Instrumented Pilot Plant Lyophilizer: Its Versatility in Developing and Programming Production Cycles

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Abstract □ A detailed evaluation of a pilot plant lyophilizer provided valuable information for the development and programming of drying cycles for production equipment. Temperature–time and pressure–time curves from the initial freeze-drying runs and a knowledge of the conductivity behavior of three products were found to be almost essential in the development of optimum lyophilization cycles.

Keyphrases □ Lyophilizer, instrumented—pilot plant □ Production cycle development—instrumented pilot plant lyophilizer □ Instrumentation—pilot plant lyophilizer □ Temperature–resistivity measurement, pilot plant lyophilizer—application to production equipment

In previous investigations (1–7), the various parameters believed to influence significantly the freezing and drying processes during the lyophilization of pharmaceuticals were elucidated. From these studies, it was also concluded that certain instrumentation and controls on a conventional freeze-dryer were essential equipment for optimum lyophilization. The purposes of this report are to describe the necessary instrumentation and controls and to demonstrate how the information obtained from both preliminary and pilot studies can be utilized to program cycles for a production scale operation. Most of the instrumentation is of the standard type and can be easily incorporated into most existing freeze-dryers.

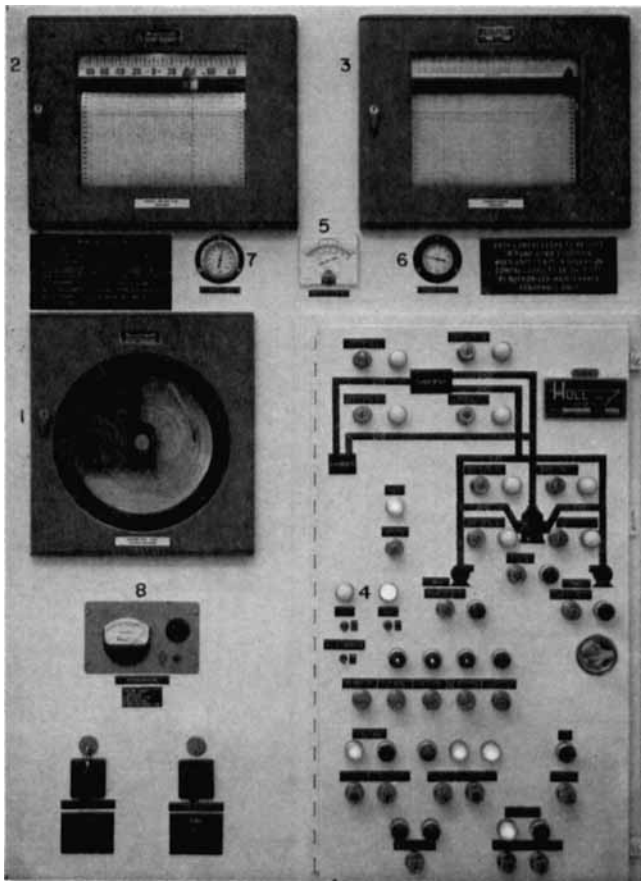

Figure 1—*Instrumentation and control panel. Key: 1, program controller; 2, temperature recorder; 3, vacuum recorder; 4, alarm system controls; 5, pyrometer; 6, vacuum and pressure gauge; 7, air pressure gauge; and 8, thermocouple vacuum gauge.*

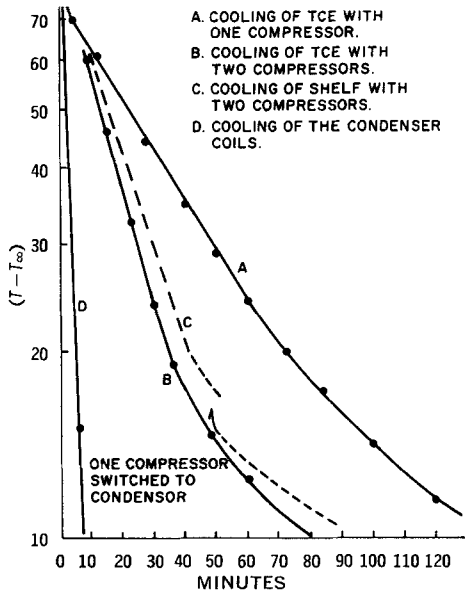

Figure 2—*Capability of the refrigeration system. The temperature controller was set at −50°.*

Essential in the development of a lyophilization cycle is a knowledge of the capabilities of the various systems comprising a freeze-drying unit. Once the capabilities of the various systems are evaluated, it is possible to use a more systematic approach to conducting preliminary studies and designing a cycle. Although the information resulting from such evaluation usually cannot be utilized directly with another lyophilizer in another plant, the methods employed to determine the necessary information will be described and the significance of the values obtained will be discussed. To make this presentation more meaningful, a brief description of the equipment will be presented.

DESCRIPTION AND EVALUATION OF EQUIPMENT

The unit[1] consists basically of a freeze-drying chamber, an external condenser, heating and chilling systems for the chamber shelves, a condenser refrigeration system, a vacuum pumping system, and a control and instrumentation panel.

Freeze-Drying Chamber—The chamber is provided with a suitable wall flange so that it could be sealed into the wall of a sterile

[1] A Hull Corp. model 653-F30 freeze-dryer.

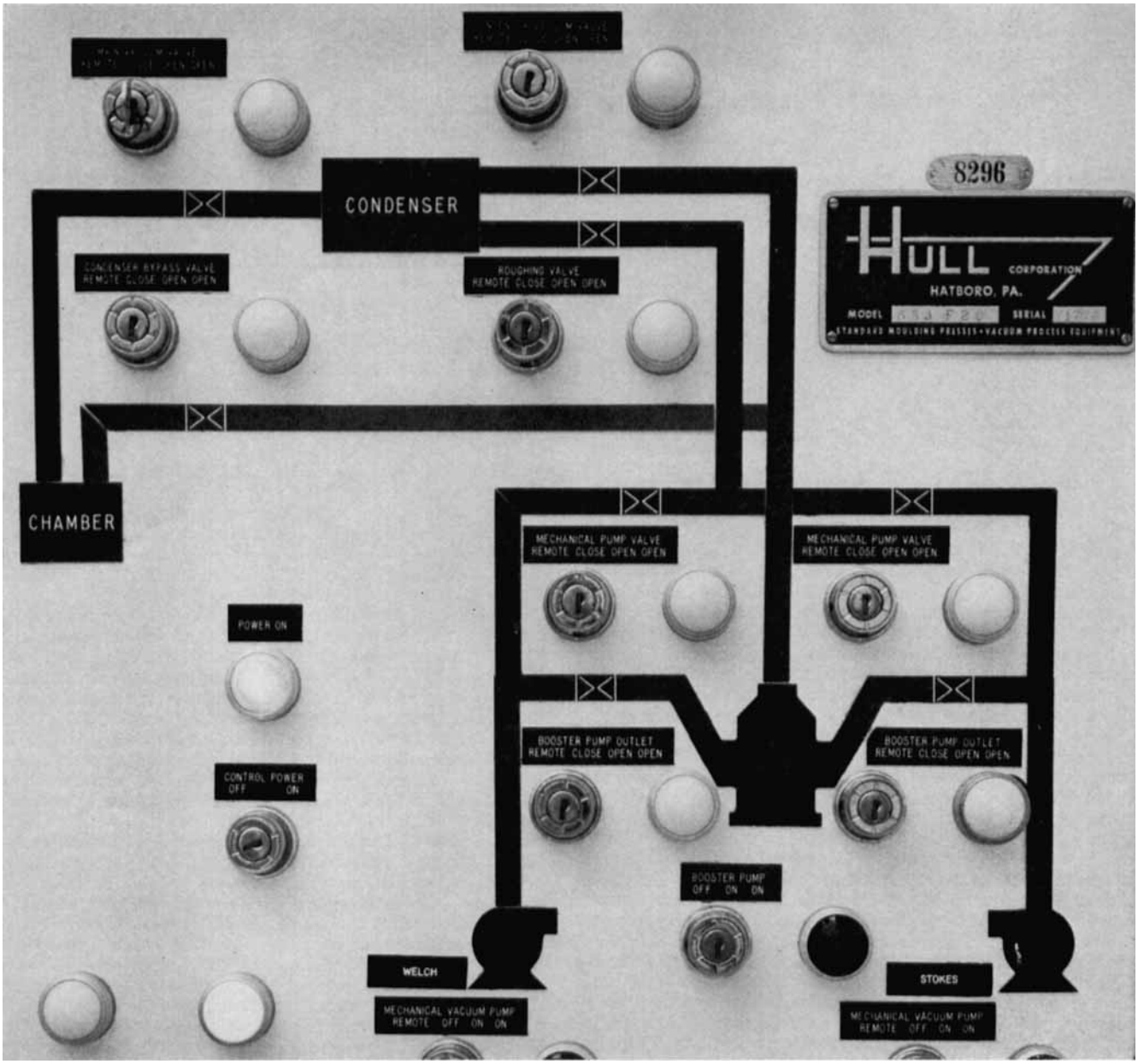

Figure 3—*Graphic representation of the vacuum system.*

room. During a run, handwheels equipped with Teflon washers seal the door of the chamber and two Thermopane-type windows permit visual observation into the chamber. A manual break valve, equipped with a stainless steel filter holder on the chamber door, allows for connection of an inert gas service in order to pressurize the chamber upon termination of the drying cycle.

The interior of the drying chamber is equipped with eight thermocouple probes for monitoring the temperature of selected samples throughout the chamber. A plug-in jack-panel arrangement permits easy connection of thermocouples which can be designed for specific purposes. Four 0.61 × 0.91-m. (2 × 3-ft.) product shelves provide a total product shelf area of 2.22 m.^{2}. The shelves are coated with a black phenolic resin for high emissivity, and stainless steel bumper strips along the front edge of each shelf preclude chipping of the coating during loading and unloading. For production purposes, stainless steel shelves are highly recommended.

External Condenser—The condenser is of a special design and, for maximum flow, is closely coupled to the drying chamber by a 25.4-cm. (10-in.) vacuum line. A 25.4-cm. (10-in.), remotely actuated, high vacuum valve isolates the drying chamber from the external condenser. The large condenser surface area in the form of plate coils, widely spaced, and the special internal baffling of the condenser contribute to a high rate of vapor removal and a good distribution of ice on the condensing surface. The unit was designed to handle at least 30 l. of moisture in the form of ice before defrosting is required.

Instrumentation and Controls—Figure 1 shows the instrumentation and control panel for the unit; a similar arrangement, either entirely or in part, can be adapted to almost any lyophilizer. This unit is equipped with a dual-control panel; one panel is located in the sterile drying room adjacent to the recording instrumentation and the door of the dryer, and the other is in the nonsterile machinery area. Key-type switches with local or remote positions permit operation of the equipment either from the sterile area or from the machinery area. To be operative from the machinery area, the key switches in the sterile room must be set to remote position.

The instrumentation includes a 24-point strip chart temperature recorder, calibrated for −85 to +85°, to record and monitor temperatures throughout the system such as product temperatures within the chamber, shelf temperature, recirculated trichloroethylene temperature, and condenser temperature. A strip chart vacuum recorder is provided, having a range of 1–1000 μ for recording the pressure within the drying chamber. This instrument is equipped with alarm switches set at 200 and 300 μ. Should the pressure within the drying chamber rise to 200 μ, a pilot light goes on and a buzzer alarm sounds. If the pressure within the chamber rises above 300

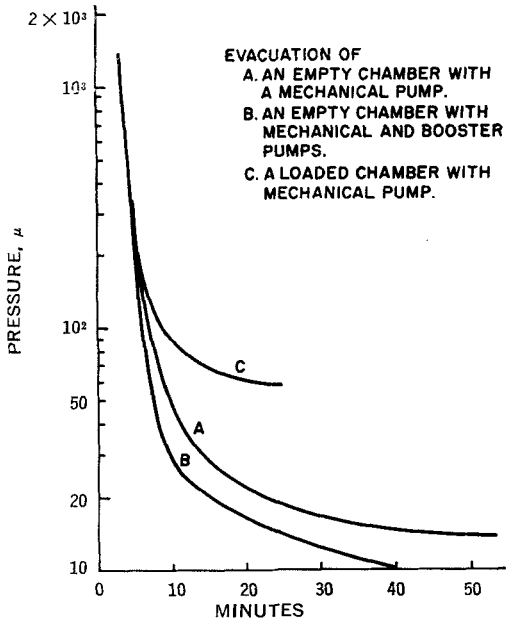

Figure 4—*Capability of the vacuum system.*

μ, a second pilot light goes on, an alarm bell sounds, the valve between the external condenser and the vacuum chamber closes, heat to the trichloroethylene is cut off (if the unit is on the heat cycle), the vacuum pump stops, and the shelf refrigeration starts. The pressure at which the alarms are set depends to some extent on the size of the chamber. Slightly higher settings can be used in larger production units of similar capability.

The panel also has a dial readout pyrometer to indicate the temperature of the defrost water leaving the external condenser; a combination dial-type vacuum pressure gauge to indicate whether the chamber is under vacuum or being pressurized during sterilization; a dial-type air pressure gauge for the pneumatically operated vacuum valves; and, lastly, a 5-station indicating-type thermocouple gauge. This gauge will permit reading the vacuum within the drying chamber, in the external condenser, at the inlet to the oil booster pump, and at the inlet to each mechanical high vacuum pump.

A contact recording program controller programs the drying cycle by controlling the temperature of the trichloroethylene recirculated through the drying chamber. The instrument, calibrated from −85 to +85°, moves a set point index through a time *versus* conditions pattern. The controller consists of a motor-driven plastic cam cut to the desired process pattern; a cam follower connected by cable to a standard control index, in this case, the temperature of the trichloroethylene entering the chamber; and a circular chart

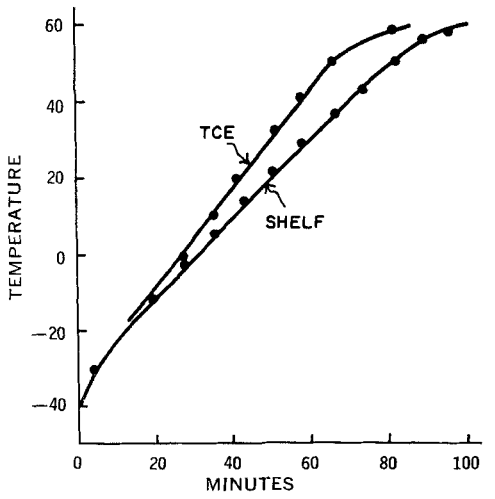

Figure 5—*Heating curves for the circulating trichloroethylene fluid and the surface of a shelf. The temperature controller was set at 60°.*

Table I—Rates of Cooling and Heating of Circulating Fluid and Shelves and Rate of Cooling of the Condenser Coils

	Cooling Rate, min.$^{-1}$	Heating Rate, deg./min.
Circulating fluid	0.0177[a]	1.35
Shelf 1	0.0165[a]	1.09
Shelf 2	0.0171[a]	1.12
Shelf 3	0.0169[a]	1.07
Shelf 4	0.0162[a]	1.05
Condenser coils	0.121[b]	—
Circulating fluid	0.00824[c]	1.35

[a] Both compressor units of trichloroethylene to shelf refrigeration. [b] One compressor on condenser. [c] One compressor on shelf refrigeration.

recorder which records the temperature of the trichloroethylene entering the chamber shelves.

Key-type stepping switches are used to control the chamber sterilization cycle and the external condenser defrost cycle. These switches, of course, preclude the accidental introduction of steam or water to either the drying chamber or the condenser during a drying run and make the sterilization and defrosting processes essentially foolproof. For sterilization, the steam enters *via* a remote-operated steam inlet valve on the chamber, and sterilizing conditions can be allowed to exist as long as desirable. A condensate remote-operated valve opens and permits the steam trap to discharge condensate from the chamber to the drain to maintain a level of 15 psig. in the chamber.

Defrosting of the condenser is accomplished by admitting 3 psig. steam *via* a remote-operated inlet valve on the condenser. The steam, of course, begins to melt the ice on the plates, and a valve to a steam condensate drain opens, permitting discharge of condensate. The defrosting may be observed through two sight glasses located at the rear of the condenser. When the ice is essentially melted, and before the discharge drain temperature rises too high, the steam inlet is closed and the water inlet valve is opened by the key stepping switch. Water fills the condenser and overflows *via* a drain at the top. When all the ice is melted and the condenser plate temperature is reduced, the key switch knob is turned to another position which closes the water inlet valve and opens a main drain valve, permitting the condenser to drain.

Refrigeration, Heating, and Vacuum Systems—The shelves in the drying chamber are chilled and heated by circulating trichloroethylene. The unit is equipped with two integral two-stage Freon 22, 10-hp. compressors. One of these maintains the temperature of the circulating trichloroethylene at any level from −40 to 70° for any length of time during the drying cycle without removing refrigeration from the external condenser. The second compressor normally serves the external condenser during the drying cycle. However, a provision exists for diverting the refrigeration from the condenser to the trichloroethylene heat exchanger to assist in the initial chilldown of the shelves. Therefore, the trichloroethylene can be initially chilled by means of the shelf compressor or by both shelf compressor and external condenser compressor, where each compressor is expanding Freon to the trichloroethylene heat exchanger, thus permitting rapid cooling of the trichloroethylene and freezing of the product. The initial chilldown of trichloroethylene and freezing of the product might be started while the external condenser is defrosting. To protect the refrigeration and heating systems from damage, an electrical interlock is provided to prevent heating or chilling of the trichloroethylene unless the pump that recirculates the trichloroethylene is operating.

The cooling capability of the refrigeration system was determined by measuring the cooling rates of the condenser coils and of the circulating fluid using either a single compressor or two compressors on the trichloroethylene. In Fig. 2, the cooling rates for the circulating fluid, condenser coils, and a shelf are illustrated. The log $(T - T_\infty)$ was plotted against time to account for the capacity of the compressor, which was rated at −50°, T_∞ being equal to −50°. Such a plot produces a straight line down to −32°, about 18° above the rated capacity of the compressor. Starting from ambient conditions, it requires over 120 min. for the trichloroethylene to reach −40° using one compressor and approximately 80 min. when both are employed.

Since the condenser coils are cooled by direct expansion of Freon, the cooling, as demonstrated by Curve C, was quite rapid.

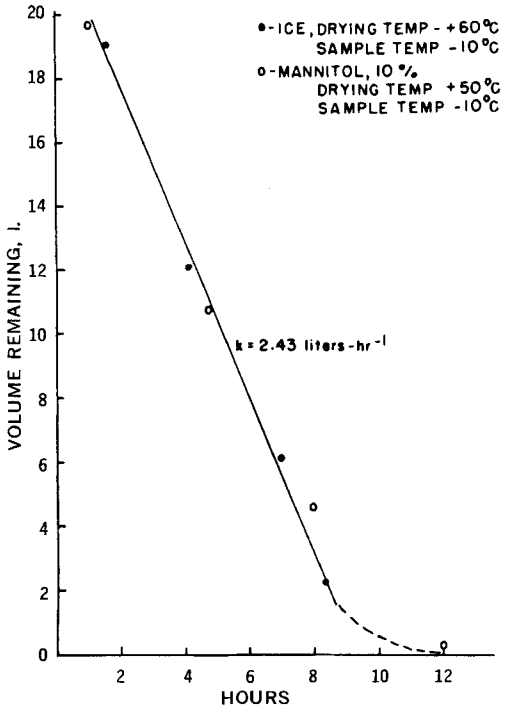

Figure 6—*A plot of the quantity of water remaining as a function of time. The rate of sublimation is represented as* k.

Within 6 min., the temperature of the condenser fell to −35°; within 15 min., it reached −50 to −55°. The temperature was lowered further with the application of vacuum.

The broken segment of Curve B resulted when the condenser was switched from shelf to condenser refrigeration. Using this procedure, the condenser coils can be cooled to below −50° within 15 min., as shown by D. Curve C represents the cooling of one of the shelves, and the heat transfer through the shelves is demonstrated by the difference between B and C.

Figure 3 shows the graphic representation of the vacuum system on the control panel. The vacuum system consists of a two-stage compound-type air-ballasted high vacuum pump, a single-stage high vacuum pump as a spare or standby, and an oil booster pump with associated piping and remote actuated valves. Thus, it is possible to evacuate the chamber, the condenser, or both together by either the single-stage or two-stage mechanical pump or by making use of the oil booster pump in conjunction with a mechanical pump to obtain a low chamber pressure. Low pressures during the drying cycles are often essential in the lyophilization of biologicals.

The capability of the vacuum system is illustrated in Fig. 4 where curves are plotted for the evacuation of an empty chamber using only the mechanical pump (Curve A) or using both the mechanical pump and the booster pump (Curve B), and for the evacuation of a chamber with a full load using only the mechanical pump (Curve C). By pulling on the condenser directly, the mechanical pump has the same efficiency in lowering the pressure to approximately 400 μ of Hg as it does when it is pulling through the booster pump. Below 400 μ, the rate of evacuation was greater when both the

Table II—Defrosting of Condenser Coils

Minutes	Condenser Temperature	Remarks
0	−60°	Steam injected into the condenser
8	3°	
26	42°	
32	Above 80°	Observation of condenser showed coils completely free of ice Water injected into condenser
38	20°	Water drain opened
44	21°	Water drain closed; compressor turned on to refrigerate coils
50	−35°	
62	−54°	

Table III—Sterilization of Chamber

Minutes	Chamber Temperature	Chamber Pressure, psig.	Remarks
0	40°		Steam injected
10	105°	3	
16	115°	10	
20	117°	12	
35	121°	15	
50	121°	15	Steam discontinued Drain opened
55	90°		Two compressors on shelf refrigeration
60	65°		Chamber opened and loaded

mechanical and booster pumps were employed simultaneously. In this manner, pressures as low as 5–10 μ can be attained; with the mechanical pump alone, pressures of approximately 13–15 μ are attainable. As illustrated by Curve C, with a charge of about 20 l. of frozen water at −30° in the chamber, the mechanical pump was capable of lowering the pressure to below 100 μ in less than 10 min.

During drying, the recirculating trichloroethylene is maintained at the desired temperature throughout the cycle in the range from −40 to 70°, as programmed by the cam controller. This controller will call for either refrigeration from the trichloroethylene compressor or the addition of heat from an electric immersion heater.

The capability of the heating system is demonstrated by the curves in Fig. 5. The circulating fluid was heated at the rate of 1.35° per minute over a range of −30 to 50°. Heating of the shelves was at a rate of approximately 1.1°/min.; within 80 min., a shelf temperature of 50° was attained. Although the increase of temperature with time deviates slightly from linearity, linear rate constants were calculated for the heating of the shelves, and these are summarized with the cooling rates in Table I.

Once the capabilities of the various systems are established, it is possible to utilize this information for the development of a cycle. During the freezing stage, if a rapid chilldown is desired, both compressors can be employed for cooling the circulating trichloroethylene fluid to the shelves. Once the desired temperature is reached, the condenser compressor can be switched to the coils in the freeze-trap. As illustrated by the broken line segment of Curve B in Fig. 2, a single compressor on the trichloroethylene is capable of maintaining this temperature or, if desired, it can be used to effect additional cooling. In the meantime, the condenser coils can be cooled to below −50° in approximately 15 min.

For the drying stage, the capabilities of the heating and vacuum system are considered. Generally, it is common practice to evacuate a chamber to a low pressure before applying heat to the shelves. But if it is possible to reduce the pressure in the chamber to below 100 μ in less than 10 min., as illustrated in Fig. 4, it is apparent, from a consideration of the curves in Fig. 5, that heat can be applied simultaneously with the vacuum. After 10 min. of heating, the trichloroethylene temperature is increased by approximately 15°, and there should be no risk of overheating before a sufficiently low

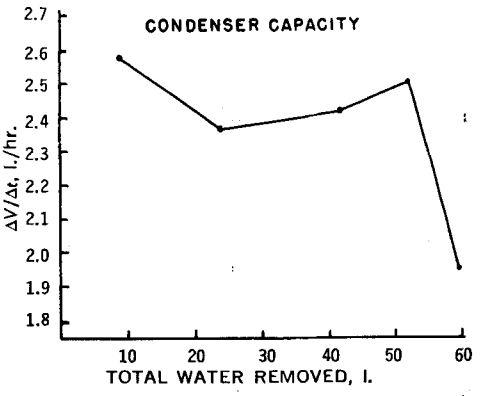

Figure 7—*A plot of the rate of sublimation as a function of total ice sublimed.*

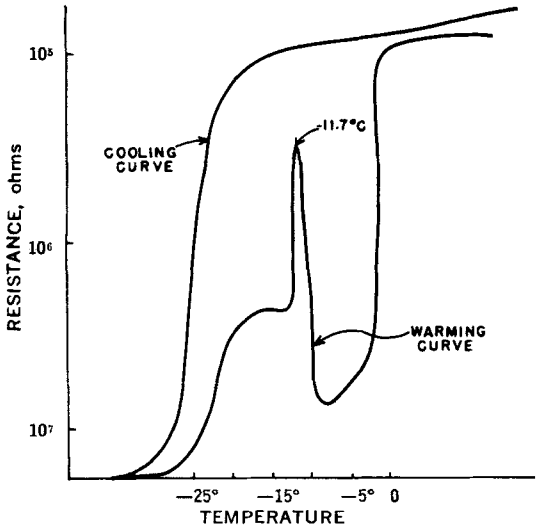

Figure 8—*Cooling and warming curves for a 0.3 molal methylphenidate HCl solution (0–10⁸-ohm decade).*

pressure is attained in the chamber. In fact, if a product necessitates cooling to a low temperature because it exhibits considerable supercooling, it might be advisable to initiate the heating phase 5–10 min. before applying vacuum.

Vaporization Rate and Condenser Capacity—The vaporization rate from pure ice and frozen solutions was determined by measuring the removal of water from crystallizing dishes during a drying cycle. The chamber was loaded with 20 l. of water or 10% mannitol solution by introducing 300-ml. quantities into the dishes and then interrupting the drying cycle at certain times during the period of maximum drying to measure the water remaining. A pressure–time curve, similar to that in Fig. 9, was used to determine the time periods for interruption of the cycle. For each subsequent time period, the water that was vaporized was replaced in the dishes. A plot of the quantity of water remaining as a function of time is illustrated in Fig. 6. The vaporization rate is almost linear during the primary drying stage; the maximum rate that can be expected during the drying cycle, using the indicated conditions, is approximately 2.43 l./hr. The open circles represent points obtained during the drying of a frozen 10% solution of mannitol; the rate of vaporization is similar to that for pure ice. This is due to the fact that for pure ice, although it has a low coefficient of thermal conductivity, the vapor flow is not obstructed by a front of dry material, while for the frozen mannitol solution, which has a higher coefficient of thermal conductivity, the obstruction to vapor flow continually increases as drying proceeds.

The capacity of the condenser is illustrated in Fig. 7, where the amount of water vaporized over specific intervals of time is plotted against the total amount of water sublimed. The capacity of the

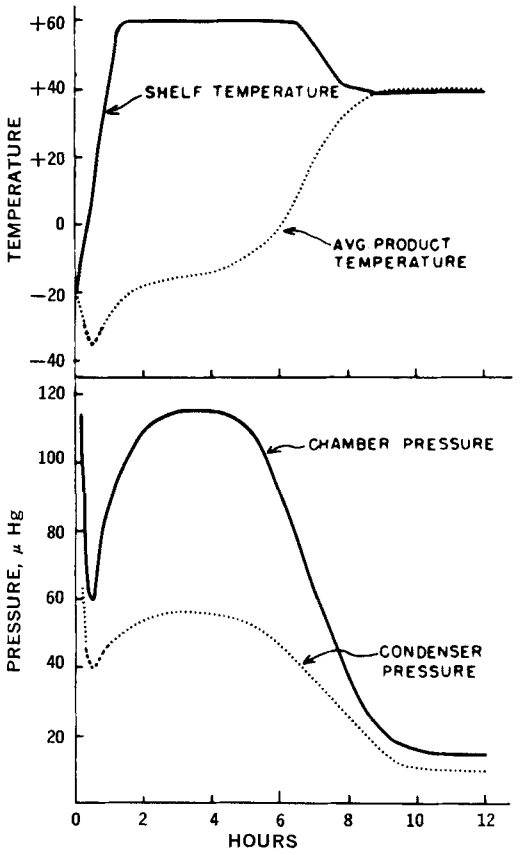

Figure 9—*Temperature–time and pressure–time curves characteristic of the drying cycle for methylphenidate HCl.*

condenser is in excess of 60 l., and an efficient rate of vaporization is maintained up to approximately 52 l.

Defrosting of Condenser Coils—With approximately 60 l. of water frozen on the condenser coils, the experiment to determine the condenser capacity was terminated and the coils were defrosted. Table II lists the details of the defrosting cycle. From Table II, it can be seen that it is possible to defrost 60 kg. of ice from the coils and have the condenser ready for another run within 1 hr.

Sterilization of Chamber—During sterilization of the chamber, spore strips of *Bacillus stearothermophilus* were located on each shelf and below the bottom shelf. Sterilization consisted of injecting steam into the chamber and allowing a gauge pressure of from 12 to 15 p.s.i. to be maintained in the chamber for a 30-min. period. Culturing of the spore strips on trypticase media showed no discernible growth after incubation for 1 week. The sterilization schedule is illustrated in Table III.

Table IV—Details of Lyophilization Cycle for Methylphenidate HCl

Hours	Shelf Temperature	Average Product Temperature	Pressure Differential (Chamber–Condenser), μ	Remarks
0	20°	25°	—	Both compressors on trichloroethylene shelf refrigeration
1	−20°	−17°	—	Condenser compressor switched to freeze trap
1.5	−20°	−20°	—	Vacuum applied; heat applied at a rate of 1°/min.
1.75	−5°	−33°	40	
3	60°	−22°	90	
5	60°	−15°	95	
6	60°	−11°	90	
7	60°	−5°	85	
8.5	60°	11°	25	Heat reduced to 40° at rate 0.25°/min.
10.5	40°	41°	13	
20	40°	40°	8	Run terminated

Table V—Details of Lyophilization Cycle for an Antibiotic Preparation

Hours	Shelf Temperature	Average Product Temperature	Pressure Differential (Chamber–Condenser), μ	Remarks
0	23°	27°	—	Both compressors used to cool samples to −20°
1	−20°	−20°	—	One compressor switched to condenser
1.25	−20°	−20°	—	Vacuum and heat applied; temperature increased at 1°/min. to 40°
2.5	40°	−22°	105	
6	40°	−12°	90	
8	40°	−8°	50	
12.25	40°	20°	20	Temperature increased at a rate 0.5°/min. to 60°
14.5	58°	42°	8	
16.75	60°	55°	5	Temperature decreased to 40°
21	40°	41°	5	Run terminated

DEVELOPMENT AND PROGRAMMING OF CYCLES

Prior to the development of a lyophilization cycle, of course, is the development of a suitable formulation. The optimum formula will permit the overall cycle to be carried out in the least amount of time, while providing a stable and efficacious product which contains a low moisture content, undergoes rapid reconstitution, and possesses the desired appearance. The potency of many pharmaceutical agents is of such a magnitude that relatively small amounts are required for the lyophilized injectable dosage form. Therefore, the need for a suitable filler or bulking agent is often indicated. Unfortunately, the list of materials to select from for this purpose is rather limited; even more important, selection of an agent is usually on an arbitrary basis.

For this paper, it will suffice to establish the importance of selecting the proper additives and to proceed with describing the development and programming of a cycle for an existing formula. For this purpose, three examples were selected, each representing or containing a relatively highly soluble medicament. Although this selection does not do justice in representing the many types of products being processed as lyophilized dosage forms, it demonstrates the approach for designing a cycle.

The first example is a drug intended for administration at a concentration of 10 mg./ml. Although the solution to be freeze dried contained 34 mg./ml. of active ingredient, a filler was required to add stability to the finished cake. A quantity of mannitol equal to that of the active substance was added. The eutectic temperature of the solution was determined by temperature–resistivity measurements (6), and Fig. 8 shows the conductivity behavior of this product during cooling and warming. In the liquid state, the resistivity of the solution is very low; but when solidification occurs, the resistivity increases sharply. The lowest temperature at which liquid can exist is the eutectic temperature. Since it is difficult to determine eutectic temperature during freezing because of supercooling, the warming curve is used. For methylphenidate HCl, the eutectic temperature was found to be −11.7°. During the freezing stage, the degree of supercooling should be considered when deciding to what temperature the samples are to be lowered to ensure complete solidification. Qualitatively, this information can be obtained from the cooling curve. For methylphenidate HCl, complete solidification occurred at −20°.

With a knowledge of the eutectic temperature and the supercooling characteristics of the product, a preliminary run consisting of one full tray of vials was then conducted. Temperature and pressure curves for the drying cycle are illustrated in Fig. 9. With the circulating fluid temperature set at 60°, all the probed samples passed through 0° within 6.5 hr. The heat was lowered gradually to 40° and allowed to remain at this temperature until the run was terminated. From the temperature and pressure curves, it can be seen that maximum drying took place between 1 and 6 hr. The max-

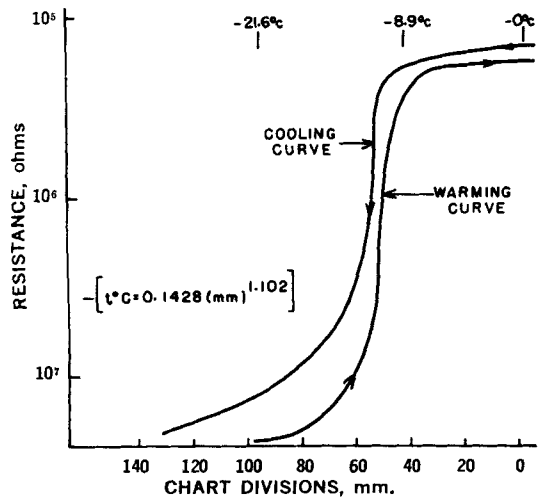

Figure 10—*Conductivity behavior during the freezing of 30% antibiotic solution and thawing of the frozen mass.*

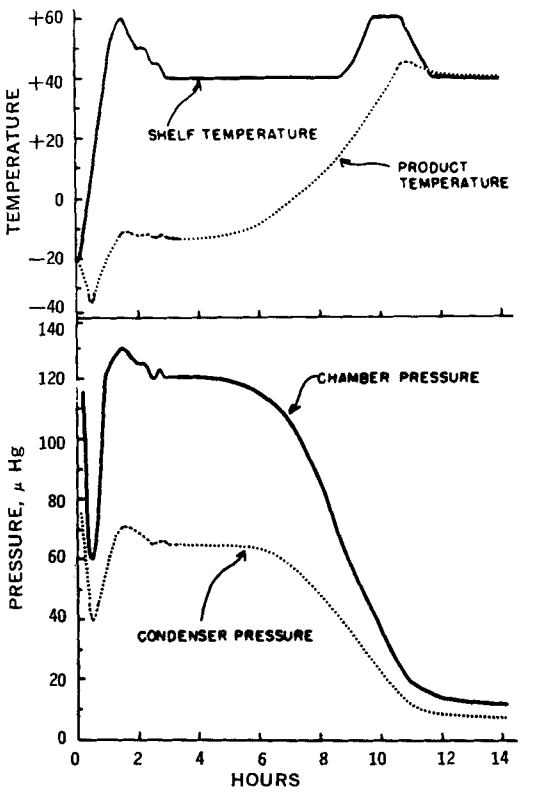

Figure 11—*Temperature–time and pressure–time curves characteristic of the drying cycle for an antibiotic preparation.*

Table VI—Details of Lyophilization Cycle for Pralidoxime Chloride

Hours	Shelf Temperature	Average Product Temperature	Pressure Differential (Chamber–Condenser), μ	Remarks
0	26°	26°	—	Both compressors on shelf refrigeration
1.5	−40°	−11°	—	
2.5	−40°	−35°	—	Compressor switched to condenser refrigeration; cam temperature set at −35°
2.75	−35°	−35°	—	Vacuum and heat applied with temperature set at 14°
6	14°	−22°	30	
20	14°	−20°	30	
40	14°	−20°	30	
52	14°	2°	20	Temperature increased at rate of 2°/hr. up to 24°
58	24°	15°	10	
64	60°	55°	8	Temperature lowered to 40°
66	40°	43°	5	Run terminated

imum vapor pressure difference between chamber and condenser occurred between 2 and 5 hr., with the chamber pressure reaching a minimum value of 15 μ after 10 hr. The leveling off of the product temperature below the eutectic point during the primary drying phase was an indication that the heat applied was not excessive.

With a knowledge of the drying characteristics of the product obtained from the temperature and pressure curves, a full chamber run of about 3500 vials (16 trays holding a total volume of 10.36 l. of solution) was made using a programmed drying cycle; the details are summarized in Table IV. Both compressors were put on shelf refrigeration to freeze the samples quickly. When the temperature of the lowest sample reached −20°, a specially cut plastic cam was attached to the programmer and the condenser compressor was switched to the freeze-trap. Within 30 min., all the samples were equilibrated at −20° and the condenser was at −55°. The plastic cam was designed to allow for a 1° rise per minute from −20 to 60° at the onset of the drying cycle.

Although the product temperature was at 40° after 10 hr., the product was allowed to remain in the chamber for an additional 10 hr. before terminating the run because the production operation is on a single shift basis. Moisture determination on 16 samples (one vial from each tray) gave results varying from 0.15 to 0.65%.

The cycle for the first production-size batch can then be programmed in the production lyophilizer by using the plastic cam designed and tested in research and development on the pilot plant lyophilizer. Slight modifications may be required at times to optimize the cycle further for the production-size batches but this is to be expected when scaling up an operation (8).

The second example is an antibiotic preparation, intended for freeze-drying as a 30% solution. Because of the high solid content, there is no need for a bulking agent. From the temperature–resistivity curves presented in Fig. 10, the eutectic point was found to occur at −8.9° and only negligible supercooling was encountered, with complete solidification at approximately −10°.

The temperature and pressure curves obtained from a preliminary run are illustrated in Fig. 11. The curves demonstrate the necessity of maintaining a temperature of 40° to eliminate the risk of melting the samples. With the drying temperature set initially at 60°, the product temperature increased steadily to −11°. Since it is desirable to hold the drying temperature at least 4–5° below the eutectic point, the trichloroethylene temperature was lowered, stepwise, until the product temperature was leveled at −13°. After the major drying had taken place and the product temperature was above 10°, the shelf temperature was increased to 60°.

On the basis of the temperature and pressure curves from the preliminary run, a drying cycle was designed for a full chamber load of 2400 units, each containing 4.5 ml. of solution. The details of this run are included in Table V. Moisture determination varied from 0.4 to 0.85%.

The third example is pralidoxime chloride, which has a eutectic temperature of −10.8° and exhibits a considerable degree of supercooling. From the temperature–resistivity curves presented in Fig. 12, complete solidification was not effected until a temperature below −30° was reached. Because of the high dosage, 5.0 g./20-ml. vial, a supporting material was not needed.

The preliminary run indicated that the drying temperature must be kept low due to the formation of a resistant film on the surface of the cake during the early stages of drying. The film obstructs

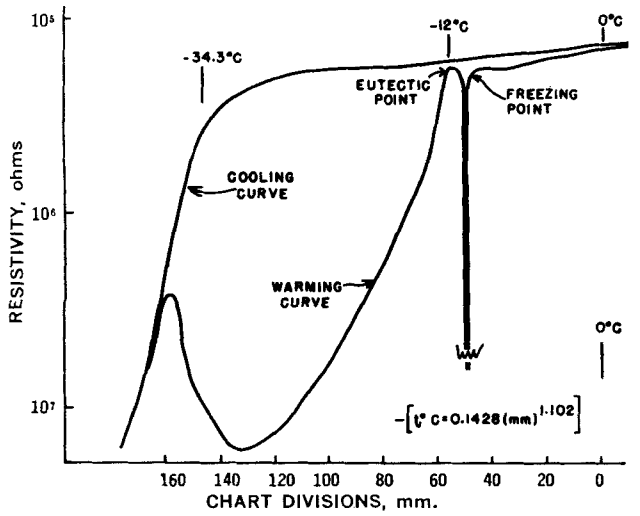

Figure 12—*Cooling and warming curves for a 2.9 M solution (50% w/v) of pralidoxime chloride.*

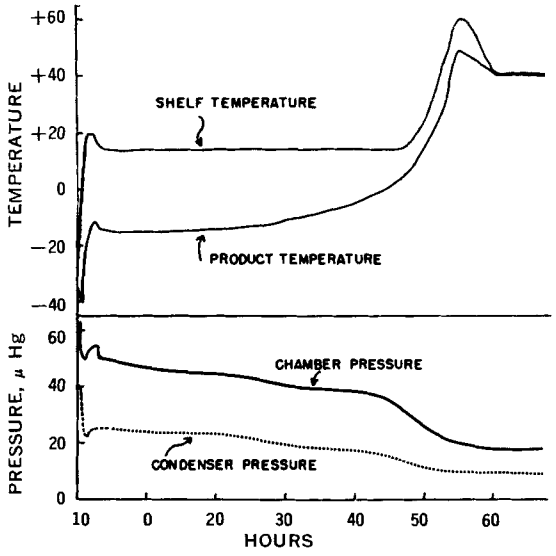

Figure 13—*Temperature–time and pressure–time curves characteristic of the drying cycle for pralidoxime chloride.*

vapor flow; as the drying front descends, this obstruction to vapor flow increases. If too much heat is applied, the sample will liquify. Figure 13 illustrates that, for this product, it was necessary to maintain a low drying temperature throughout the primary drying phase.

With the drying temperature set at 20°, the product temperature rose to within half a degree of the eutectic point. The drying temperature was lowered to 14°, and the product temperature dropped to −15°. The temperature of the product remained essentially constant for over 20 hr. before it began to rise gradually. However, it took over 45 hr. before the product passed through zero. The drying temperature was increased gradually to 60° and subsequently lowered to 40° when the product temperature passed through 40°. From the curves in Fig. 13, it was decided that the initial drying temperature should be no higher than 16°.

A full chamber load consisting of 1500 units, each containing 15 ml. of solution, was frozen to −35°, and the cycle proceeded as summarized in Table VI. With this product, it was necessary to run the cycle beyond 60 hr. to obtain a 100% acceptable yield. Moisture determination on the dried samples ranged from less than 0.1 to 0.25%.

An example for a poorly soluble drug has been omitted because eutectic temperatures for such medicinals are usually slightly below 0°, and the degree of supercooling is generally negligible. In such cases, the design of a cycle is dependent on the properties of the additive or bulking agent employed and not on the properties of the drug. A knowledge of the thermal conductivity of the frozen mass would be another refinement in the design of the freeze-drying cycles.

SUMMARY

A pilot plant lyophilizer was designed to permit versatility for the research and development of lyophilization cycles. The unit contains the necessary instrumentation to control temperatures accurately and to monitor temperature and pressure.

The equipment and instrumentation were described, and a procedure employed for evaluating the various systems comprising the unit was outlined. An explanation and examples were given to illustrate how the information obtained could be utilized to the greatest advantage in conducting preliminary studies and programming cycles.

Through the utilization of temperature–resistivity measurements obtained during preliminary screening and temperature–time and pressure–time curves from the initial freeze-drying runs on the pilot plant unit, a procedure was developed for programming drying cycles for production equipment.

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NOTES

Spectrophotometric Determination of Anthramycin

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Abstract □ A quantitative UV spectrophotometric method for the determination of a new antitumor antibiotic, anthramycin, is described. The method is shown to be applicable for the determination of anthramycin in fermentation beers of *Streptomyces refuineus* var. *thermotolerans* (NRRL 3143) and in various preparations containing this antibiotic.

Keyphrases □ Anthramycin—spectrophotometric determination □ *Streptomyces refuineus* var. *thermotolerans*—spectrophotometric determination of anthramycin in fermentation beers □ Fermentation beers—spectrophotometric determination of antibiotic, anthramycin

Anthramycin (I*a*), an active antitumor antibiotic, produced by *Streptomyces refuineus* var. *thermotolerans* (NRRL 3143), was recently isolated in the form of its crystalline methyl ether (I*b*) and as anhydroanthramycin (I*c*) (1, 2).

Anthramycin possesses a characteristic UV spectrum essentially identical with that of anthramycin methyl ether when measured in methanol (Fig. 1). Studies showed that the UV spectrum of anthramycin methyl ether conforms to Beer's law at concentrations of 2.0–10.0 mcg./ml. These data were used to establish an assay procedure for the quantitative determination of anthramycin based on the evaluation of UV absorption measurements.

Anthramycin can also be determined by *in vitro* biological assay, *e.g.*, *versus Bacillus* sp. TA (NRRL-B-3167).

EXPERIMENTAL AND RESULTS

Determination of Standard Curve—Anthramycin methyl ether (10.0 mg.) was dissolved in methanol and brought to 100 ml. in a volumetric flask. This stock solution was then used to prepare 10 different concentrations of anthramycin methyl ether in the range of 2–10 mcg./ml. Absorbances were read in the Beckman DU spectrophotometer at 335 nm. Figure 2 shows UV absorption curves of anthramycin and its methyl ether in methanol.

Ethanol and *n*-butanol may also be used in place of methanol.