozen material.

The *desorption phase or secondary drying* starts when ice has been distilled away and a higher vacuum allows the progressive extraction of bound water at above zero temperatures. This, again, is not an easy task since overdrying might be as bad as underdrying. For each product, an appropriate residual moisture has to be reached under given temperatures and pressures.

Final conditioning and storage begins with the extraction of the product from the equipment. During this operation great care has to be taken not to lose the refined qualities that have been achieved during the preceding steps. Thus, for vials, stoppering under vacuum or neutral gas within the chamber is of current practice. For products in bulk or in ampoules, extraction might be done in a tight gas chamber by remote operation. Water, oxygen, light, and contaminants are all important threats and must be monitored and controlled.

Ultimate storage has to be carried according to the specific "sensitivities" of the products (at room temperature, +4°C, –20°C). Again uncontrolled exposures to water vapor, oxygen (air), light, excess heat, or nonsterile environment are major factors to be considered. This obviously includes the composition and quality of the container itself, i.e., glass, elastomers of the stoppers, plastic or organic membranes.

At the end, we find the *reconstitution phase*. This can be done in many different ways with water, balanced salt solutions, or solvents either to restore the concentration of the initial product or to reach a more concentrated or diluted product. For surgical grafts or wound dressings, special procedures might be requested. It is also possible to use the product as such, in its dry state, in a subsequent solvent extraction process when very dilute biochemicals have to be isolated from a large hydrated mass, as is the case for marine invertebrates.

Figure 1 summarizes the freeze-drying cycle and indicates for each step the different limits that have to be taken into consideration. Figure 2 gives an example of a typical freeze-drying cycle.

II. INSIGHT INTO THE BEHAVIOR OF PRODUCTS AT LOW TEMPERATURE

A. Thermal and Electric Properties

A fundamental paradigm of freeze-drying is to understand that, in almost every case, there is no direct correlation between the structure of a frozen product and its temperature since all structural features depend essentially from the "thermal

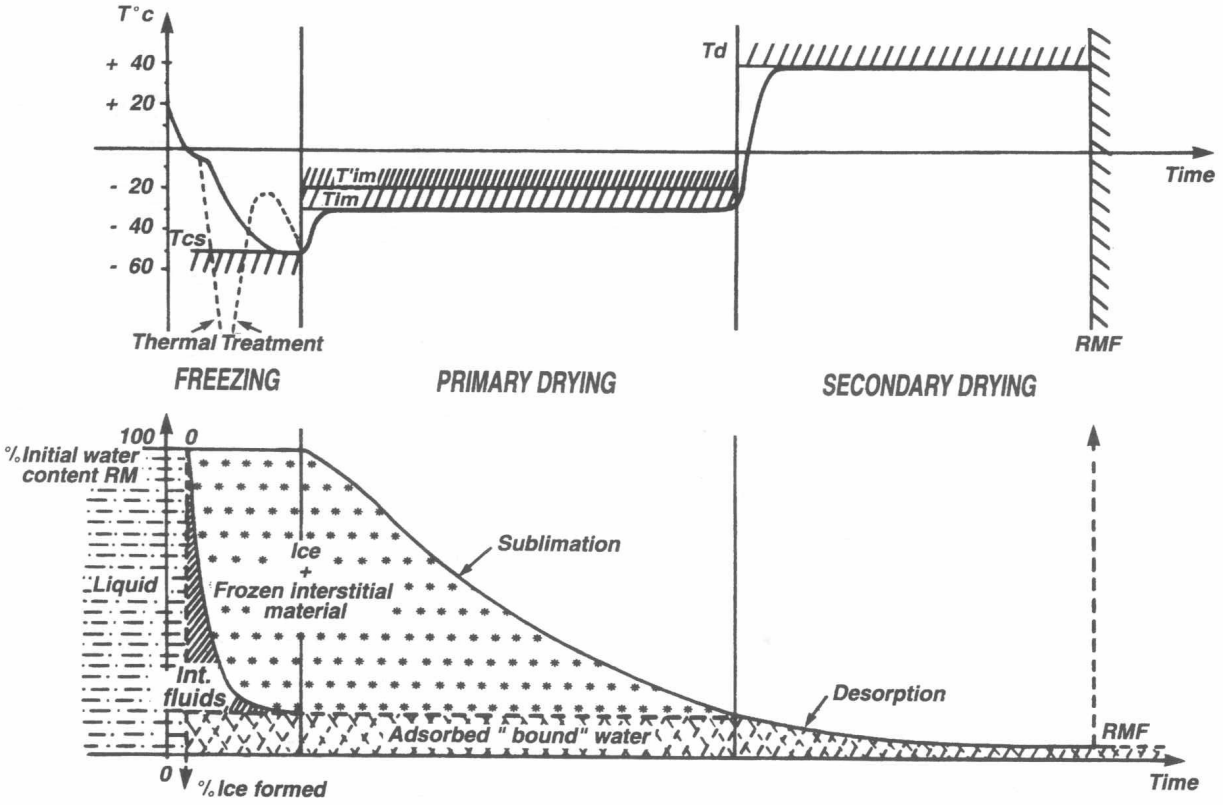

Figure 1 Schematic evolution of the freeze-drying process. Temperatures (upper curve) and water content (lower curve) are indicated versus time. In the temperature diagram T_{CS} = maximum temperature of complete solidification; T_{im} = minimum temperature of incipient melting; T'_{im} = absolute limit for fast process; T_d = maximum allowed temperature for the dry product; RMF, final requested residual moisture.

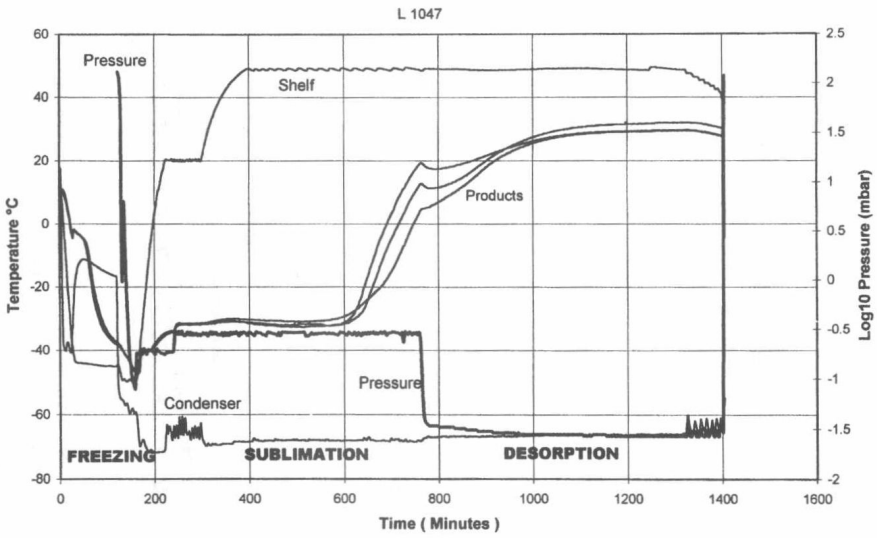

Figure 2 A typical freeze-drying cycle. Note that the pressure is raised to 0.3 mbar to increase the heat transfer during sublimation and then lowered to 0.02 mbar for desorption.

history" of the material. In other words, the knowledge of the temperature of a frozen solution is not enough to allow the operator to know its structure since this latter depends, essentially, upon the way this temperature has been reached, i.e., upon the freezing cycle.

For instance, we did show in 1960 [4] that an aqueous solution of sodium chloride at –25°C could be

> Either a sponge-like ice network soaked with highly concentrated fluids if the system has been cooled progressively from +20°C to –25°C
>
> Or else a totally frozen solid where all the interstitial fluids have crystallized as eutectics if the system has been cooled, first to –40°C and then rewarmed to –25°C

Actually, when water separates as pure ice, as is the case for diluted solutions, there might be a considerable degree of supercooling in the remaining interstitial fluids. It is, then, compulsory to go to much lower temperatures to "rupture" these metastable states and provoke their separation as solid phases. This, indeed, has a great significance because it is precisely within those hypertonic concentrated fluids that the "active substances" lie whether they are virus particles, bac-

teria, or delicate proteins, and where they can undergo serious alterations in this aggressive environment. This is the reason why we advised to cool the product at sufficiently low levels to reach what we have called T_{cs}: the maximum temperature of complete solidification [4,5]. However, when frozen, the material will only start to melt when it reaches either eutectic temperature or what we called T_{im}, the minimum temperature of incipient melting [4,5].

Differential thermal analysis (DTA) and differential scanning calorimetry (DSC) are useful techniques to proceed to this determination [4,5]. They can be very advantageously coupled with LF electric measurements since the impedance of the frozen system drops in a spectacular way when melting occurs (see Figs. 3 and 4). In other terms, a high electric impedance is always related to a state of utter rigidity. Moreover, the electric measurements are more reliable than DTA/DSC alone. Indeed, when we are dealing with a complex system—more particularly when it contains high molecular weight compounds—the material hardens progressively during freezing and, often, in the course of rewarming shows incipient melting only at relatively high temperatures. Until that point the DTA curve remains "silent." Unfortunately, this is not always a sign of absolute stability since, quite often, a "softening" of the structure appears much earlier. It is our experience that a sharp decrease in electric impedance is a clear warning for an operator who should try and maintain the product during primary drying at temperatures below

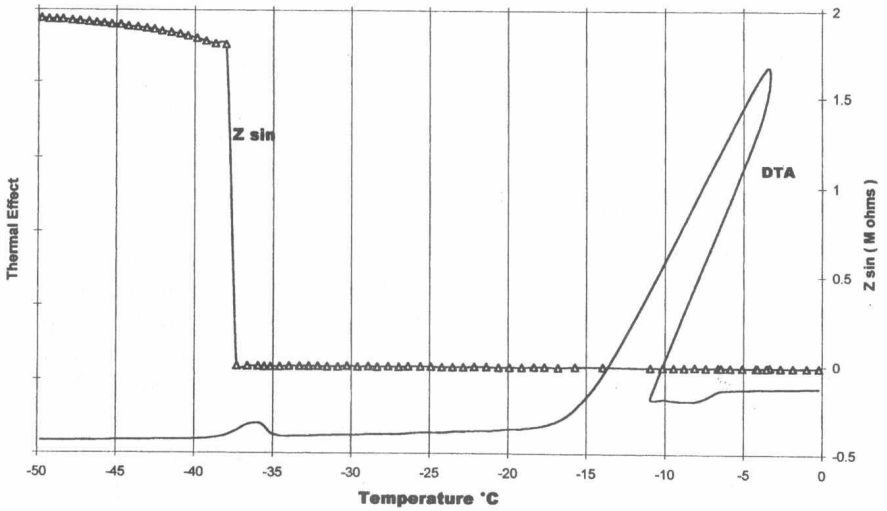

Figure 3 Differential thermal analysis (DTA) and impedance (1000 hz–Z sin φ) of a 2 p. 100 solution of Cl Na in water during controlled freezing.

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