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Freeze-Drying Equipment for Large-Scale Laboratory Use

BY B. R. RECORD AND R. TAYLOR

Microbiological Research Establishment, Porton, nr. Salisbury

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The principles and practice of the freeze-drying process have been reviewed by Greaves (1946) with special reference to the large-scale drying of human serum and plasma, and the field has been surveyed in detail by Flosdorf (1949). More recently these authors and others have contributed authoritative articles on the subject (see Harris, 1954). Simple apparatus for the freeze-drying of small quantities in the laboratory has been described by various workers, e.g. Greaves (1956). However, for laboratory freeze-drying on the large scale more elaborate apparatus is desirable, and it was to meet such a demand that the equipment described in this paper was designed. Except for certain ancillary items such as vacuum pumps and compressors, the construction of the machines has been carried out entirely in our engineering section. The main aim in design was to provide a freeze-drying service which could be operated without any special skill, with the minimum of attention and handling of material, and which would provide a satisfactory end-product whether in small ampoules or large bulk volumes of 10 l. or more. The equipment has given good service in this Establishment with the minimum of trouble over a number of years.

The three types of machine described all operate on the vacuum spin-freeze principle introduced by Greaves (1944), in which an aqueous solution is caused to freeze rapidly by its evaporation in the vacuum chamber in which it is subsequently dried, any foaming or frothing of the material during

freezing being suppressed by centrifugal force. Various proprietary makes of laboratory freeze-driers make use of this principle by placing the small ampoules or bottles in inclined holes in a rotating plate, so that the material freezes as a wedge of varying thickness at one side of the ampoule. It is clear that the extension of this method to deal with greater volumes of material contained in larger vessels would result in a wedge of material of considerable thickness with a proportional increase in drying time such as to render the method too slow to be practicable. Thus with the larger size of container it becomes imperative to spin each vessel in the vacuum chamber about its longitudinal axis at a speed sufficient to distribute the contents in a uniformly thick layer around the walls. Although more elaborate equipment is required to achieve this end, certain advantages are gained over the usual methods involving pre-freezing. Prefreezing in a blast of cold air is a slow process which produces ice crystals of large size and an unsatisfactory dried product that is difficult to reconstitute. Even when the liquid is clean enough to allow supercooling to take place one has often to reckon with the possibly deleterious effects of exposure of proteins, etc., to increasingly high salt concentrations for long periods during the later stages of freezing. Freezing can, of course, be completed more rapidly by rotation of the container, especially if it is of thin glass, in a low-temperature bath but it may then become necessary to wipe clean the outside of the con-

tainer before loading into the vacuum chamber in order to avoid contamination of the pump oil. There is also the danger, should there be any tendency to supercooling, of sudden freezing of the mass on one side of the vessel. With either method, and especially with small bottles, rapid loading into the freeze-drying chamber is essential in order to establish the vacuum before any thawing of the material has had time to occur.

Satisfactory evaporative freezing depends upon the following conditions:

(1) The production of sufficient centrifugal force by rotation of each container about its longitudinal axis in the vacuum chamber to ensure (a) suppression of foaming, which according to Dr R. I. N. Greaves (see Harris, 1954) requires only about 7 g., and (b) distribution of the contents as a uniformly thick layer, the magnitude of the force required then depending on the height of the container.

(2) A vacuum pump of sufficient capacity in relation to the size of the vacuum chamber to reduce the pressure from a few mm. Hg, when freezing commences, to about 0.1 mm. (corresponding to the vapour pressure of ice at the coil temperature) within a few minutes.

(3) A compressor capable of dealing at -30° or lower with the rapid evaporation and condensation of water vapour in a quantity sufficient to cool the liquid (and container) to 0° , to remove the latent heat of fusion and to cool the ice so formed to -25° or lower, i.e. at least 110 cal./g. of water to be dried. During this stage, the evaporation and condensation of about one-sixth of the total amount of water to be dried must therefore take place in as short a time as possible and the compressor is here called upon to deliver its maximum duty. If adequate duty is not available, freezing will be unduly prolonged. The demand could be met by circulating cooled methanol from a large refrigerated storage tank, but we have preferred to use large compressors which then run 'light' during the remainder of the freeze-drying procedure.

TYPES OF MACHINE

To meet the demand for a wide variation in the volume of material to be freeze-dried, it has been found desirable to construct three different types of machine, each designed for spinning the individual containers in the vacuum chamber. The drive is obtained by gear wheels throughout in order to avoid any risk of slip, but to simplify manufacture they are of the spur type, noise and wear being reduced by constructing some of the gears of Tufnol (Tufnol Ltd., Perry Barr, Birmingham). The main drive is supplied via a central shaft, through a special vacuum seal, from a motor mounted beneath the chamber. The refrigerated coil is located in the lower part of the chamber, and presents a sufficiently large surface to accommodate, as a fairly thin layer, the maximum quantity of ice likely to be condensed

upon it during the drying of a batch of material. Fig. 1 shows a typical layout of one of the machines (Type 2) with control panel. The compressor and vacuum pump are housed in a sound-proof compartment behind the panel.

Type 1 machine designed to accommodate 60 of the 1 oz. (30 ml.) screw-cap type of bottle or the 1 oz. ampoule

This has already been briefly described and illustrated (Record & Taylor, 1953). Each of the sixty bottle carriers (int. diam., $1\frac{1}{4}$ in.) is machined in one piece from stainless-steel rod and runs in two ball bearings located in the carrier head. This head is readily removed by withdrawing six studs and can be interchanged with a solid rotor for use with smaller ampoules for drying in the wedge form. A steel gear on the central driving shaft meshes with each of six bronze gear wheels on the inner ring of carriers and each one of these drives the remaining nine (Tufnol) gear wheels in its corresponding sector. The first and last members of the gear train in each sector are in mesh with two of their neighbours, there being ample backlash in the intermediate gears. The Monel tubular heating element located beneath the carrier head can be loaded to a maximum of 0.5 kw, control being effected by a Variac transformer, and a chromium-plated reflector throws the heat upwards to metal disks on the gear wheels whence it is conducted through to the carriers. A small sealed thermostat, which is pre-set to switch off at 30° , to prevent overheating the product, is inserted in a hole situated in the side of the carrier head. The central shaft is gear-driven by a 0.5 h.p. motor to give a final carrier speed of 2000 rev./min., which is more than adequate in suppressing foaming and gives ample centrifugal force to distribute the liquid uniformly round the walls of the small containers used. The vacuum seal on the central shaft is of similar design

Fig. 1. A general view of one of the machines with control panel.

Fig. 2. General arrangement of type 2 machine in section.

and construction on all three types of machine, and is described in detail below. The condensing coil is maintained at about -45° by a 1 h.p. water-cooled compressor with Freon 12 as refrigerant, and the vacuum is obtained by the use of a two-stage rotary oil pump of 150 l./min. displacement.

The volume of liquid in a 1 oz. bottle is restricted to 15 ml. since loss of liquid past the shoulder of the bottle is likely to occur if this is exceeded. Although this loss is not a problem with 1 oz. ampoules, the same limit of volume is observed to minimize the risk of cracking the glass during the freezing stage. As the 1 oz. bottles taper slightly towards the base, a thin rubber band round this portion helps to centre the bottle.

When operating the machine, spinning is necessary for the first 20–30 min. only, by which time the vacuum in the chamber has reached 0.1 mm. Hg and the product temperature is -25° . With the thin liquid layer, freezing takes place very rapidly, there being no discontinuity in the recorded product temperature to show when freezing actually occurs. Throughout the main part of the drying procedure the product assumes a temperature of about -40° with open-necked bottles, or about -35° when aseptic precautions are taken by the fitting of sterile-filter caps. With 15 ml. of water/bottle, i.e. a total load of 0.9 l., drying is complete in about 6 hr. without the application of heat. Two batches, i.e. 120 bottles or 1.8 l., can be conveniently processed every 24 hr. on this machine, with overnight running.

Type 2 machine to accommodate 18 fruit-bottling jars

In selecting a container suitable for the freeze-drying of moderate quantities of material, the standard fruit-bottling jar with glass lid, rubber sealing ring and screwed-metal closure has much to commend it, since such bottles have proportions ideally suited to freeze-drying, are cheap and readily obtainable, are made to close tolerances in size, withstand moderate temperature changes and are fairly robust mechanically. The glass lid with narrow rubber-ring seal offers advantages for storage of the dried material in having the minimum amount of rubber through which diffusion of water vapour can occur. The 3 lb. size (capacity approx. 1 l.) was chosen as the most suitable compromise for our present requirements.

The general arrangement of the machine is shown in Fig. 2. The vacuum chamber is of welded construction and was rolled from $\frac{1}{2}$ in. mild-steel plate, the base and lid being of $\frac{3}{8}$ in. steel. The chamber is opened by raising the lid by means of the screw jack and swinging it to one side. The bottle carriers are made of drawn-steel tube (int. diam., $4\frac{1}{2}$ in.) which allows for the fitting of thin rubber-ring cushions around the top and bottom of each jar. The tubes are welded to a steel base and spigot, and chromium-plated on the outside, except for the underside which has a matt-black finish. A 1 h.p. motor drives the central shaft, which enters the chamber through a vacuum seal. At the upper end the shaft terminates as a keyed socket which engages a steel spur gear driving the inner ring of six Tufnol gears, each of these driving two bronze gears in the outer ring. The complete carrier and gear assembly may be removed from the chamber by withdrawing six bolts. This part of the machine is shown in Fig. 3. The condensing coil is cooled by the direct expansion of Freon 12, a 4 h.p.

water-cooled compressor being used, and the vacuum line is connected to a two-stage rotary oil pump of 150 l./min. displacement. Fig. 4 is a view of the inside of the open chamber showing some filter-capped jars in position. The thermocouple pickup on one of the jars is also shown.

Heat is applied during drying by a circular disk-type heater (with a maximum loading of 125w each) situated under each carrier, all the eighteen heaters being connected in parallel and controlled by a Variac. An earlier model had provision for switching into circuit only those heaters actually required, but conduction of heat through the carrier head makes this unnecessary. With the varied load of material, both in respect of quantity per jar and nature of material, usually dealt with in this machine the use of a large heat input is precluded and it is then advisable to use only a nominal heat input of about 200w, which raises the final temperature of the dried material to about 35° . With a uniform load this heat input could be

Fig. 3. Type 2 machine: carrier head and gear assembly

Fig. 4. Type 2 machine: view from above. Six filter-capped jars are shown in position.

Fig. 5. Type 3 machine: sectional view of general arrangement.

greatly exceeded, provided that an automatic device was incorporated to switch off the heat as the product approached dryness. However, with the low heat input specified, and a load of 200 ml./jar (a total load of 3-6 l.), a comfortable margin of time is allowed for a 24 hr. turnover, and any risk of cracked jars is avoided.

The jars are spun at 1000 rev./min., which produces a centrifugal force on the surface of a $\frac{1}{4}$ in. thick layer of liquid (200 ml.) of about 45 g. This far exceeds the force necessary to suppress foaming but is required in order to produce a uniformly thick layer of liquid round the wall of the jar. The inner layer surface will then be inclined to the vertical by about 1 in 45, corresponding to a gradation in thickness of about $\frac{1}{8}$ in. over the 6 in. height of the liquid layer. Where it is necessary to handle the material aseptically, the metal-screw caps of the jars can be fitted with filters, consisting of a cotton-wool pad sandwiched between layers of muslin, and wired to the metal-screw caps. This presents a slight obstruction to the flow of water vapour from the jar, and raises the drying temperature by about 5° under normal conditions (200 w heat input).

The bottling-jar type of closure is well suited for storage of the dried material, but as with other devices has not provided a perfect seal against moisture over long periods. For such purposes we have canned the jars, the vacant space around the top of the jar being filled with silica gel, to provide a double barrier with a dry space between.

It is sometimes an advantage to be able to dry simultaneously 5-10 ml. amounts of material in the 1 oz. screw-cap bottle in this machine, and for this purpose adaptors each holding four bottles can be placed in the carriers as required. The material is then, of course, dried in the wedge form.

Type 3 machine to hold one large container

This machine was designed for the freeze-drying of a large volume of solution in one operation. From Fig. 5 it is seen that the general layout, vacuum chamber, condensing coil, etc., are similar to that of the type 2 machine. The carrier, however, consists of a single large mild-steel cylinder welded to a stout base to accommodate a cylindrical container 18 in. diam. by 18 in. high. The base of the carrier is welded to a 2 in. shaft, the whole being turned true in the lathe and carefully balanced before assembly. Adequate bearings and robust construction are essential with the large liquid load (maximum of 15 l.) carried. The container is made of anodized spun aluminium, and Fig. 6 shows the container together with its perforated lid, to which a cotton-wool cover may be attached when sterile conditions must be maintained.

The drive is transmitted by twin V belts from a $\frac{1}{4}$ h.p. motor to a pulley on the 2 in. shaft, which is drilled throughout its length in order to convey the thermocouple leads from the carrier to the slip rings at the lower extremity of the shaft, a wax seal being arranged at this point. Heat is supplied by a concentric chromium-plated cylinder which carries nine vertical strip heaters symmetrically disposed, each strip having a maximum loading of 250 w. A horizontal metal reflector beneath the carrier restricts heat exchange with the condenser. To cut off the supply of heat as the product approaches dryness, a radiation thermostat (an adaptation of the usual domestic hot-water type) is fixed at the centre of rotation and set to switch off the heater load at a suitable temperature (usually about 20°). These features are seen in Fig. 7, which is a view from above

with the lid swung aside. The thermostat, although somewhat crude, is simple and certain in action. The heat input is controlled by a Variac. The compressor, condenser and vacuum pump are similar to those used in the type 2 machine.

With a container of this height having vertical walls, the speed of rotation is determined by considerations of uniformity in the thickness of the liquid layer, the required centrifugal force being far in excess of that needed to suppress foaming. At the speed adopted, i.e. 500 rev./min. (centrifugal force of 65 g at the wall of the container) the difference in the thickness of the liquid layer between the top and the bottom of the vessel does not exceed $\frac{1}{4}$ in. Lower speeds could, of course, be used if the containers were made with the appropriate profile, tapering inwards towards the base.

With these large metal containers it is an advantage to maintain spinning throughout the freeze-drying process to ensure good thermal contact between the large mass of ice and the wall of the vessel. The more positive contact with the wall of the metal container thus achieved offers another marked advantage over the orthodox tray drier, where the mass tends to dry from beneath, as well as at the upper surface, thereby causing inclusion of ice within a cocoon of dried material and greatly hindering completion of the process. See, for example, R. Gane (see Harris, 1954, p. 189) and Bradish, Brain & McFarlane (1947).

Fig. 6. Type 3 machine: container and lid.

Fig. 7. Type 3 machine: view from above.

A container of the size used in this machine has the equivalent area of a tray drier of nearly 6 ft.², 13.5 l. producing a liquid layer with an average depth of 1 in. With a heat input of 1 kw and a test load of 13.5 l. of pure water, drying is complete in about 18 hr., which corresponds to an overall average drying rate of 0.75 l./hr., or 39 $\mu\text{g./cm.}^2\text{/sec.}$ Allowing for the rapid initial loss of water during the evaporative freeze, the steady rate during freeze-drying is about 32 $\mu\text{g./cm.}^2\text{/sec.}$ At this rate, with an open container, the temperature of the ice adjacent to the wall of the vessel is only about 1° higher than that of the condenser, and the drying rate can be substantially increased by raising the heat dissipated further to, say, 1.5 kw. This rate of drying compares very favourably with that quoted by Bradish *et al.* (1947) for a tray drier in which the condenser was adjacent to the drying material. It seems doubtful whether such close proximity of the condensing surface to the product does in practice result in any appreciable gain in drying rate, in view of the very small temperature differential between the subliming ice and the condenser surface in our equipment.

Adaptation for large-scale ampoule drying. The versatility of this type of machine is well illustrated by its application to ampoule drying. In place of the aluminium container, the carrier will accommodate several tiers of ampoule racks. Fig. 8 shows three tiers of racks, each rack holding 150 ampoules of the 1 oz. size. Each ampoule is inclined slightly to the centre so that its contents freeze in the wedge form. With 450 such ampoules, each containing 10 ml. of serum, i.e. a total load of 4.5 l., primary drying was completed in 48 hr. with a heat input of only 200 w. The speed of rotation (500 rev./min.) is more than sufficient to suppress foaming, even with ampoules arranged in the

inner ring, although the drying of these ampoules in the middle of the centre rack is hindered somewhat by the restriction of their heat input by neighbouring ampoules. Racks cut from solid aluminium should offer some improvement in this respect.

Drying above the freezing point. In certain cases, where cell suspensions, etc., are required to be dried, freezing may not be tolerated without damage to certain enzymes, etc., and yet the suspension is required to be dried at the lowest possible temperature, e.g. just above 0°. The type 3 machine has been used for such a purpose by introducing an air leak, controlled by a needle valve, in the vacuum line to the pump. This maintains a constant pressure in the system, and at 5 mm. Hg, for example, and with continuous spinning to suppress foaming, 2 l. of a cell suspension was dried successfully overnight at +2° ($\pm 0.5^\circ$) without the application of any heat, to yield a compact, dry and friable product which still retained most of its original enzyme activity.

Special features of design

All three machines have certain features in common which have been arrived at by experiment and found, by actual use over a number of years, to be sound.

Lid seal. A very effective seal is achieved by a projecting lip on the underside edge of the lid, bearing upon a flat rubber ring cut from $\frac{1}{4}$ in. thick rubber sheet and located in a groove machined in the top rim of the chamber (Figs. 2, 5). When the chamber is evacuated the atmosphere exerts a pressure of about 3.7 tons on the lid, or 100 lb./in. length on the rubber ring.

Mainshaft seal. This is an adaptation of the orthodox type of seal employed on refrigerator-compressor crankshafts, and consists of a fixed portion (a spring-loaded metal bellows sweated to a phosphor-bronze ring) which seats upon the rotating portion (a case-hardened steel cup enclosing a Duprene rubber ring fitted snugly on the rotating shaft). This type of seal is effective only when running in oil and it is therefore enclosed in an oil reservoir which is connected by a pipe to an external sight glass, which may be topped up with oil occasionally as required. In order to ensure retention of oil in this reservoir, a second identical bellows seal is fitted back to back with the first one. A small air bleed, closed by a plug, at the top of the reservoir ensures complete filling with oil on assembly. Any vacuum leak at this shaft seal can occur only after the oil has first been drawn into the chamber, and this will be evident in the sight glass. The arrangement has given service over a period of many years without trouble, requiring topping up with oil at intervals of several months only.

Refrigerator-coil inlet and outlet seals. The inlet and outlet points of the low-temperature condenser from the vacuum chamber have often caused trouble in past designs owing to poor thermal insulation, and in some the expansion valve has been placed inside the chamber. This is undesirable for two reasons: (i) screwed refrigeration joints inside the vacuum chamber may allow leakage of gas which would affect the vacuum, contaminate the pump oil and be difficult to locate and repair; (ii) final adjustment of the regulator valve under working conditions is impossible. The difficulty has been overcome in the present designs by leading the two pipes into the chamber through Tufnol blocks of large dimensions, sealing the pipe by a rubber washer closed in on the pipe by a screwed collar. Although

Fig. 8. Type 3 machine: ampoule racks.

the rubber washer probably becomes quite brittle at low temperature, no vacuum leaks have occurred at these points, and the seals have been undisturbed over a period of many years.

Vacuum inlet pipe. If the pumping of much water vapour and a poor vacuum are to be avoided this pipe must end in a region of low temperature in some position such as shown in Fig. 5.

Before opening the chamber after primary drying, air is admitted slowly through a needle valve. Freeze-dried materials, being in a fine state of subdivision, are usually very hygroscopic in nature and the admitted air is therefore dried by fitting the needle valve in the vacuum line to the pump so that the air entering the chamber first passes over the refrigerated coil, where most of its contained moisture is removed.

Measurement of temperature. A continuous record of the temperature of the material being dried (or of a representative sample) is important since the condenser temperature and the vacuum do not by themselves give sufficient indication of the temperature of drying and give negligible information about the rate of freezing, the final product temperature or the completion of drying. Multipoint recorders are commercially available which are robust yet sensitive enough to operate directly from copper-constantan thermocouples, and the simplicity, convenience and reliability of this method of temperature recording makes it the method of choice for freeze-drying purposes. Each machine has its own three-point thread recorder to give a continuous record of the temperature of the inlet and outlet of the condenser, and of the material being freeze-dried. The recorders are compensated for changes in ambient temperature, and no constant temperature ('cold') junction is required.

The temperature of the refrigeration-coil inlet and outlet is obtained by strapping the thermocouple, including several inches of the thermocouple leads, to the pipe at its point of entry into the chamber, the whole being well lagged. It is important that the thermocouples be insulated electrically from all metal parts of the equipment, as small stray earth currents can introduce serious errors.

In the present equipment, the measurement of product temperature presents a more difficult problem, since the current produced by the thermocouple e.m.f. must be conveyed from the rotating container via slip rings. The constantan line is picked up by the spring contact of a floating constantan brush bearing lightly against a central constantan rod turned to a point. In an earlier design the copper line was picked up by a copper brush bearing against a copper slip ring, and provided that the slip-ring diameter was kept small (0.25 in.) tolerable results were obtained with types 1 and 2 machines. In the type 3 machine, the thermocouple slip rings cannot conveniently be arranged inside the vacuum chamber, and it was found that at atmospheric pressure rapid oxidation and fouling of the copper slip ring rendered the device impracticable, especially as the container in this type is spinning throughout the drying operation. By the use of an auxiliary copper disk running freely in light ball bearings and in rolling contact with the copper slip ring, it is possible to make a point contact with the copper brush in the same way as employed for the constantan. This improved design (Figs. 9 and 4) is now giving excellent results on all three types of machine (Prov. pat. 12661/57).

At their point of exit from the vacuum chamber, thermocouple and heater leads are sealed by passing them through a cup containing vacuum wax (see, for example, Greaves, 1946). That part of each wire embedded in the wax must be stripped of its insulation over a short length and, where stranded conductors are used, the bared strands must be separated to avoid air channels in the wax. A layer of oil on the surface of the wax is effective in sealing minute cracks. Large temperature changes must be avoided by thermal insulation of the cup by the insertion of a short length of thick-wall rubber tube (see, for example, Fig. 5, type 3 machine). Although proprietary glass-to-metal seals are now available, they are unsuitable for thermocouple lines.

Measurement of vacuum. A continuous record of the pressure inside the freeze-drying chamber may be obtained by the use of the Pirani type of gauge, which is well suited to cover the pressure range concerned. Such a gauge must be calibrated against a standard, and even when calibrated the reading obtained depends to some extent on the ratio of air:water vapour in the system. With a continuous record of product temperature (or of a representative sample in types 1 and 2 machines), together with well-designed equipment in which vacuum troubles seldom occur, the continuous recording of vacuum is not essential. Reliable readings at intervals are nevertheless desirable and for this purpose an absolute gauge, the McLeod gauge,

Fig. 9. Thermocouple slip-ring assembly.

has obvious advantages. Its use in an air-water-vapour system, however, is beset with pitfalls, and many controversial statements concerning its use on such systems are to be found in the literature. For example, Flösdorf (1949) states that provided that the measured pressure is below that of the 'condensation point' of the gauge, a true reading will be obtained. On the other hand, Greaves (1946) states that this type of gauge will neglect the water-vapour pressure altogether. In fact, the McLeod gauge reading obtained is somewhere between these two extremes, and it can be shown that the low readings obtained with this type of gauge on an air-water-vapour system are due mainly to adsorption of water vapour on the glass wall of the gauge during compression of the gas for measurement. Errors arising from deviations from the ideal gas laws in water vapour approaching saturation are of a relatively small order. The magnitude of the water-vapour adsorption error appears to depend on the condition of the glass; it is increased by cleaning the glass bulb and capillary with chromic acid, and a gauge head cleaned in this manner commonly gives readings of only 0.01 mm. Hg on an air-free system in which the ice-vapour pressure is known to be 0.055 mm. Hg (condensing coil temperature -45°). On the other hand, an identical gauge in which the bulb and capillary have been coated with molten paraffin wax *in vacuo* gives a true reading of 0.055 mm. Hg when connected to the same system. If reliable McLeod-gauge readings are to be obtained on an air-water-vapour system, such as that occurring in freeze-drying equipment, it therefore becomes imperative not only to use a gauge of such dimensions that no condensation of water vapour occurs when the gas is compressed for measurement, but further that adsorption of water on the glass of the gauge should be eliminated by treatment of the glass surface with a smooth water-repellent coating.

The occurrence of water-vapour adsorption errors appears to have been recognized by Ede (1947), who excluded moisture entirely from the gauge and measured only a balancing air pressure. Unfortunately, as the author points out, such a gauge will fail if the amount of air in the system falls below a certain level. By introducing a small quantity of glass wool into the upper part of the bulb of a McLeod gauge, we have been able to obtain, by a direct reading on the gauge used in a normal manner, a fair idea of the partial pressure of air in the freeze-drying chamber. Almost all the water vapour is adsorbed on the glass wool during compression of the gas for measurement, the gas being virtually dry by the time the mercury has risen into the capillary for measurement. The total pressure is determined on a separate identical gauge previously coated with paraffin wax as just described.

Both gauges were shown to give practically the same reading when connected to the same moisture-free system. On the other hand, typical readings when short lines were used for connexion to the vacuum chamber during freeze-drying were as follows: condenser temperature, -45° ; total pressure (waxed gauge), 0.060 mm. Hg; air pressure only (glass-wool gauge), 0.002 mm. Hg. It is evident that practically all air has been swept out of the chamber during the drying process.

Defrosting procedure. For rapid defrosting of the refrigerated coil, provision is made for filling the lower half of the vacuum chamber from a hot-water supply. The removal of 15 l. of ice from the coil can be carried out in about 10 min. by this procedure.

Vacuum-pump exhaust. In the early stages of pumping down large chambers, the vacuum pumps, even when fitted with complex baffles or filters, throw up a fine aerosol of oil. Consequently, the exhaust from all pumps is piped outside the building.

PERFORMANCE

Evaporative spin-freeze. In evaporative freezing, in contrast with prefreezing where heat is abstracted via the wall of the containing vessel, cooling takes place at the free surface of the liquid and the ice layer starts to form at this interface, advancing outwards to the wall of the vessel at a rate depending on the surface temperature of the ice and limited by the thermal conductivity of the ice layer already formed. Thus for a given surface temperature, the rate of advance of the ice boundary will decrease progressively as freezing proceeds and complete and instantaneous 'snap' freezing can occur only with very thin liquid layers. With a condensing-coil temperature of -30° , and a vacuum of 0.29 mm. Hg equal to the vapour pressure of ice at this temperature, a 1 cm. thick ice layer is advancing at the rate of 1 mm./min. (a value of 0.005 cal./sec./cm.²/°C for the thermal conductivity of the ice already formed being accepted). The freeze-drying of thick-liquid layers may therefore be undesirable with substances sensitive to concentrated salt solutions. It is, however, important to avoid the other extreme of too thin a liquid layer if drying from the frozen state is to be assured. It has already been shown that about one-sixth of the water in the product to be dried must be evaporated in order to cool the remainder from room temperature to -25° . This estimate makes no allowance for the heat capacity of the container and if this is large in relation to the volume of material to be dried, it may even be possible for all, or most, of the water to evaporate before freezing takes place. It is then desirable to increase the volume of the material by dilution. Some solutions, especially those containing material at high concentration or of high viscosity, hinder evaporative freezing owing to the formation of a surface skin of dried material; evaporation then practically ceases at the outset and the solution does not freeze. The remedy lies in diluting the solutions, where this is feasible, or in prefreezing where it is not.

Fracture of the containing vessel. One of the practical difficulties arising from the direction of ice formation in evaporative freezing is the tendency to cause cracking of the glass containing vessels, as a result of the expansion on freezing of the liquid trapped by the inner cylinder of ice first formed. Fracture occurs only with a certain minimal thickness of liquid layer in relation to the

dimensions and mechanical strength of the container, e.g. 200 ml./jar appears to be a safe volume for 3 lb. bottling jars under all conditions. With volumes in excess of 200 ml., fracture is most prone to occur with dilute solutions or suspensions in the absence of salts. Under the normal conditions of operation of the type 2 equipment, the presence of sodium chloride in solution affords complete protection with 400 ml./jar even at concentrations as low as 0.025 %, evidently by weakening the ice structure by the presence of a liquid layer between the ice crystals, and it is usual to find evidence of slight bulging of the ice surface at one or two points when freezing has been completed. The protection afforded by various salts appears to depend on their eutectic points in relation to the conditions of freezing. For example, in tests carried out in the type 2 machine under normal operating conditions, potassium chloride, with a eutectic point at -11.1° , was far less effective than sodium chloride (eutectic point at -21.2°) in preventing fracture. At a slower rate of freezing, on the other hand (e.g. by the introduction of an air bleed into the system), potassium chloride may prove equally effective.

The tendency of ampoules to fracture when material is dried in the wedge form depends on the tenacity with which the ice layer can key itself to the glass wall. For this reason solutions of gelatin are very prone to cause trouble, owing to the strong adherence of gelatin film to a glass surface. Such a film would be formed at the edges of the wedge of material in the early stages of evaporative freezing, and would serve to key the ice layer firmly to the ampoule. It was found that the trouble could be eliminated by pretreatment of the ampoule interior with one of the organosilanes. This particular hazard with gelatin concerns only the wedge form of evaporative freezing and does not arise when the container is spun about its own axis.

'*Spill*.' 'Spill' (Cowan, 1951), or ejection of the dried material from the container as drying proceeds, is another hazard which depends upon the nature of the material being dried. Since 1 g. of water vapour at a pressure of 0.1 mm. Hg has a volume of about 10⁴ l., the rate of evolution of water vapour from a single 3 lb. bottling jar in the type 2 machine amounts to over 50 l./sec., and this may cause portions of the dried outer layer to be ejected with almost explosive violence. In the freeze-drying of a 1 % sodium chloride solution about half of the salt is ejected in this manner, whereas serum appears to suffer no loss. The usual sterile-filter caps reduce the effect by decreasing the vapour flow as a result of the raised water-vapour pressure inside the bottle. Such filters also trap most of the material that is ejected, but are not

completely effective. By drying under the influence of centrifugal force, as in the normal operation of the type 3 machine (force 65 g), 'spill' is almost completely suppressed, a 1 % sodium chloride solution drying under these circumstances with negligible loss.

Size, shape and distribution of the ice crystals. When moderate concentrations of proteins, etc., are properly freeze-dried, i.e. from the completely solid state, the resulting dried product retains not only the volume of the original solution but also the detail of the ice-crystal pattern laid down during the freezing stage. Microtome sections of the dried material, after embedding in paraffin wax under vacuum, reveal a network lattice of dry protein delineating the boundaries of the original ice crystals (Fig. 10). Except for a random distribution in the surface layers, the bulk of the ice is laid down as a mass of thin laminae perpendicular to the wall of the container and thus parallel to the temperature gradient during freezing. In this respect the pattern closely resembles that observed in freezing by ordinary methods of cooling (see, for example, Dorsey, 1940, p. 411). Under standard conditions of freezing in the type 3 machine, a 10 l. load of 5 % ovalbumin showed that the resulting ice-crystal laminae had a fairly uniform thickness of about 0.006 mm.

Fig. 10. Microtome section of freeze-dried 5 % ovalbumin.

A typical drying cycle. Fig. 11 shows a routine temperature record obtained during the freeze-drying of 10 l. of 5% ovalbumin in the type 3 machine. The temperatures of the inlet and outlet of the condensing coil are almost coincident and are shown by the lower trace. The product temperature is recorded on the upper trace. Evacuation of the chamber began at time 0, with the coil temperature at -45° and the product at room temperature (20°). Reduction of the chamber pressure to a few mm. Hg was marked by a rapid fall in product temperature to 0° , near which it remained for the next 20–25 min., until the freezing of almost all the water was complete. The temperature of the mass of ice then dropped sharply until it was superimposed on that of the condenser. Measurements with a thermocouple which, together with about 12 in. of the thermocouple leads, was embedded in a layer of ice frozen on to the condensing coil inside the chamber, showed that the actual condenser temperature was in fact approximately 2 degrees lower than that indicated by the coil temperature on the chart. The product temperature in the absence of any applied heat other than from stray radiation and conduction derived from the surroundings was therefore about 2 degrees higher than that of the condenser.

After switching on the heaters (1 kw loading) at 2 hr., steady-state conditions were attained, with a product temperature adjacent to the wall of the container some 5–6 degrees higher than that of the condenser inlet, i.e. 7–8 degrees higher than the true condenser temperature. As drying proceeds the temperature of the product gradually rises as a result of the impedance to vapour flow presented by the increasing thickness of the network of dried material. The magnitude of the impedance is dependent not only on the thickness of the dried layer above the ice surface but also on the size of the ice crystals or laminae laid down in the freezing stage, which govern the size of the channels in the dried mass through which the water vapour makes its escape. The final stages of drying are completed relatively quickly, there being no evidence of the prolonged second phase described by Bradish *et al.* (1947) in the orthodox tray drier. The product,

Fig. 11. Temperature record of freeze-drying of 10 l. load in type 3 machine.

when removed from the machine at the end of the recording, showed complete absence of wet spots.

An indication of the amount of obstruction to vapour flow in the absence of a layer of dried material may be obtained by drying a 10 l. load of pure water under the same conditions of vacuum, applied heat (1 kw), etc. The subliming ice then assumes a constant temperature within only 1° of the actual condenser temperature; this suggests that the gain in rate of drying which would be obtained with a condenser in close proximity to the product would be negligible. Whereas the drying temperature for water is markedly lower than that for 5% ovalbumin solution, the total drying time is approximately the same. These results depend, of course, on the maintenance of a vacuum equal to that of the vapour pressure of ice at the temperature of the condensing coil; any rise of pressure in the chamber would at once lead to a rise in the product temperature.

SUMMARY

1. Equipment is described for the routine freeze-drying of material on a large laboratory scale with the minimum of handling and attention. The evaporative spin-freeze principle is used throughout.

2. Details are given of various special features of design, and of the methods used for the measurement of the temperature of drying material in rotating containers, and of the vacuum in low-pressure air-water-vapour systems.

3. The practical advantages and disadvantages of this type of equipment are discussed and the performance is illustrated by a typical freeze-drying cycle.

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