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Development of freeze-drying cycles for pharmaceutical products using a micro freeze-dryer

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Abstract

This paper aims to investigate how a small-scale freeze-dryer can be used for process design. The system encompasses a temperature controlled metallic ring that surrounds a small batch of vials, in contact with the external vials through removable thermal conductors. The temperature of the ring can be modified to keep a constant difference with the temperature of one or more vials of the batch. In this paper an extensive validation of the system is given, considering 10% w/w sucrose and 5% w/w mannitol solutions, processed in different types of vials (6 R and 20 R) and in different operating conditions. The micro freeze-dryer was also shown to be able to provide accurate estimates of the overall heat transfer coefficient from the shelf to the product in the vials (K_v) and of the resistance of the dried cake to vapor flux (R_p): both values appeared to be very close to those obtained for the same case studies in a pilot-scale unit. Finally, the use of the micro freeze-dryer to control product temperature and drying time values to simulate a pilot-scale unit was addressed, thus demonstrating the adequacy of this system for process scale-up.

Keyword

Freeze-drying, pharmaceuticals, micro freeze-dryer, mathematical modeling, processing.

Introduction

Freeze-drying is a key step in the production process of many pharmaceutical products, as it allows stabilizing drugs produced as liquid solutions, through the removal of the solvent at low temperature.[1-4] The process is particularly important for biopharmaceuticals, whose market is rapidly growing (in the last four years, over 40 new biopharmaceuticals were approved, representing about 30% of the new drugs approved[5-6]) as these molecules are highly unstable in the liquid phase.[7-9] Quite recent data published by LaTorre-Snyder[10] point out that 16 of the top 100 drugs (based on sales) are freeze-dried. Therefore, it is of outmost importance the development of new systems that allow fast and efficient development of a freeze-drying process for a given drug, thus shortening the time-to-market and providing benefits to the patients.

Freeze-drying does not significantly impair the critical quality attributes of the product only when the temperature of the product remains below a threshold value, that is a characteristic of the formulation being processed. In the case of amorphous products, the goal is to prevent the collapse of the dried cake (thus obtaining an "elegant" product, that can be rapidly reconstituted), while in the case of crystalline products the goal is to remain below the eutectic temperature, to avoid melt-back.[11-15] Besides, the time required to completely remove the water has to be minimized, to maximize the plant productivity, a target value of residual moisture has to be reached at the end of the process, to maximize the shelf-life, and the sublimation flux has to be compatible with the freeze-dryer duct features, to avoid the occurrence of sonic flow[16,17], and with the condenser capacity. The operating conditions, namely the temperature of the heating fluid (T_{fluid}) and the pressure in the drying chamber (P_c), have thus to be carefully selected. During the primary drying stage, when ice sublimation occurs, this is crucial as the threshold temperature is lower and the sublimation flux is higher.

Several approaches may be used to get this result, and all of them require carrying out

several experiments with the drug. This is obvious when a design of experiment approach is used to assess the effect of T_{fluid} and P_c on product temperature, sublimation flux and drying time. In case mathematical modeling is used for *in silico* process simulation and design space calculation, several experiments are as well needed to estimate the values of model parameters.[18-20]

Experimental investigation is time consuming and expensive, since it has to be carried out processing the active drug and not just the excipients. Beside the true freeze-drying process, batch preparation/loading/unloading, condenser defrosting, equipment sterilization, and other operations have to be considered when evaluating the time required for a single experiment. The cost and the availability of the drug is another important concern. This motivates the development of new technological solutions for experimental investigations.

A possible solution is represented by processing a small number of vials in the lab or pilot-scale unit used for process design. This way, valuable drug product is saved, as well as the time for batch preparation. However, the results obtained when processing a small-scale batch, e.g. drying time, may be quite different from those achieved processing a full (larger scale) tray batch. This is mainly related to the difference of transferring heat to the product: in a large batch, most of the vials (the "central" ones) are heated by the heating fluid flowing in the shelf, and a small fraction of the batch (about 1-5%, the "edge" vials) are heated also by radiation from chamber walls and, above all, as recently evidenced by Scutellà and coworkers, by conduction in the gas that surrounds the vials.[21,22] In addition, edge vials are missing the inter-vial heat transfer mechanism created by the surrounding vials. The result is that in the edge vials drying time is shorter than in central vials, and product temperature is higher. In a small-scale batch most of the vials are actually edge vials: e.g. in a batch of 19 vials, arranged according to a hexagonal array in a compact way, 7 vials are in central position, while 12 are edge vials. As a consequence of this, the drying time of the small batch will be shorter than that

achieved in a larger batch, and the mean temperature of the product will be higher.[23] This motivated the development of small-scale (micro) freeze-dryers, where few vials containing the drug are processed.[24] The main goal of these units is to obtain homogeneous drying conditions, as close as possible to those of the central vials of a pilot/commercial units, in such a way that drying time and product temperature are representative of the larger units. The possibility of replicating in the micro freeze-dryers the evolution of the product in the edge vials is yet another objective, as the product in these vials exhibits the highest temperature.

Obeidat and coworkers[25] proposed a system where the temperature of the chamber wall can be modified aiming to control the heat received by the edge vials. Looking for better results, they modified this system by introducing in the chamber a metallic cylindric device, whose temperature could be arbitrarily modified: also in this case they acted on the amount of heat transferred to the edge vials by radiation (or, at least, on a part of this), but they did not affect the heat transferred through conduction in the gas (that is even more important than radiation, as shown in Refs. 21 and 22). The system was tested with 7 (20 mL) vials and, in any case, an edge vial effect could be observed, and batch uniformity with respect to temperature appeared to be perfectible.

A different system was proposed by Thompson et al.[26] and investigated by Goldman et al.[27] and by Fissore et al.[28]. This device (MicroFD®) uses a completely different approach to get uniformity (of temperature and drying rate) in the small-scale batch, minimizing (and even cancelling) the edge effect. In this system, the batch of vials is surrounded by an aluminum ring in contact with the external vials of the batch. Removable thermal conductors are used to arrange different types of vials, e.g. 20 R vials[27], 6 R vials[28], and others, to ensure the contact between the edge vials and this external ring system. The goal of this ring is to mimic the presence of an additional row of vials where sublimation is occurring, in such a way that the edge vials of the small-scale batch will behave as central vials of a larger batch. Product

temperature in some vials of the batch is measured through thin thermocouples, and the temperature of the ring (LyoSim®) is set on the basis of these measurements. In the present configuration of the micro freeze-dryer a control system acts to maintain a constant offset temperature between the ring and the product (defined as the difference between the temperature of the ring and that of the product in the vials). Goldman et al.[27] considered a batch of 7 (20 mL) vials, and pointed out that it was possible to reproduce in the micro freeze-dryer the drying conditions of the edge or of the central vials of a pilot-scale unit. Fissore et al.[28] considered a batch of 19 (10 mL) vials, evidencing the role of the ring in obtaining uniform drying conditions in the micro freeze-dryer.

This paper aims to deepen the study focusing on three main issues:

(i) An extensive experimental study of the role of the ring to get uniform drying conditions, focusing on the effect of the temperature offset, considering different products and types of vials with respect to previous studies;

(ii) The use of the micro freeze-dryer to estimate the overall heat transfer coefficient from the shelf to the product in the vials (K_v) and of the resistance of the dried cake to vapor flux (R_p), in such a way that it becomes possible to evaluate *in silico* product temperature, sublimation flux and drying time for the same case study in a pilot/commercial-scale unit;

(iii) The use of the micro freeze-dryer to get values of product temperature and drying time close to those of a pilot/commercial-scale unit. In this framework it has to be taken into account that drying time is not the same in all the vials of the batch and, thus, the focus should be on the central vials, those lower in temperature. With respect to product temperature, the uncertainty of temperature measurement, and also of thermocouple positioning, beside the non-uniformity of product temperature even in vials of the same group, has to be considered when evaluating this result. This means that our goal is to get an acceptable equivalence of product temperature in the two units (i.e. a maximum difference

in product temperature lower than a given threshold, e.g. 1°C), and an acceptable equivalence of the batch drying time (i.e. a maximum difference in drying time lower than a given threshold, e.g. 1 h).

By this way it will be possible to properly asses the adequacy of the system for process design and optimization.

Materials and methods

Freeze-drying units

Experiments were carried out in two devices, namely the small-scale freeze-dryer MicroFD® and the pilot-scale freeze-dryer REVO® by Millrock Technology Inc. (Kingston, NY, USA). The first device has a chamber with a circular 6" diameter shelf, where the selected vials are loaded, together with the removable thermal conductors that ensure the contact between the external vials of the batch and the temperature-controlled aluminum ring (LyoSim®). The temperature of the ring is based on the mean temperature of the product during the process, ranging from -15°C to +15°C offset with respect to this value. The temperature of the shelves may range from -60°C to +60°C in the MicroFD® and from -70°C to +65°C in the REVO® freeze-dryer.

In both systems, we were able to monitor product temperature, using T-type thermocouples, and chamber pressure, using a capacitive (Baratron type) and a thermal conductivity (Pirani type) pressure gauge. The ratio between the signals of the two pressure gauges was used to identify the ending point of the primary drying stage[29] as this curve exhibits a constant trend during the primary drying stage and then, when the ice sublimation is almost completed, it decreases towards a lower asymptote. When the lower asymptote is achieved, the ice sublimation is completed, but experimental tests[29] pointed out that both the onset, the offset and the mid points of the decreasing part of the pressure ratio curve may be representative of

the ending point of the primary drying stage. Therefore, the whole curve will be evaluated in this study. An additional system was available in the micro freeze-dryer for process monitoring, namely AccuFlux®.[30,31] It consists in a thin film differential thermopile, placed in contact with the shelf and the bottom of the vials, that provides a direct measurement of the heat flux from the shelf to the vials.

Products and vials

Tests were carried out using sucrose and mannitol aqueous solutions. Both sugars were purchased from Sigma Aldrich (≥99.5%) and used as received. Solutions were filtered, after preparation, using a 0.2 μm PES membrane, and processed in either 6 R (3 mL per vial) or 20 R (5 mL per vial) tubing vials (Schott Pharmaceutical Packaging, Inc., Lebanon, USA).

As results concerning the homogeneity of the system when processing a 5% w/w sucrose solution in 6 R vials were discussed by Fissore et al.[28], here we focused on 5% w/w mannitol solutions processed in 6 R vials, and on 10% w/w sucrose solutions processed in 20 R vials, aiming to assess the effect of the product and of the vial type on the system performance. Experiments carried out in the micro freeze-dryer involved either 19 6 R (10 mL capacity) vials, or 7 20 R (20 mL capacity) vials. Experiments carried out in the REVO® freeze-dryer involved 84 vials arranged according to a hexagonal array (8 rows, containing either 11 or 10 vials, thus resulting in 33 edge vials and 51 central vials).

All vials were partially stoppered using an igloo stopper (NovaPure Chlorobutyl Igloo Stoppers, West Pharma, Exton, USA) apart from those where product temperature was measured, both in the micro freeze-dryer and in the REVO® freeze-dryers: in this case the stopper was replaced by a holder that enabled to carefully control (and maintain) the position of the T-type thermocouple used to measure product temperature. Both edge and central vials were monitored, placing the thermocouples in close contact with the vial bottom.

Model parameters calculation

Simple one-dimensional models, assuming negligible temperature and composition gradients in the radial direction, appear to be adequate to calculate the dynamics of the temperature of the product and the time required to complete the ice sublimation.[32] They assume that the heat flux to the product is proportional to the difference between the temperature of the heating fluid and the temperature of the product at the vial bottom (T_b):

$$J_q = K_v \left(T_{fluid} - T_b \right) \tag{1}$$

while the mass flux of water vapor from the interface of sublimation to the drying chamber is proportional to the water partial pressure difference between the interface of sublimation ($p_{w,i}$) and the drying chamber ($p_{w,c}$), being the latter almost coincident to the total pressure in the chamber (P_c) as the gas in the chamber is about 100% water vapor:

$$J_w = \frac{1}{R_p} \left(p_{w,i} - p_{w,c} \right) \tag{2}$$

The one-dimensional model here used[33] is based on the energy balance at the sublimation interface:

$$J_q = \Delta H_s J_w \tag{3}$$

and on the mass balance for the frozen product:

$$\frac{dL_f}{dt} = -\frac{1}{\rho_f - \rho_d} J_w \tag{4}$$

Model equations may be solved once the values of the two parameters K_v and R_p are known. With respect to the overall heat transfer coefficient K_v, a gravimetric test may be carried out.[34] Fist, vials are filled with water, or with a solution, weighed and loaded into the drying chamber. Then, the product is frozen and, finally, chamber pressure and shelf temperature are set to the desired values for the drying test, thus promoting ice sublimation. Before the completion of the

ice sublimation, chamber pressure is restored to the atmospheric value and the weight loss (Δm) in each vial is measured. The total amount of heat received by the product (Q) is given by:

$$Q = \Delta m \Delta H_s \tag{5}$$

and it may be expressed also as:

$$Q = K_v A_v \int_0^{t_d} \left(T_{fluid} - T_b \right) dt \tag{6}$$

being t_d the drying duration of the gravimetric test and A_v the cross-section area of the vial. By equating eqs. (5) and (6), in case T_{fluid} and T_b are measured it is possible to easily determine K_v. The coefficient is mainly a function of the type of vial used and of the chamber pressure, being poor the effect of the heating fluid temperature.[34]

If no gravimetric run is carried out, K_v may be obtained at the end of a full primary drying cycle. In fact, at the end of the drying process Δm corresponds to the amount of water in each vial (m_0), and eq. (6) may be used to get K_v provided that Δm is replaced by m_0, and t_d corresponds to the duration of the primary drying stage.

The MicroFD® allows estimating K_v also in-line through AccuFlux®. This sensor is able to measure the heat flux from the shelf ($J_{q,shelf}$), that is a fraction of the heat received by a vial in a freeze-dryer. Using the measurement of product and shelf temperature it is thus possible to evaluate $K_{v,shelf}$:

$$K_{v,shelf} = \frac{J_{q,shelf}}{T_{fluid} - T_b} \tag{7}$$

In this case it is thus required to carry out a preliminary gravimetric test and to compare the total amount of heat received by the product to the heat flux from the shelf, to evaluate the correction factor for obtaining K_v from $K_{v,shelf}$.

With respect to the coefficient R_p, its determination requires: (i) carrying out a test with the product, (ii) measuring product temperature during this test, and (iii) carrying out a preliminary gravimetric test to get the value of K_v for the selected type of vial and operating

conditions. Calculations are straightforward: at a given time instant during the test with the product it is possible to get the value of J_q simply using eq. (1), as all variables and parameters are known and/or measured, and the mass flux J_w is simply calculated by means of eq. (3). Once J_w is known, taking into account that $p_{w,c} \cong P_c$ and that $p_{w,i}$ is a well-known function of T_i, then R_p can be calculated simply using eq. (2), being all variables and parameters known and/or measured. The same approach may be used both in the micro freeze-dryer and REVO® freeze-dryers in case the User aims to estimate R_p in line. As previously stated, it is possible to obtain K_v at the end of the sublimation test without any need to carry out a preliminary gravimetric test and, thus, also R_p can be evaluated, at the end of the primary drying step, without any need to carry out additional tests.

Design of experiments

The first group of experiments was carried out to assess the efficacy of LyoSim® to get uniform drying conditions in the micro freeze-dryer. Experiments were carried out using aqueous solutions containing either 10% w/w sucrose or 5% w/w mannitol. Sucrose-based solutions were processed in the 20 R vials, 5 mL per vial, while mannitol-based solutions were processed in 6 R vials, 3 mL per vial (as the drying of 5% and 10% w/w sucrose solutions in 6 R was investigated in Ref. 28).

Operating conditions in the micro freeze-dryer were set according to a 2^N design of experiments, where N, the number of parameters considered, is equal to 2, namely T_{fluid} and P_c. This particular type of design of experiments was selected as it allowed to greatly reduce the number of experiments needed to assess the effect of a certain operating parameter on the target variables, considering a “high” and a “low” value for each operating parameter. For the sucrose-based solution the values of heating fluid considered were -20°C and 0°C, while those of chamber pressure were 60 mTorr and 90 mTorr. For the mannitol-based solution the values of

heating fluid considered were 0°C and 10°C, while those of chamber pressure were 100 mTorr and 200 mTorr. The target variables considered are (i) the mean value of weight loss and its standard deviation in the whole batch, (ii) the difference between the mean weight loss in the edge and in the central vials, and (iii) the temperature trend in edge and central vials, all being representative of the homogeneity of the batch.

Product temperature was measured in 4 vials in each run, and the mean value was used to set the ring temperature: in case of the 7 20R vials batch we measured the temperature in the central vial, which is the reference one, and that in 3 of the edge vials, and calculated the mean value from all the measurements. The rational for this choice is that in case the batch is homogeneous, then the temperature exhibited by the vials in different positions has to be very close. Besides, it is possible to compare the measurements in vials in different positions to assess if the batch is really homogeneous. Experiments using just the measurement of the temperature of the central vial were also carried out, and no difference was observed with respect to the case of multiple temperature measurements. A similar analysis was carried out in Ref. 28 for the 6R vials, where it was shown that it was essentially irrelevant which thermocouples arrangement was used in the system, i.e. just in the central vials (with 6R vials we have 7 vials that may be considered as “central”) or in both central and edge vials.

With respect to the ring temperature, only negative values of the offset were investigated. The rational for this selection lies in the fact that it is necessary to counterbalance the extra-amount of heat reaching the edge vials, with respect to the central ones, to simulate central-batch conditions and, thus, heat has to be removed from the edge vials. This may be accomplished by using a ring temperature lower than the temperature of the product in the vials. The first offset value investigated was thus -1°C. Aiming to minimize the experimental effort, additional tests were carried out at -3°C and -5°C, thus neglecting -2°C and -4°C and lower values. These selected offset values were able to satisfactorily address the effect of this

parameter. It has to be remarked that in all tests the apparatus was able to keep the value of temperature offset with a maximum variation +/-0.2°C.

In all the tests the liquid solution was poured into the vials, then loaded into the chamber where the temperature of the heating fluid was set at +20°C for 20 minutes. Then, the set point of the heating fluid temperature was set at -40°C, with a cooling rate of -1°C/min. After 2 hours at -40°C, vacuum was started in the chamber and the set point of T_{fluid} was modified to the value desired for the drying test. In case of freeze-drying of mannitol-based solutions an annealing stage was carried out: when the temperature of the cooling medium reached the target value of -40°C, it was kept at this value for 10 minutes and then increased to-15°C in 25 minutes. The annealing temperature was maintained in the system for 2 hours and, then, again the temperature of the cooling fluid was brought to -40°C (in 25 minutes) and maintained for 1 hour at this value before moving to the primary drying stage.

The second part of the study was focused on the possibility of using MicroFD® to estimate K_v and R_p. Experiments previously carried out in the same operating conditions (type of vials, pressure, ring temperature offset) were compared, with the aim to evaluate the values of K_v, which is expected to be the same if the type of vials, the pressure and the ring temperature offset are not changed. In this framework the role of the ring temperature offset was investigated. Gravimetric tests were also carried out in the REVO® freeze-dryer, using the same type of vials and chamber pressure of the test in the micro freeze-dryer. These tests were done aiming to determine the mean value of K_v (and its standard deviation) for both central and edge vials, and to compare results obtained in the two pieces of equipment.

With respect to R_p, which is expected to depend mainly on the type of product and on the filling height, the trend observed in the micro freeze-dryer with the same type of product (10% w/w sucrose solution), processed in different operating conditions and even type of vial (6 R and 20 R), were compared. Similarly, the curve of R_p vs. L_d obtained in the REVO® freeze-

dryer with the same product was compared to those obtained in the micro freeze-dryer.

Finally, aiming to assess the possibility of obtaining in the micro freeze-dryer values of drying duration and product temperature representative of a pilot/commercial scale unit, a REVO® freeze-dryer in this case, product temperature measurements obtained with thermocouples, and pressure values measured by Pirani and capacitance gauges, in the two devices were compared. These experiments were carried out using 5% and 10% w/w sucrose solutions, processed in 20 R vials at 60 mTorr and 0°C.

Results and discussion

Study of the effect of LyoSim®

The first part of the study was focused on the extension of the validation of the efficacy of LyoSim® to get uniform drying behavior in the micro freeze-dryer. Preliminary results were published by Fissore et al.[28] about 5% and 10% w/w sucrose solutions processed in 6 R (10 mL) vials. We used a simple gravimetric test and evaluated both product temperature trends in edge and in central vials, and the weight loss in each vial of the batch after 6 hours of drying. For each of the operating conditions of the design of experiments considered, three values of the temperature offset were considered. With respect to product temperature, it is certainly required that the trends in edge and in central vials are as close as possible. With respect to the sublimation rate, i.e. the weight loss in each vial during the test, the target is to get a mean value of weight loss in edge vials as close as possible to that of the central vials and, in general, a standard deviation of the weight loss in the whole batch close to the values typical of a large-scale unit. With respect to this, data of the distribution of K_v may be found in the literature[35,36] and may be considered representative of the non-uniformity of drying rate as the sublimation flux is proportional to the heat flux and the driving force for the heat transfer calculation is assumed to be the same in all the batch. Values ranging from 3 to 7% of the relative standard

deviation of K_v values are not unusual for a glass tubing vials batch[36], being even higher for molded vials. Fissore et al.[28] found that a temperature offset ranging from -3°C to -5°C was able to provide good results with respect to drying rate homogeneity and, in particular, with -5°C also the temperature profiles in edge and central vials were in excellent agreement.

The study was extended, using the same approach, considering at first a different type of solute, namely mannitol, in the same type of vial (6 R), and then the freeze-drying of a product (the 10% w/w sucrose solution) in a different type of vial (20 R). By this way, it was possible to assess if in these case studies the system is also able to provide batch homogeneity and which is the optimal ring temperature offset value.

Results obtained for the 5% w/w mannitol solution are shown in Figure 1, showing the temperature profiles in edge and in central vials for two set of values of T_{fluid} and P_c, namely those resulting in higher (10°C and 200 mTorr) and lower (0°C and 100 mTorr) drying rates and product temperatures. In Table 1 the weight losses measured (i.e. the sublimation rate) for all of the operating conditions tested are presented. Focusing on the sublimation rate, it appears that when the sublimation rate is higher, namely at 10°C (both 100 and 200 mTorr), the values of standard deviation of the distribution of the sublimation rate are very close for all ring temperature offset values investigated, while for the test carried out at 0°C (both 100 and 200 mTorr) a minimum of the standard deviation is clearly identified for a ring temperature offset of -3°C. This value may thus be indicated as the optimal one when processing the 5% w/w mannitol solution in 6R vials. This statement is also confirmed by the evaluation of the temperature profiles shown in Figure 1: for a ring temperature offset of -3°C the agreement between the edge and central vials temperature profiles is excellent for both couples of operating conditions evaluated (graph B and E). In any case, it has to be highlighted that when evaluating the temperature profiles the measurement error of the thermocouples has to be taken into account: when using T-type thermocouples this error may be ±1°C. Thus, apart from results

shown in graph D, in all other cases the difference between the temperature profiles in edge and central vials falls inside the uncertainty range of the measurement system.

The investigation was then moved to a different type of vial, namely 20 R type (20 mL) using a 10% w/w sucrose solution. In this case only 7 vials are loaded into the micro freeze-dryer. As in the previous case, all the results obtained concerning the weight loss in the gravimetric tests are shown in Table 2, while the temperature trend in the central vial and in one of the external vials for the test at -20°C and 60 mTorr are shown in Figure 2. Results concerning the drying rate evidence that, in this case, the values of standard deviations obtained are lower than those obtained when processing the 6 R vials: this is reasonable if we consider that when using the 6 R vials the batch is composed by 19 vials (7 in the central part and 12 at the edge), while when processing the 20 R vials only 7 vials are loaded (1 in the central position and 6 at the edge). Therefore, the contribution of the central vial to the non-uniformity of the system is lower. Considering the values of ring temperature offset investigated in this case, the definition of the optimal value is not so easy: looking at the sublimation rate, the optimal value at 60 mTorr appears to be -3°C, as at -20°C and 90 mTorr, while it should be increased at -1°C for the high temperature-high pressure conditions tested. Looking at the temperature trends, here shown just for the low temperature-low pressure case, the optimal ring temperature offset appears to be -1°C, although acceptable results are obtained also for -5°C (as the difference between the temperature values in edge and central vial is always below the uncertainty of the temperature measured by the thermocouple).

In conclusion, the lessons we learned are the following:

1. LyoSim® is able to get uniform drying behavior in both 6 R and 20 R vials, both for sucrose and for mannitol-based solutions (these solutes were considered in this study as they are representative, respectively, of amorphous and crystalline excipients, besides being the most frequently used drug stabilizer in a freeze-drying process);

2. When using the 20 R vials, ring temperature offset ranging from -1°C to -5°C were shown to provide acceptable results, taking into account the uncertainty of temperature measurement (when evaluating the difference between temperature of the product in the central and in the edge vials) and the usual degree of heterogeneity in a batch (when evaluating the distribution of drying rate values);
3. When using the 6 R vials, the ring temperature offset has to be optimized, in particular in case of low temperature cycles. In this case and offset value of -3°C appears to be adequate for the mannitol solutions.

If we compare these results with those shown in Ref. 28 for the sucrose solutions (5% and 10%) processed in the 6R vials we may evidence that the optimal value of the ring temperature offset depends on both the type of vial (6R or 20R), and on the type of product being processed. In fact, depending on the product a different sublimation rate is obtained in the system, and this implies a different rate of "heat consumption" in each vial. This affects the heat balance in the system and, thus, the rate of heat removal that has to be achieved with the ring to get a uniform batch. As a consequence, it is not unexpected that in case of 5% and 10% sucrose solutions processed in 6R vials the optimal ring temperature is different from that obtained when processing 5% mannitol solutions in the same type of vials, and is also different from that obtained when processing the sucrose solutions in a different type of vial (20R). In case of a product different from those previously considered, the simple gravimetric test previously described should be carried out to optimize the ring temperature offset, using values of the ring temperature offset in the range -1°C to -5°C as starting value for the analysis.

Determination of model parameters K_v and R_p

The interest in carrying out experiments in a small-scale freeze dryer is also to evaluate the overall heat transfer coefficient from the shelf to the vial (K_v) and the resistance of the dried

product to vapor flow (R_p). In this framework we have to investigate two issues: the first one is the effect of the ring temperature on the calculated values of K_v and R_p, and the second is to evaluate how close these values are to those obtained in a different unit as, obviously, the interest of the freeze-drying practitioner is to use the MicroFD® to get values of K_v and R_p representative of a larger equipment.

Figure 3 shows the values of K_v obtained using the gravimetric test described in the Materials and Methods section with the 20 R vials. Results are shown for all the operating conditions tested, namely chamber pressure (60 mTorr, upper graph, and 90 mTorr lower graph) shelf temperature (0°C and -20°C) and, evidently, the ring temperature offset. It has in fact to be taken into account that K_v is an effective heat transfer coefficient, that takes into account all the heat exchanged by the product (J_q), divided by a driving force given by the difference between T_{fluid} and T_b. Therefore, when manipulating the ring temperature offset we affect the heat exchanged between the freeze-dryer and the edge vials: this affect the value of K_v value of the edge vials and, in turns, the mean value of K_v. As it is expected, when chamber pressure is increased the value of K_v is higher (due to the higher thermal conductivity of the gas in the gap between the bottom of the vial and the shelf surface), while the influence of the temperature of the heating fluid is lower (being K_v higher for higher values of T_{fluid}). It is remarkable that, when modifying the ring temperature offset also the mean value of K_v changes, being lower for lower values of the offset, although for the range of offset values considered, -1°C to -5°C, the difference in the value of K_v is not so relevant, in particular at the lower temperature of the shelf heating fluid.

With respect to the values of R_p *vs* L_d Figure 4 shows the results obtained in the micro freeze-dryer for a 10% w/w sucrose solution processed in the same operating conditions (0°C and 60 mTorr) but in different types of vials (6 R and 20 R) and for different values of the temperature offset. It is well known that the resistance of the dried product to vapour flux

depends mainly on the type of product, on the freezing conditions, and on the thickness of the product itself. Therefore, using different types of vial should not result in any difference of the R_p *vs* L_d curve, provided that the same product and freezing conditions are used, and that no collapse or microcollapse occurred (and this is the target of every freeze-drying process). Figure 4 shows that the curves of R_p *vs* L_d for the sucrose solutions considered in this study in the 6 R and 20 R vials are almost overlapping. Moreover, the curves obtained are almost independent from the value of ring temperature offset. This is reasonable as, using the algorithm described in the Materials and Methods section, in all tests the true product temperature is measured with the thermocouple, and the specific measurement (that is clearly affected by the ring temperature offset) is used in the calculations. Results shown in Figure 4 evidences two important issues:

1. If we need just to evaluate the R_p *vs* L_d curve for a given product, processed in a vial whose K_v value was has been previously determined, we may use the MicroFD® (and the algorithm previously described) without any need to optimize the ring temperature offset, thus strongly saving time and raw material;
2. The previous experiment may be carried out in the 20 R vials, as the consumption of raw material is lower with respect to the 6 R vials (being required to load only 7 vials instead of 19 vials), and it is much easier to get uniform drying conditions.

The second issue related to the evaluation of K_v and R_p in the micro freeze-dryer is assessing if these values are close or not to those of a different unit, e.g. the REVO® freeze-dryer considered in this case. This is due to the fact that, obviously, *in silico* simulation of the process is aimed at optimizing the drying conditions in the pilot/commercial scale unit and not in the micro freeze-dryer.

Figure 5 compares the values of K_v obtained in the micro freeze-dryer for the 20 R vials and different values of the ring temperature offset with the mean value obtained in the REVO® freeze-dryer for the same type of vial and at the same pressure. Taking into account the

uncertainty range it appears that for the three values of ring temperature offset tested the mean value of K_v in the micro freeze-dryer falls in the range of values obtained in the REVO® freeze-dryer (D), and in case the ring temperature offset is -5°C, the agreement between the mean values obtained in the two pieces of equipment is very good. If we focus on central (E) and edge (F) vials in the REVO® freeze-dryer we may notice that the K_v value obtained in the central vials is quite close, taking also into account the uncertainty range, to those obtained in the MicroFD®. The mean value of K_v in the REVO® freeze-dryer (D) is obviously a "mean" value of those in the central (E) and in the edge (F) vials. Similar results were obtained for other values of chamber pressure (results not shown here for sake of brevity).

Figure 6 compares the R_p *vs* L_d curves for a 10% w/w sucrose solution processed in 20 R vials in the REVO® freeze-dryer, using several temperature measurements, thus resulting in several R_p *vs* L_d curves, with the curves obtained with the same type of vials in the micro freeze-dryer, for different values of ring temperature offset. In both freeze-dryers freezing conditions were the same. The agreement between the two groups of curves is excellent, thus showing that the R_p *vs* L_d curve obtained in the micro freeze-dryer is representative of the trend obtained in a larger-scale unit (provided, naturally, that the freezing conditions are the same, and that in both cases no collapse/microcollapse occurred).

We thus learned the following lessons:

1. If we carry out a gravimetric test in the micro freeze-dryer to get K_v, using ring temperature offset in the range -1°C to -5°C the value we obtain is close to the mean value of a larger-scale unit;
2. If we carry out also a test in the pilot/commercial scale unit to get the value of K_v it is possible to tune the ring temperature offset in the micro freeze-dryer to replicate with higher accuracy the product dynamics. This issue was discussed in Ref. 27 and, thus, is not further discussed here.

Equivalence between MicroFD® and a larger-scale freeze-dryer

Finally, in order to confirm the equivalence between the results obtained in the micro freeze-dryer and in a larger-scale freeze-dryer, a freeze-drying cycle was carried out till the end both in the MicroFD® and in the REVO® freeze-dryer. The case study is the 10% w/w sucrose solution, processed in 20 R vials at 0°C and 60 mTorr. Tests in the micro freeze-dryer were carried out for 4 values of ring temperature offset, adding -7°C to the three values (-1°C, -3°C and -5°C) previously considered. The upper graph shows the trend of the Pirani pressure signal, being the measure of the capacitance manometer at the set-point value (60 mTorr). The Pirani trend, or the ratio between the Pirani and capacitance sensor signals, may be used to assess the drying time: in this framework it is crucial to define the criteria to identify the ending point. It is in fact possible to use the onset, offset or mid points, as extensively discussed in Ref. 29, as well as to check when the difference between the Pirani and Baratron signals falls below a given threshold (e.g. 1 mTorr). If we consider the mid-point as representative of the ending point of the primary drying stage, we find a value of 12 h and 40 minutes for the REVO® freeze-dryer while in the micro freeze-dryer the calculated drying time are 12 h and 25 min, 11 h and 40 min and 11 and 25 min for the ring offset temperature values of -5°C, -3°C and -1°C respectively. A test with an offset equal to -7°C was also carried out and, in this case drying time is 13 h and 25 min. Therefore, when carrying out the experiment in the micro freeze-dryer the difference in the drying time calculated as mid-point of the Pirani curve ranges from 15 min to 2 h, being the difference negligible (15 min) for the value of ring temperature offset (-5°C) resulting in the best agreement between the K_v values in the two pieces of equipment. Similar conclusions are obtained using other criteria to define the ending point of the primary drying stage on the basis of the Pirani curve. In any case, taking into account that, to be sure that drying is completed in all the batch before increasing the heating fluid temperature to promote the water desorption

(secondary drying), freeze-drying practitioners wait until the difference between the Pirani and Baratron signals is lower that a target threshold, the drying time calculated in this way is not so much different in the case studies considered. In fact, this time is related to the drying of the coldest vial, which is for sure a “central” vial and, thus, the ring temperature scarcely affects its dynamics.

If we consider product temperature, the difference between the values measured in the REVO® freeze-dryer and in the micro freeze-dryer in central vials is very small in all cases (lower than 1°C), at least until the thermocouple measurement is representative of the temperature of the frozen product (i.e. until the final part of the first asymptote of the curve, reached after heating starts from the freezing temperature).

Previous conclusions were confirmed also for a test where a 5% w/w sucrose solution was processed, at 0°C and 60 mTorr, both in the micro freeze-dryer (just results obtained with the ring temperature offset of -5°C are shown in Figure 8) and in the REVO® freeze-dryer. As far as the Pirani curves are considered, the difference in the mid points is about 20 minutes, very close to the onset and offset points. Also in this case the agreement between the temperature profiles in the two units is excellent, as long as the thermocouples were still in ice inside the product and, thus, their reading is accurate. It is in fact well known that the temperature reading obtained through a thermocouple is reliable just in the first part of the drying stage, until a sharp change in the slope of the curve appears: this is due to the fact that the thermocouple is no longer in perfect contact with the bottom of the vial (placement error), or to the loss of contact between the thermocouple and the ice. In any case, this does not affect the quality of the results and the conclusions that may be obtained, as the reliable measurement is that of the first part of the trend, and as shown in Figures 7 and 8 the trends obtained in the MicroFD® and in the REVO® freeze-dryer differ for less than 1°C, that is the uncertainty of a T-type thermocouple.

In conclusions, the lessons we learned are the followings:

1. If we carry out a freeze-drying cycle in the MicroFD® using a ring temperature offset value in the range -1°C to -5°C it is expected that product temperature is very close to that obtained in the REVO® freeze-dryer for the same operating conditions (product, type of vial, freezing and drying conditions). With respect to drying time, the value obtained in the micro freeze-dryer can be close to that obtained in the REVO® freeze-dryer.
2. If the goal of the study is to get an accurate estimate of the drying time in the MicroFD®, then the offset ring temperature has to be optimized in such a way the heat transfer conditions to the product are as much close as possible to those of the REVO® freeze-dryer. This requires carrying out few gravimetric tests in the micro freeze-dryer, modifying the offset ring temperature in each test and stopping the run 4-6 hours after the onset of the primary drying (and, in any case, before the completion of the ice sublimation), and one gravimetric test in the pilot-scale unit, where the duration of the run is the same of that in the MicroFD®. After each test, in both freeze-dryers, the weight loss in each vial has to be measured, and the optimal offset temperature is evaluated looking for the best agreement between the mean weight losses in both units. Obviously, this approach is time consuming, and in most cases the results obtained according to the previous point are satisfactory.

Conclusions

The results of a large experimental investigation involving both a micro freeze-dryer (MicroFD®) and a pilot-scale unit (REVO®) have been presented and discussed in this paper. The goal was to assess if such micro freeze-dryer could be really helpful to freeze-drying practitioners facing the challenge of designing the process for a new drug. Results obtained with different products, type of vial, temperature of the heating shelf and pressure in the drying chamber evidenced that it is effectively possible to get in the micro freeze-dryer uniform drying

conditions (product temperature and drying rate). Additionally, it is possible to use the micro freeze-dryer to get the K_v values for the vial of interest close to that obtained in a larger unit, to get the R_p values for the product of interest also close to those obtained in a larger unit. Finally, it is possible to get drying time and product temperature values for a given formulation representative of those obtained in a larger freeze-dryer. When a preliminary optimization of the offset ring temperature of the micro freeze-dryer is carried out, by running one gravimetric test in the larger unit and few gravimetric tests in the micro freeze-dryer, the accuracy of the pieces of information obtained in the micro freeze-dryer is very high. Otherwise, if values of the offset ring temperature are not optimized, then the accuracy of the pieces of information obtained in the micro freeze-dryer falls inside the uncertainty range of the target variables. Further investigations will address the development of an automatic system for the in-line optimization of the ring temperature, thus saving time related to the optimization of this parameter. Besides, the analysis of the equivalence of the results obtained in the two units will be extended to other critical quality attributes, e.g. rehydration rate, cake structure, specific surface area, drug activity, although it is usually admitted that in case freezing conditions in two freeze-dryers are the same, and product temperature during primary drying is the same, then also the other critical quality attributes are equivalent.

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Declaration of interest: Role of the funding source

Millrock Technology, Inc, has supported the study by loaning the MicroFD® used in this study

to the research group of the Politecnico di Torino, and by helping the researchers to solve practical issues related to the experimental investigation.

Research data

Research data may be requested to the authors.

List of Symbols

A_v	cross-section area of the vial, m^2
ΔH_s	enthalpy of ice sublimation, J kg^{-1}
J_q	heat flux to the product, W m^{-2}
$J_{q,shelf}$	heat flux from the shelf to the product, W m^{-2}
J_w	mass flux, kg $s^{-1}m^{-2}$
K_v	overall coefficient of heat transfer to the product in the vial, W $m^{-2}K^{-1}$
$K_{v,shelf}$	heat transfer coefficient from the heating shelf to the product in the vial, W $m^{-2}K^{-1}$
L_d	thickness of the dried product, m
L_f	thickness of the frozen product, m
Δm	weight loss in a vial during a gravimetric test, kg
m_0	mass of water in a vial, kg
P_c	pressure in the drying chamber, Pa
$p_{w,c}$	water vapor partial pressure in the drying chamber, Pa
$p_{w,i}$	water vapor partial pressure at the interface of sublimation, Pa
Q	heat transferred to the product, J
R_p	resistance of the dried product to vapour flux, Pa h m^2 kg^{-1}
T_b	temperature of the product at the bottom of the vial, K
T_{fluid}	temperature of the heating fluid, K
t	time, s
t_d	duration of the gravimetric test, s

Greeks

ρ_d	apparent density of the dried product, kg m^{-3}

ρ_f	density of the frozen product, kg m^{-3}

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the MicroFD® for several values of the ring temperature offset (A: -1°C; B: -3°C; C: -5°C) and in the REVO® freeze-dryer (D: whole batch, E: central vials, F: edge vials) for a 20 R vials (0°C, 90 mTorr), The error bars correspond to one standard deviation.

Figure 6. Values of the resistance of the dried cake to vapor flux as a function of the thickness of the dried layer for a 10% w/w sucrose solution processed in 20 R vials at 0°C and 60 mTorr in the REVO® freeze-dryer (graph A), calculated from three temperature measurements, and in the MicroFD® (graph B) for different values of the ring temperature offset (solid line: -1°C; short dashed line: -3°C, dash-dotted line: -5°C).

Figure 7. Graph A: Comparison between the Pirani pressure measurements obtained in three tests carried out in the MicroFD® with different values of the ring temperature offset (black lines) and in the REVO® freeze-dryer (grey line). Graph B: Comparison between the temperature measured in a central vial in the MicroFD® with different values of the ring temperature offset (short dashed line: -1°C, short dash-dotted line: -3°C, dash-dotted line: -5°C, dashed line: -7°C) and in the REVO® freeze-dryer (solid line). Data refer to the freeze-drying of a 10% w/w sucrose solution processed in 20 R vials at 0°C and 60 mTorr.

Figure 8. Graph A: Comparison between the Pirani pressure measurements obtained in three tests carried out in the MicroFD® with a ring temperature offset of -5°C (black line) and in the REVO® freeze-dryer (grey line). Graph B: Comparison between the temperature measured in a central vial in the MicroFD® with a ring temperature offset of -5°C (short dashed line) and in the REVO® freeze-dryer (solid line). Data refer to the freeze-drying of a 5% w/w sucrose solution processed in 20 R vials at 0°C and 60 mTorr.

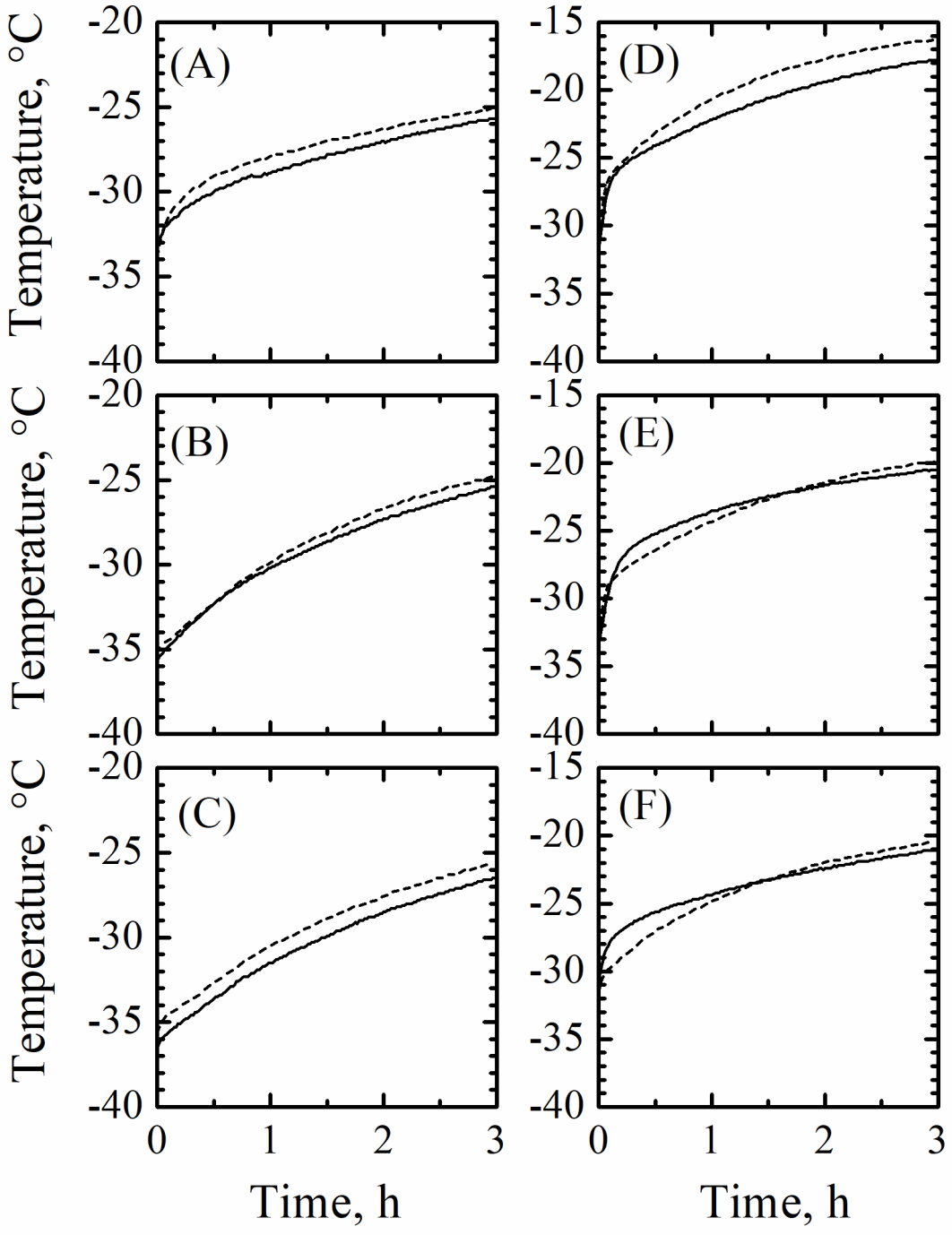
Table 1

Operating conditions	**temperature offset, °C**	**Δm, g**	**standard deviation**	**Δm external vials- Δm internal vials, g**
0°C, 100 mTorr	-1	1.06	6.56%	0.027
	-3	1.02	4.38%	-0.024
	-5	1.015	7.54%	-0.008
10°C, 100 mTorr	-1	1.30	3.26%	0.044
	-3	1.27	3.64%	0.072
	-5	1.24	3.28%	-0.012
0°C, 200 mTorr	-1	1.24	7.17%	0.034
	-3	1.19	3.71%	0.044
	-5	1.08	8.76%	-0.111
10°C, 200 mTorr	-1	1.48	4.74%	0.065
	-3	1.50	3.08%	0.042
	-5	1.48	3.27%	-0.044

Table 2

Operating conditions	**temperature offset, °C**	**Δm, g**	**standard deviation**	**Δm external vials- Δm internal vial, g**
-20°C, 60 mTorr	-1	1.42	6.64%	-0.015
	-3	1.35	5.13%	-0.126
	-5	1.27	5.21%	-0.109
0°C, 60 mTorr	-1	2.74	3.80%	0.085
	-3	2.71	2.66%	0.027
	-5	2.65	2.95%	-0.019
-20°C, 90 mTorr	-1	1.59	4.48%	0.029
	-3	1.43	4.08%	-0.041
	-5	1.41	6.14%	-0.174
0°C, 90 mTorr	-1	3.20	1.90%	-0.024
	-3	3.27	3.33%	-0.068
	-5	3.15	3.50%	-0.186

Figure 1


(A)
(B)
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Temperature, °C
Temperature, °C
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Figure 2

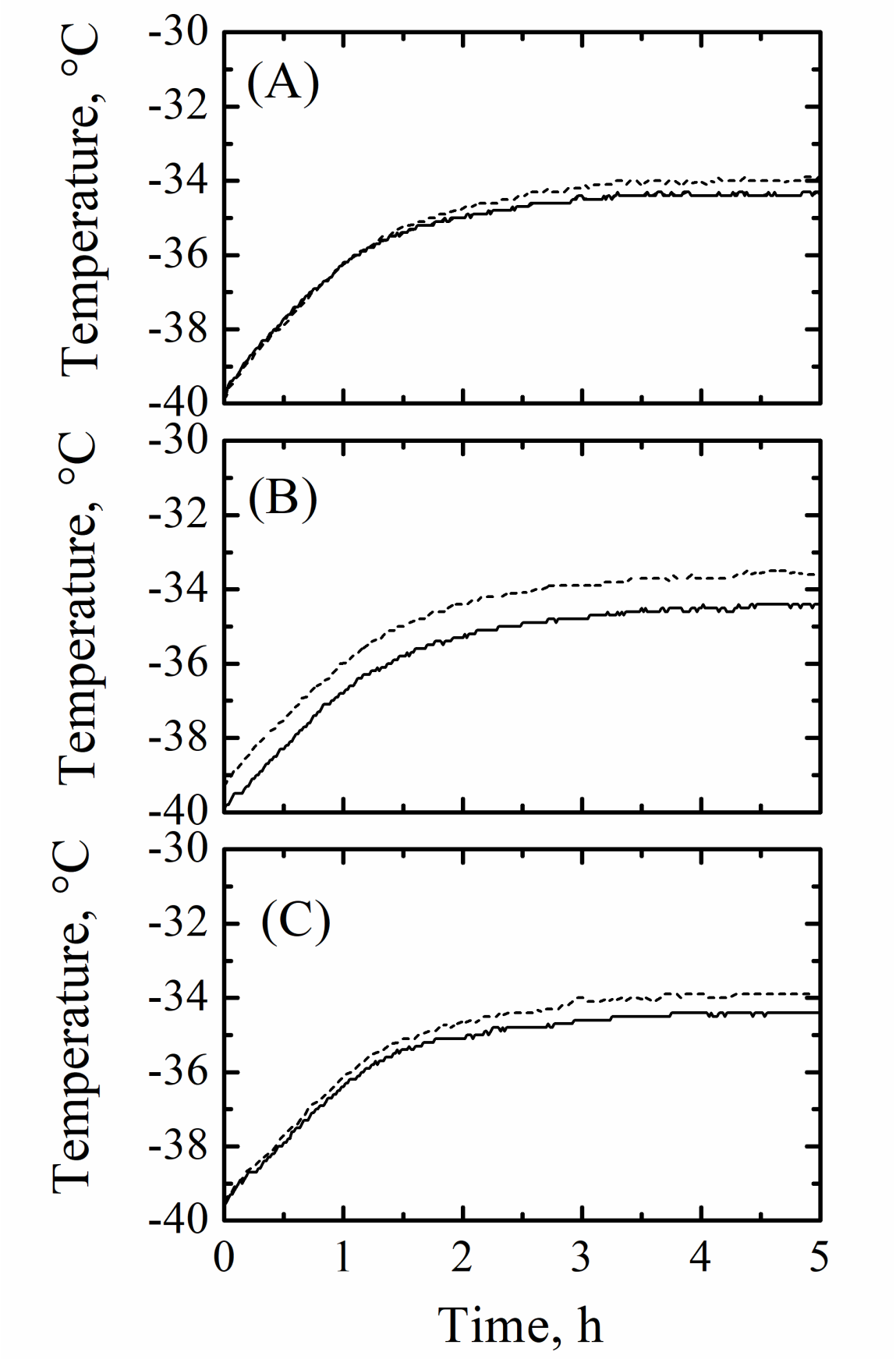
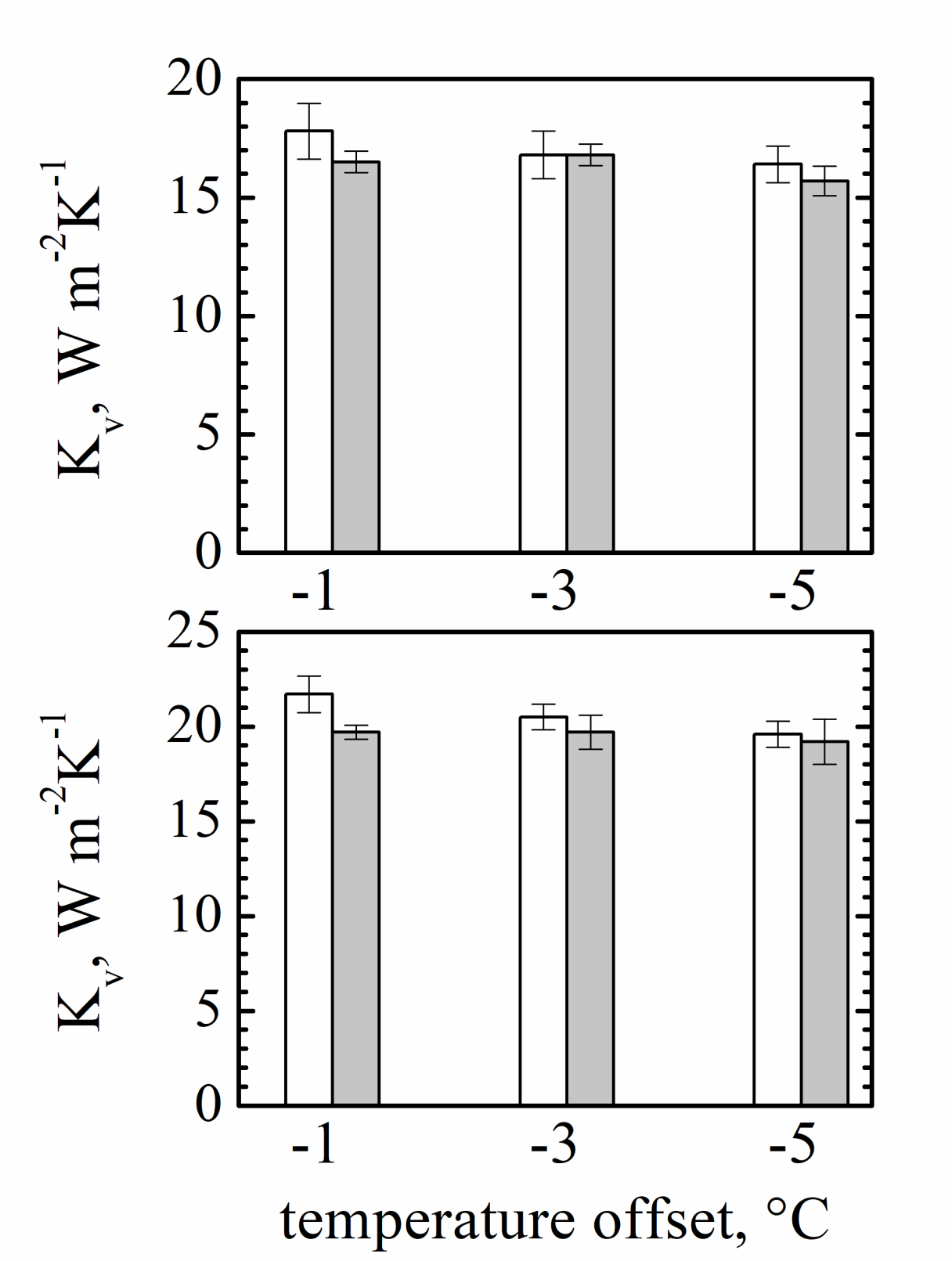


Figure 3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temperature offset, °C

Figure 4

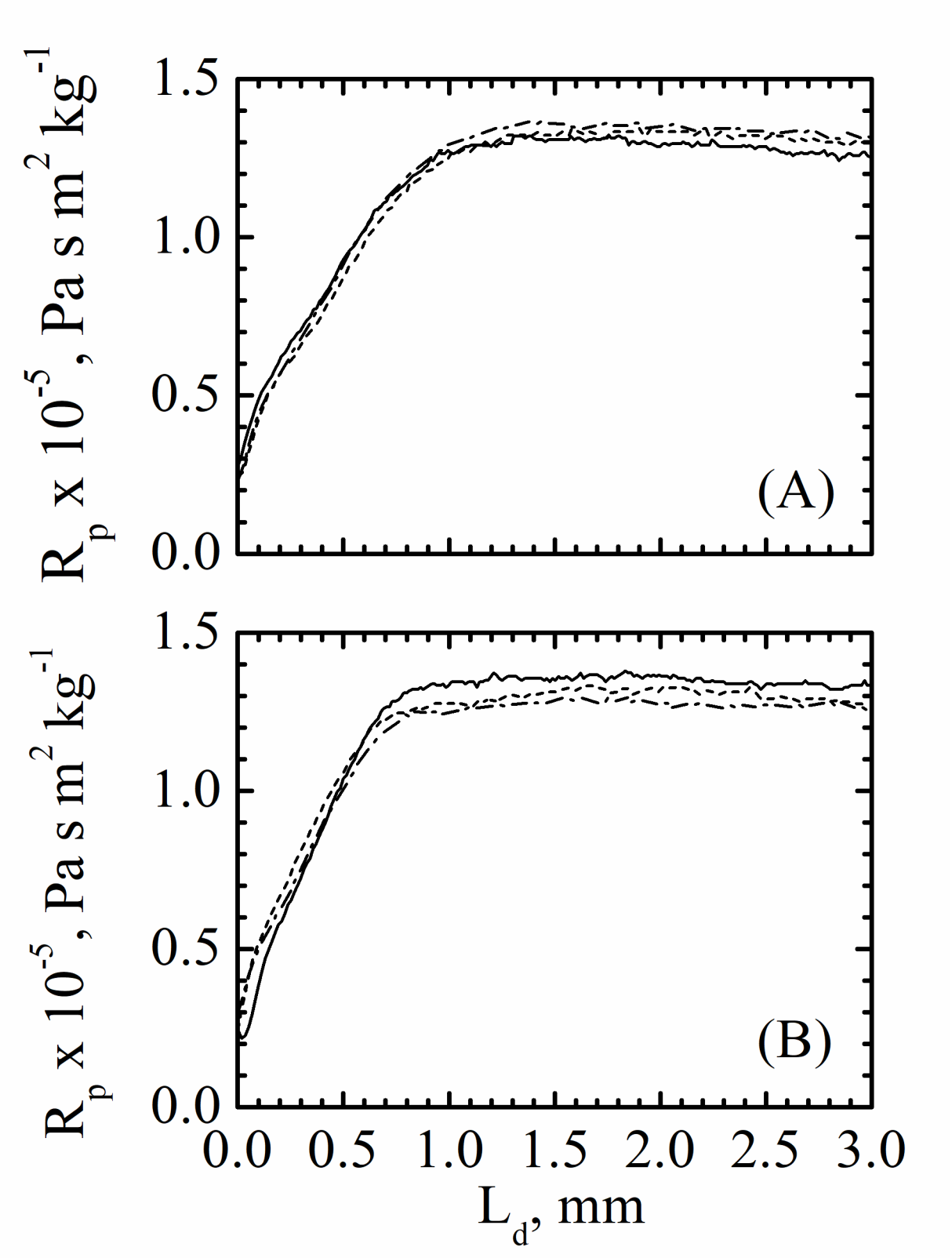


Figure 5

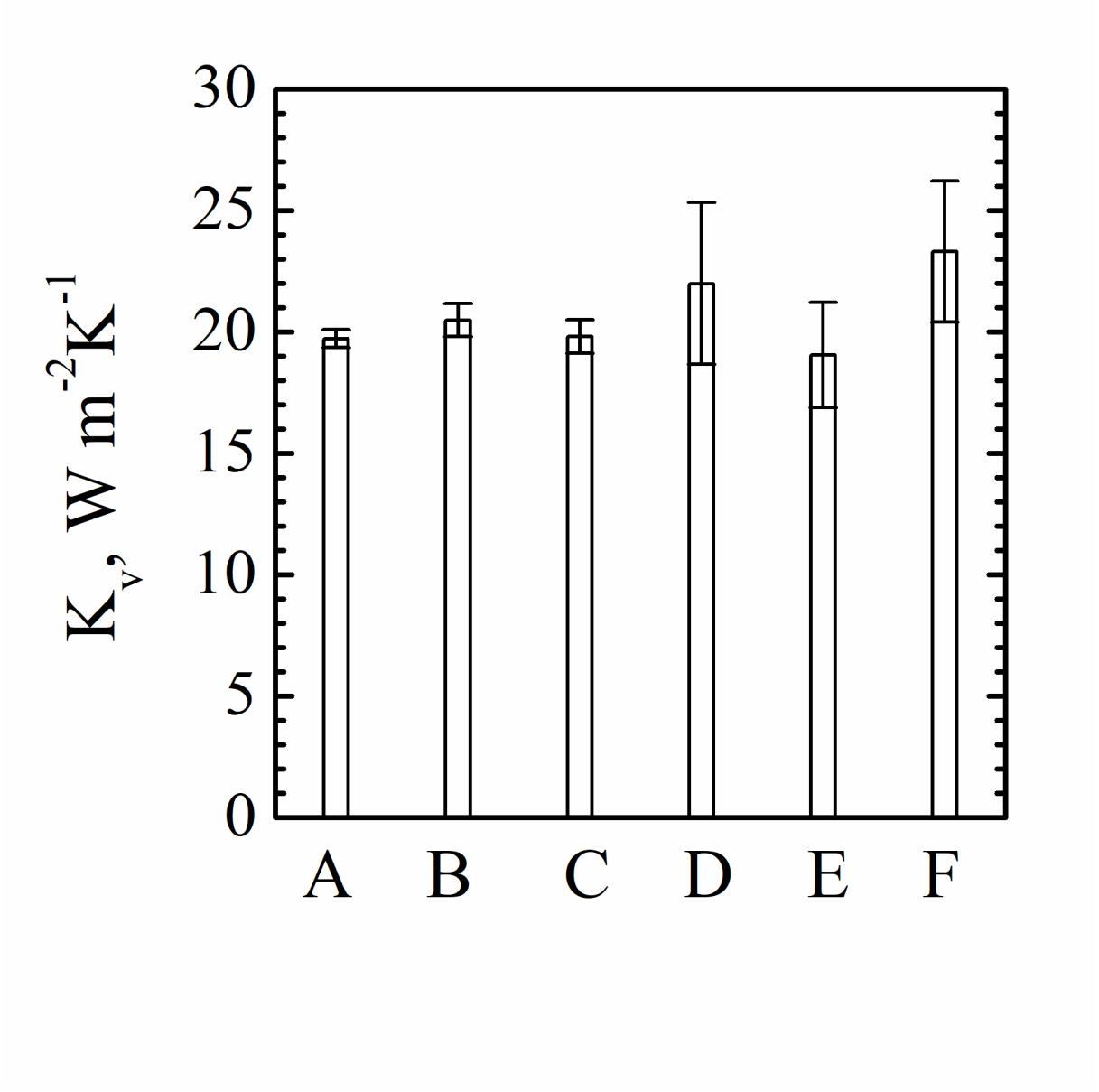


Figure 6

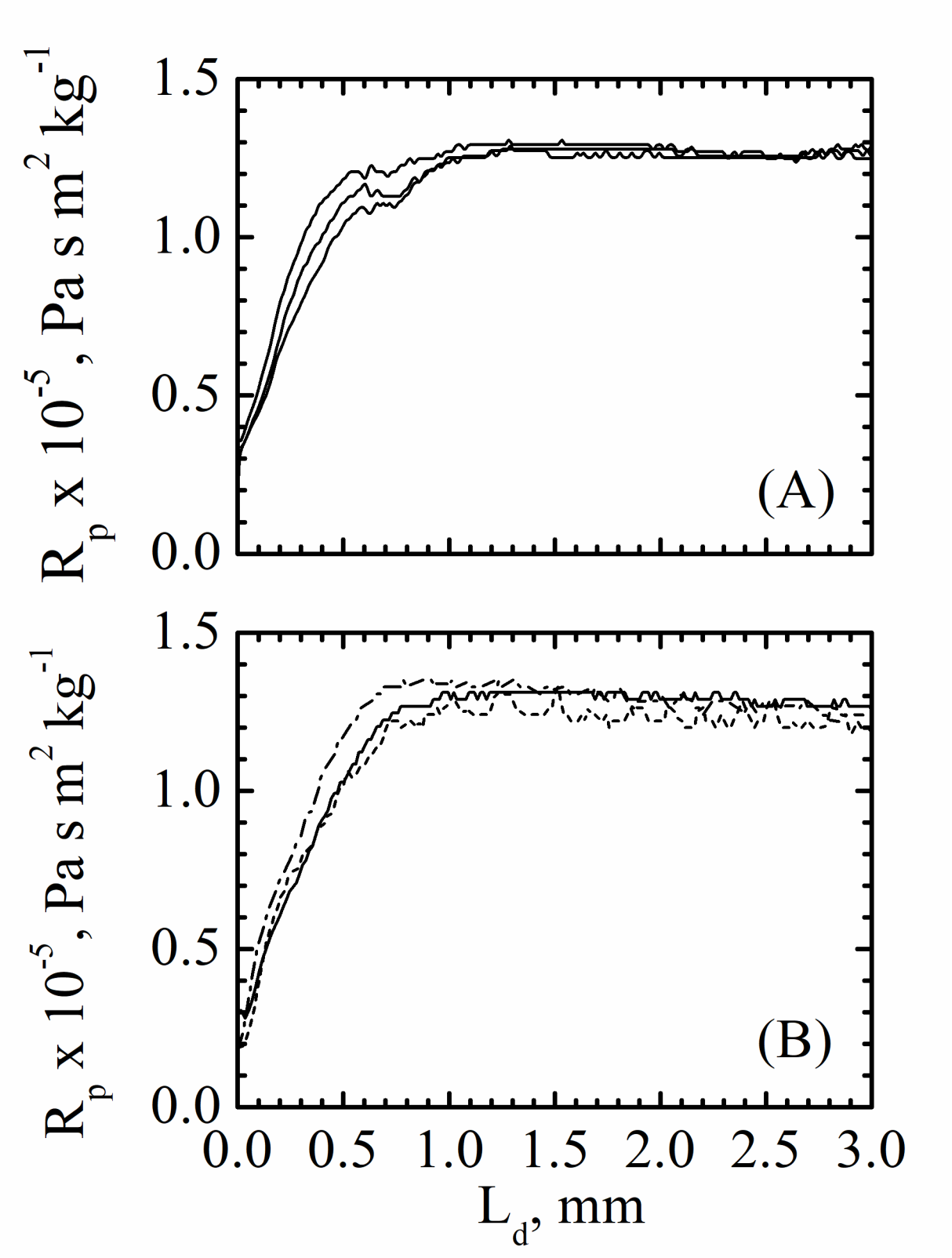


Figure 7

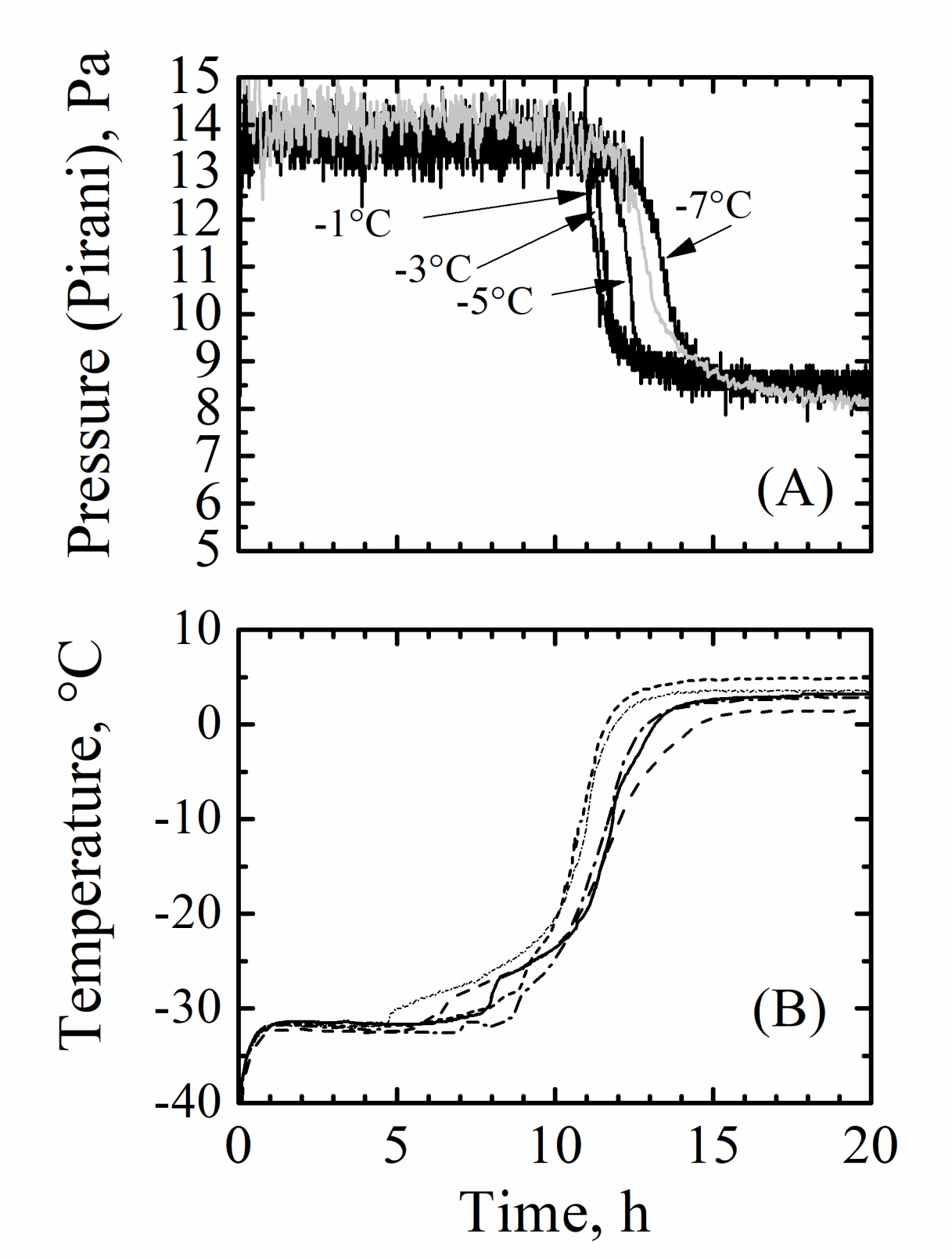
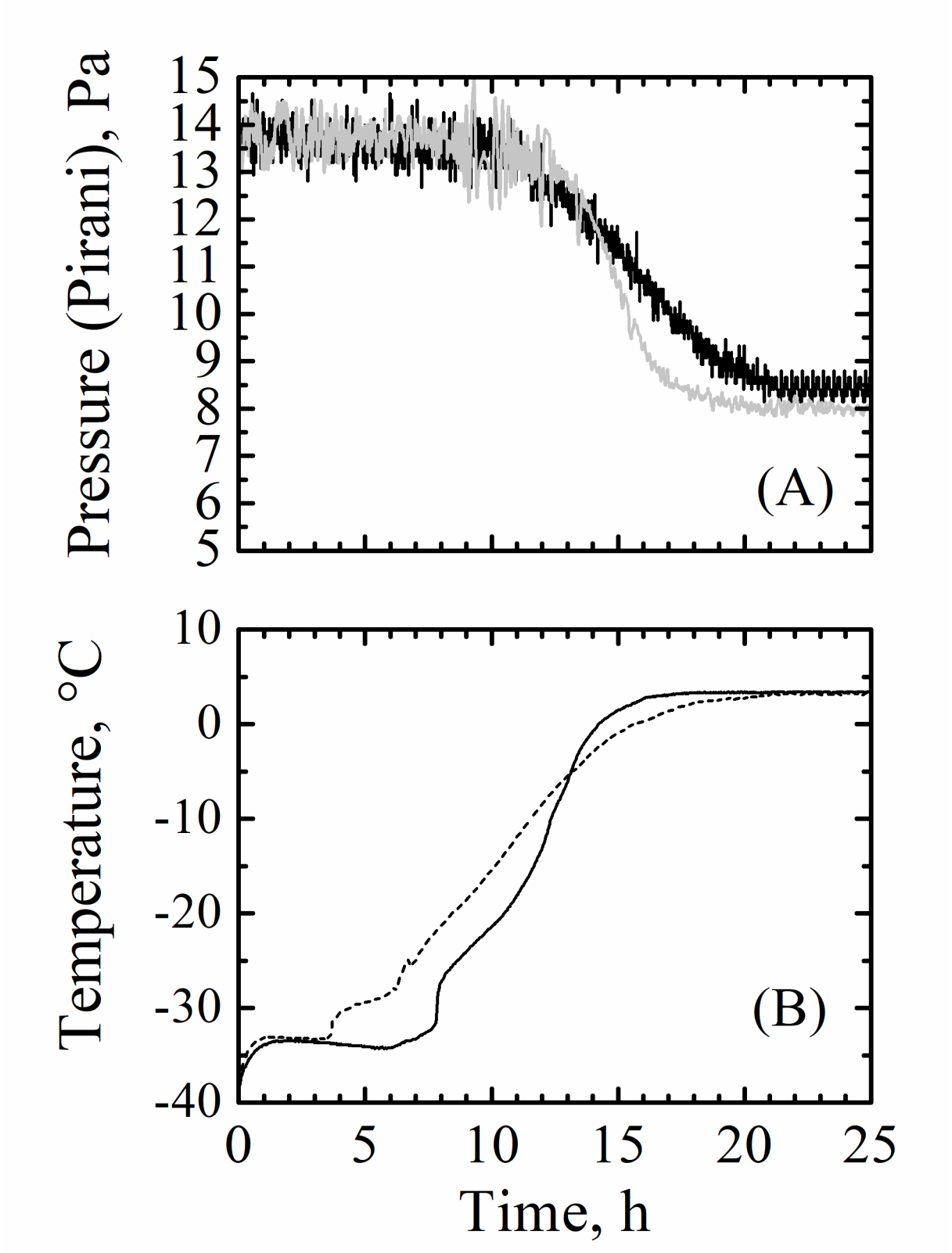

Pressure (Pirani), Pa
15
14
13
12
11
10
9
8
7
6
5
-1°C
-3°C
-5°C
-7°C
(A)
Temperature, °C
10
0
-10
-20
-30
-40
(B)
0
5
10
15
20
Time, h

Figure 8


Pressure (Pirani), Pa
15
14
13
12
11
10
9
8
7
6
5
(A)
Temperature, °C
10
0
-10
-20
-30
-40
(B)
0
5
10
15
20
25
Time, h