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Research paper

On the use of a micro freeze-dryer for the investigation of the primary drying stage of a freeze-drying process

Davide Fissore[a,*], Giuseppe Gallo[a], Angelo Emiliano Ruggiero[a], Taylor N. Thompson[b]

[a] Dipartimento di Scienza Applicata e Tecnologia, Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129 Torino, Italy
[b] Millrock Technology, Inc., 39 Kieffer Ln, Kingston, NY, USA

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ABSTRACT

This paper deals with the use of a small-scale freeze-dryer, where very few vials are loaded (e.g. 19, each 10 mL, or 7, each 20 mL), for freeze-drying cycle investigation. The system has a metallic ring surrounding the batch of vials, in contact with the external ones, and its temperature is manipulated independently from that of the shelf on the basis of the temperature of the product measured by thermocouples in some vials of the batch. The experimental study was carried out using two sucrose solutions (5% and 10% w/w), aiming to verify the homogeneity of the batch. Both product temperature and the weight loss after 6 h from the onset of the primary drying stage were selected as key parameters. Experiments were carried out according to a 2^N design of experiments, with two values of chamber pressure (60 and 90 mTorr) and two values of shelf temperature (−20 and 0 °C). Satisfactory results may be obtained by selecting a ring temperature 5 °C lower than that of the monitored samples in case of both products investigated. Besides, the system appears to be useful for the estimation of the coefficient of heat transfer to the product (K_v) and of the resistance of the dried cake to vapour flux (R_p), thus enabling the use of mathematical modelling for process design and optimization.

1. Introduction

Freeze-drying practitioners face great challenges related to the ever-increasing number of new drugs that require freeze-drying to maintain structure-function relationship and stability, to avoid aggregation, as well as physical and chemical degradation, during both manufacturing and storage [1–3]. This is especially true for biopharmaceuticals: over 40 new biologics were approved in the last four years, representing about 30% of the total new drugs approved in this time period [4,5]. Unfortunately, these molecules (e.g. antibody derived binding molecules, nucleotide delivery systems, etc.) are highly unstable in liquid phase and, thus, freeze-drying is required to increase their shelf-life by removing water [6–8].

Freeze-drying is considered a “gentle” process that makes it possible to remove the solvent without (significantly) jeopardizing the quality of the active pharmaceutical ingredient. However, the operating conditions have to be carefully selected in order to get the target value of residual moisture in the final product, and to keep product temperature below a threshold value, characteristic of the formulation being processed, during the drying stages [9–11]. Additional constraints may be related to equipment characteristics, such as the condensing capacity and the occurrence of sonic flow in the duct connecting the condenser to the drying chamber when the sublimation flux reaches a critical value [12,13].

A large number of experiments are typically required to identify the optimal values of the operating conditions, in particular during the primary drying stage, when ice sublimation occurs, as the critical temperature is low, and the mass flow is high. This is true both in case a trial-and-error approach is used for process design, and also when using mathematical modeling [14–18]. Usually, one-dimensional models, neglecting radial gradients of temperature and composition, are adequate to evaluate *in silico* the evolution of the mean product temperature and of the residual amount of ice [19]. They require solving the energy balance at the interface of sublimation:

$$J_q = \Delta H_s J_w \tag{1}$$

and the mass balance for the frozen layer:

$$\frac{dL_f}{dt} = -\frac{1}{\rho_f - \rho_d} J_w \tag{2}$$

J_w and J_q are, respectively, the mass flux of water vapor from the interface of sublimation to the drying chamber and the heat flux to the product. J_q is assumed to be proportional to the difference between the temperature of the heating shelf (T_s) and that of the product at the

* Corresponding author.
E-mail address: davide.fissore@polito.it (D. Fissore).

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Nomenclature

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 test, 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, °C
T_s	temperature of the heating shelf, °C
t	time, s
Δt	time interval, s
t_d	duration of the primary drying stage, s

Greeks

ρ_d	apparent density of the dried product, $kg\,m^{-3}$
ρ_f	density of the frozen product, $kg\,m^{-3}$

bottom of the vial (T_b):

$$J_q = K_v(T_s - T_b) \tag{3}$$

J_w is assumed to be proportional to the difference between the partial pressure of water at the interface of sublimation ($p_{w,i}$) and that in the drying chamber ($p_{w,c}$), that can be considered to be equal to the total chamber pressure (p_c), being the gas in the chamber about 100% water vapor:

$$J_w = \frac{1}{R_p}(p_{w,i} - p_{w,c}) \tag{4}$$

Several process analytical technologies were proposed and validated in recent years to facilitate the design of a freeze-drying process (see, among the others, the recent review by Fissore and co-workers [20]). Nevertheless, a number of experiments are still required to estimate K_v, whose value is dependent on the type of vial and freeze-dryer used, on chamber pressure and, up to a lower extent, on shelf temperature [21]. Also the coefficient R_p has to be estimated experimentally [22]: its value is, in fact, dependent on the product being processed, on freezing conditions, and on the thickness of the dried product.

All the experiments required to get the values of model parameters (for *in silico* process design and optimization), or to assess the effect of the operating conditions on final product features and process duration, are highly time consuming. It has to be stressed that the time required to carry out a freeze-drying process is not only related to freezing and primary/secondary drying stages, but also to sample preparation, vial loading/unloading, and condenser defrosting, among the others. Besides, most of these tests have to be carried out processing the formulation containing the active pharmaceutical ingredient (only the tests for estimating K_v may be carried out using just the solvent, without the drug), both for process design and validation. These issues represent a relevant cost for pharmaceutical industry, that may seriously affect the time-to-market of new drugs, but also the drug development stage. This is the driving force for the development of new equipment that allow processing few vials, obtaining information (e.g. optimal operating conditions, model parameters, process duration) that can be used in a pilot-scale or in an industrial-scale unit [23].

The main challenge in this field is represented by the fact that drying conditions are not uniform in a batch: in fact, it is well known that radiation from chamber walls may affect the heat flux to the product in the external vials of the batch [21] and, thus, the temperature and the drying duration. Beside radiation, also the conduction in the gas surrounding the external vials of the batch is a phenomenon responsible of atypical drying conditions in these vials [24,25]. This is particularly true when processing a small size batch, as in this case the number of vials at the edge of the batch can be even higher than that in the central positions, while in a large-scale batch they represent only a small fraction of the batch. This issue has thus to be taken into account when processing small size batch with the goal of obtaining results representative of the drying conditions in a large-scale unit.

Obeidat et al. [26] investigated the performance of a freeze-dryer where 7 (20 mL) vials were loaded in a chamber whose wall temperature were controlled to reproduce the dynamics of edge or central vials in a large-scale unit. A second prototype was investigated, where a cylindrical temperature-controlled wall was introduced in the drying chamber, surrounding the batch of vials. In both cases it appeared that when carefully selecting the temperature of the chamber walls (or of the cylindrical wall) the mean temperature of the vials located in the mini freeze-dryer may be close to that exhibited by the vials in a large-scale unit. However, there was still an edge vial effect and the temperature uniformity across the batch was inconsistent.

A different device was proposed by Thompson et al. [27] and investigated by Goldman et al. [28]: it consists of a small chamber where a temperature controlled aluminum ring is in direct contact with the external vials of the batch. The goal of this aluminum ring is to mimic additional rows of vials and for this reason it has to be in contact with the vials of the batch. The way of controlling the heat received by the external vials of the small batch is thus significantly different from that of the device by Obeidat et al. [26] Moreover, its temperature is controlled on the basis of the product temperature in some vials of the batch, with the goal of maintaining a certain offset between the ring and the product temperature. Also in this case few vials can be loaded, e.g. 7 each 20 mL vials in the study of Goldman et al. [28], or 19 each 10 mL vials in the study of Thompson et al. [27], and an excellent agreement between both product temperature and the overall heat transfer coefficient K_v in this small-scale unit and in a large-scale one was shown in case the temperature of the metallic ring was properly selected.

This paper is focused on the investigation of the micro freeze-dryer proposed in Refs. [27] and [28] with the aim to address several pending issues. The first one is related to the homogeneity of drying conditions in the micro freeze-dryer: in fact, the greater the uniformity of the values of product temperature and sublimation rate in the processed vials, the greater their reliability when these results are used for scale-up and/or process transfer. The second one is related to the selection of the ring temperature: some practical guidelines will be given on the basis of an extended experimental investigation using different products and operating conditions, beside addressing also the problem of product temperature measurement. Finally, the estimation of the values of K_v and R_p to be used for *in silico* process simulation and design space calculation will be addressed.

2. Materials and methods

2.1. Freeze-drying unit

Experiments were carried out in the small-scale freeze-dryer MicroFD® by Millrock Technology, Inc (Kingston, NY, USA). It is characterized by a drying chamber with a 6″ diameter shelf, whose temperature can range from −60 °C to + 60 °C. In this chamber it is possible to load the selected type of vials: the external vials of the batch are in contact with an aluminum ring (LyoSim®) (see Fig. 1A and B). As an example, it is possible to load 19 each 6R (10 mL) vials, or 7 each 20 R (20 mL) vials, or a different type of vials, properly adjusting the ring structure in such a way that the contact between the external vials and the ring itself is guaranteed (thus maximizing the efficiency of this system).

The temperature of the ring is controlled independently from that of the shelf and is based on the mean value of product temperature measured through thin T-type thermocouples inserted in some of the vials of the batch, and maintained in the correct position (in close contact with the bottom of the vial), using a specifically designed device (Fig. 1C). The temperature offset of the ring, defined as the difference between the ring temperature and the mean product temperature, can range from −15 °C to + 15 °C with respect to the mean temperature of the product. By this way it becomes possible to simulate the dynamics of central or edge vials of a larger-scale unit. In fact, in case of central vials, it is expected that the ring acts as additional rows of vials in contact with the external vials of the batch: in this case the ring has to act as a "heat sink", as in each vial the heat supplied is used for ice sublimation. Therefore, the temperature of the ring will be lower than that of the vials of the batch (the offset is thus negative). The opposite situation is that when the goal is to simulate edge vials, as in this case additional heat has to be provided to the batch and, thus, the ring temperature has to be higher than that of the vials of the batch (the offset is thus positive).

Controlled ice nucleation, using an ice-fog based technology (FreezeBooster®), is also possible, aiming to further increase the homogeneity of the batch. Once the desired nucleation temperature is reached (e.g. −5°C), the system has to be maintained in the same conditions in such a way that product temperature in the vials of the batch becomes as much uniform as possible. At the same time, the temperature in the condenser reaches a very low value, e.g. −70 °C. The ice fog is then obtained by atomizing (through a pump) a certain amount of water (e.g. 5 mL) in the condenser: due to the low temperature, the water drops immediately freeze, and are then introduced in the chamber to induce ice nucleation. Using this method ice nucleation occurs at the same time and at the same temperature in all the vials of the batch [29–31].

Beside thermocouples, process monitoring is achieved through a capacitive pressure gauge (Baratron type), a thermal conductivity pressure gauge (Pirani type), and a heat flux sensor (AccuFlux®) [32,33]. The last device is a thin film differential thermopile (see Fig. 1A) in direct contact with the shelf and the vial bottom, acting as a transducer that produces an electrical signal that is proportional to the heat flowing through the surface, which allows a direct measurement of the heat flux from the shelf to the product in the vials. By using AccuFlux® it is possible to estimate immediately the heat transfer coefficient from the shelf to the vials using Eq. (3): both the values of the heat flux from the shelf (J_q) and of the shelf temperature (T_s) are in fact known (in this case, it is required to measure also product temperature at the bottom of the container).

2.2. Product and vials

All tests were carried out using sucrose aqueous solutions. The solid content was either 5% w/w or 10% w/w, and water was used as solvent. Sucrose was purchased from Sigma Aldrich (≥99.5%) and used as received. Solutions were filtered through 0.2 µm PES membrane and poured into 6R tubing vials (Schott Pharmaceutical Packaging, Inc., Lebanon, USA), 3 mL per vial. Vials were partially stoppered with an igloo stopper (NovaPure Chlorobutyl Igloo Stoppers, West Pharma, Exton, USA), then weighed and loaded directly on the shelf, in direct contact with the heat flux sensor and with the metallic external ring (the external vials of the batch). Vials whose temperature was not monitored were partially stoppered with igloo stoppers, while those where temperature was measured were stoppered with the thermocouple holder previously described (see Fig. 1B).

2.3. Design of experiments

Experiments were carried out aiming to assess the effectiveness of the ring system to achieve uniform drying conditions in the vials of the batch. Tests were carried out using both products and investigating the effect of the temperature offset (defined as the difference between the temperature of the ring and that of the product) during the primary drying stage on both product temperature and on the sublimation rate. 4 values of the temperature offset were considered, namely −1°C, −3°C, −5°C and −7°C: the rational for selecting a ring temperature lower than product temperature is that it is necessary to remove heat from the external vials, in order to get product dynamics, i.e. the sublimation rate and the product temperature, similar to that of the product in the central vials. By this way it is possible to reproduce in the system the dynamics of the majority of the vials of an industrial-scale batch.

The freezing stage was initially carried out without using controlled nucleation and/or an annealing step: both methods are in fact well known to be able to reduce batch non-uniformity, but the goal of the first part of the study was to assess the effect of the ring, and it was thus

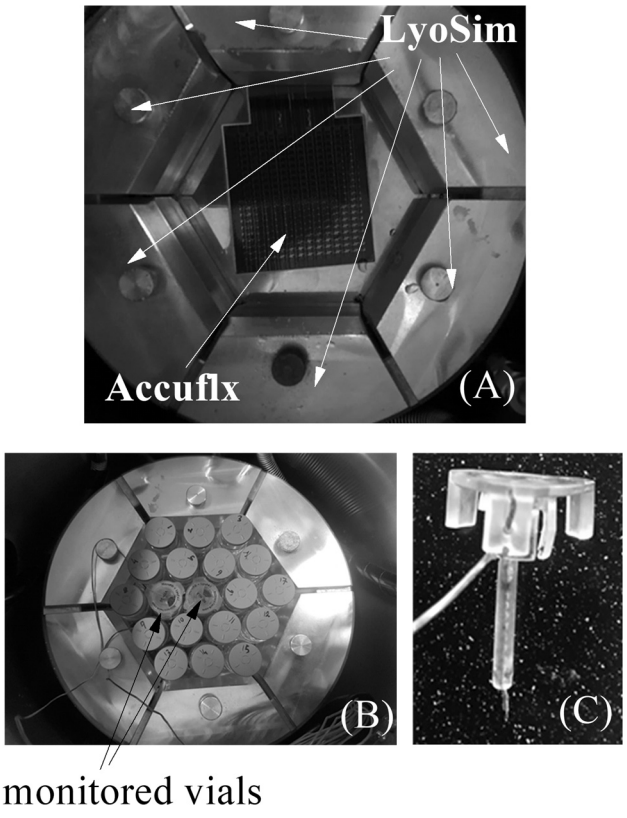

Fig. 1. (A) Drying chamber of the freeze-dryer used in this study: the ring (LyoSim®) and the heat flux sensor (Accuflux®) are evidenced. (B) Example of vials loading in the micro freeze-dryer. (C) Thermocouple holding device used in the monitored vials.

preferred to avoid any other variation that could interact with it. At the beginning of each test, a product temperature equalization step at 20 °C was carried out, for 10 min and, then, the set point of the shelf temperature was modified to a value of −40 °C, that had to be reached in 1 h, i.e. with a rate of variation of −1°C/min (a quite typical value in freeze-drying operation). The real temperature of the shelf was shown to decrease with a temperature very close to this target rate (being the measured value ranging from −0.95 to −0.98 °C/min) until a shelf temperature of −35 °C was reached and, then, when the target value of −40 °C was approaching, the control system modified the temperature of the shelf at a lower rare, about −0.25 °C/min. With respect to the product in the vials, its temperature decreased with a different dynamic, at a lower rate. In the first part, when the liquid is cooled, the rate of temperature decrease in the product was about 0.8 °C/min. After ice nucleation, and completion of the freezing stage, the ice was cooled at a very high rate (about −2°C/min) till the shelf temperature reached the value of −35 °C, and at a lower rate (about −0.1 °C/min) when also the rate of cooling of the shelf was lower. In all the tests similar trends were observed. About 2 h were required to complete the freezing stage.

Experiments using controlled nucleation were also performed successively, as previously described, considering a nucleation temperature of −5°C. No experiments with annealing were carried out: annealing is in fact known to result in a more uniform product structure among the vials of the batch, but it takes time, and as one of the advantages of the MicroFD is to accelerate the operations required to set-up a cycle (as previously stated, a low number of vials means shorter loading/unloading times, shorter defrosting times, etc.), we did not consider annealing as a valuable alternative, while controlled nucleation had no effect on the duration of the freezing stage.

With respect to the operating conditions of the primary drying stage, namely the temperature of the heating shelf and the pressure in the drying chamber, a 2^N design of experiment was set up, where N = 2 is the number of parameters considered. A 2^N design of experiment is a particularly useful type of design of experiments as it allows minimizing the experimental effort required to gain knowledge about a certain system, being based on the selection of two values, a “high” and a “low” one, for each of the parameters that are supposed to affect the dynamics of a system. For the case study under investigation, i.e. the freeze-drying of a 5% sucrose solution, shelf temperature and chamber pressure are the parameters to be investigated, and the selected values of shelf temperature were −20 °C and 0 °C, while the values of chamber pressure were 60 mTorr and 90 mTorr. Product temperature was observed in all cases to remain below (or close to) the value of −32 °C, usually considered as the limit temperature for a process with sucrose solutions, ranging from −36 °C for the lower pressure/lower temperature values considered (at 60 mTorr, −20 °C and lower temperature of the ring), till about −32.5 °C for the higher pressure/higher temperature values considered (at 90 mTorr, 0 °C and higher temperature of the ring) when the 5% sucrose solution was processed. Similar results were obtained with the 10% sucrose solution. In any case, it has to be remarked that being the goal of the study the evaluation of the effect of the ring on the homogeneity of the batch, the occurrence of cake collapse was not a concern.

After 6 h from the onset of the primary dying stage the run was stopped, and the pressure in the chamber was brought to the atmospheric value. Weight loss in all the vials of the batch was then measured and recorded: weight losses in the vials of the external row and in those in the central positions are then compared at different values of the track offset used to set the ring temperature using MiniTab®18 and carrying out an analysis of variance (ANOVA): difference about mean values in the two groups of vials were considered significant when the obtained p-value was lower than 0.05.

2.4. Calculation of model parameters

The MicroFD has the ability to estimate K_v by measuring the heat flux using the AccuFlux® sensor. AccuFlux® measures heat flow between the shelf and vials and, once the value of the flux is divided by the driving force given by the difference between shelf and product temperatures, it is possible to get the value of K_v (see Eq. (3)). It has to be stressed that each vial in the batch is heated by different mechanisms, and AccuFlux® accounts just for the heat transferred by the shelf; therefore, the value of K_v calculated through AccuFlux® is not the parameter to be used in Eq. (3), but it should be more correctly indicated as $K_{v,shelf}$, and the value of heat flux as $J_{q,shelf}$.

With respect to R_p, its value is usually retrieved from the heat balance at the sublimation interface, Eq. (1). Once J_q is known, from this equation it is possible to calculate the sublimation flux, J_w. At this point, it is possible to use Eq. to calculate R_p assuming that $p_{w,c}$ is equal to p_c and calculating $p_{w,i}$ from the measured product temperature. It is thus required to know the value of J_q, the total heat flux transferred to the product, and not just the heat flux from the shelf measured by AccuFlux®: the procedure is described in the following. Being Δm, i.e. the weight loss in each vial, measured at the end of the test, and both the temperature of the shelf (T_s) and that of the product (T_b) measured during the run, it is possible to calculate the effective value of K_v using the following equation, derived from the energy balance at the interface of sublimation:

$$K_v = \frac{\Delta m \Delta H_s}{A_v \int_0^{t_d} (T_s - T_b) dt} \tag{5}$$

This corresponds to the well-known gravimetric test, extensively described in the literature. [21] Once K_v is known, it is possible to calculate also the curve R_p vs. L_d. The procedure is quite straightforward:

- At $t = 0$, $L_d = 0$ and, thus, $R_p = 0$.
- After a time interval Δt, e.g. 1 min, it is possible to calculate the heat transferred to the product:

$$Q = K_v A_v \int_t^{t+\Delta t} (T_s - T_b) dt \tag{6}$$

and the mean heat flux:

$$J_q = \frac{Q}{A_v \Delta t} \tag{7}$$

where K_v has the value calculate through Eq. (5) as it must account for all the heat transferred to the product, and not just that exchanged with the shelf, as previously discussed.

- At this point it is possible to calculate the mass flux, using Eq. (1).
- Once J_w is known, it is possible to calculate R_p, using Eq. (4) and assuming that $p_{w,c}$ is equal to p_c and calculating $p_{w,i}$ from the measured product temperature.
- The variation of L_d can be calculated using Eq. (2).
- Previous calculations are repeated for the following time intervals Δt, thus obtaining the whole curve R_p vs. L_d.

Once the values of K_v and R_p are known, process dynamics may be simulated using the one-dimensional model previously presented (Eqs. and), thus allowing the off-line calculation of the design space and the process optimization.

3. Results

First experiments were carried out with the 5% w/w sucrose solution at −20 °C and 60 mTorr, varying the temperature offset, given as difference between the temperature of the ring and that measured by thermocouples placed in central vials. In this configuration only vials in the central position of the shelf were monitored through thermocouples, and four different temperature offsets were considered namely −1, −3, −5 and −7°C. Results are shown in Fig. 2, where the weight

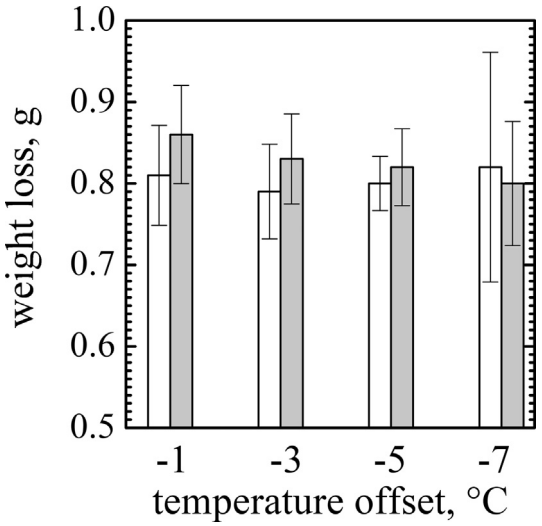

Fig. 2. Comparison between the weight loss in the edge (grey bars) and in the internal (white bars) vials for different values of temperature offset (difference between the temperature of the ring and that of the monitored vials). (Operating condition: $T_s = -20$ °C, $P_c = 60$ mTorr, 5% w/w sucrose solution, thermocouples are placed just in central vials).

loss in the central vials is compared to that measured in the external row of vials, in direct contact with the ring. The value of weight loss at the end of the test (it has been pointed out that the duration of the test was 6 h in all cases, thus obtaining a measurable water loss, without completing the ice sublimation in the batch) is shown, for each group of vials, as mean value with an error bar that corresponds to one standard deviation. The main outcome of this test is that the temperature of the ring has an effect on the dynamics of the batch: in particular, moving from a temperature offset of −1°C to a value of −5°C the homogeneity of the batch is improved. In fact, while at −1°C the mean value of weight loss in the central vials was 0.81 g and that in the external vials was 0.86 g, with an offset of −5°C the weight loss in central and external vials is 0.8 g and 0.82 g respectively, that means about 1% difference in the amount of ice sublimated. Also the standard deviation is reduced, thus evidencing a greater homogeneity of the batch. When considering the batch as a whole, the mean weight loss was 0.84 g ± 7.7% for a temperature offset of −1°C, and 0.81 g ± 5.23% for a temperature offset of −5°C, thus evidencing the increase of batch homogeneity as a decrease of the standard deviation of the values of weight loss the vials of the batch. When the temperature offset is further decreased, to −7°C, it appears that the weight loss in the external vials is lower than that in the central vials: in this case the cooling effect of the ring dominates in these vials and is responsible for the lower sublimation rate observed in the external vials. This was confirmed also by an additional test where the temperature in one of the edge vials was measured and appeared to be lower than that of the central vials. For this reason, the value of −7°C was discarded from the following investigations, where the focus was on the interval ranging from −1°C to −5°C (the absence of positive offset values in this study has been discussed in the Materials and Methods section). With respect to the results shown in Fig. 2 it has to be remarked that the statistical independence of the mean values of weight loss measured has been verified through the ANOVA analysis.

A second test was carried out to assess the effect of the number of thermocouples used to track the dynamics of the product in the vials and used to calculate the temperature of the metallic ring. In this configuration 6 thermocouples were used, 2 placed in central vials and 4 placed in vials of the external row. Results are shown in Fig. 3 for the same operating conditions used in the previous test and show a similar trend, with an optimal value of the temperature offset of −5°C. Also in this case the statistical independence of the values of weight loss was proven through the ANOVA, resulting in p-values lower than 0.05.

In any case, it should be highlighted that no annealing or controlled nucleation was used in the freezing stage, thus pointing out the role of the temperature ring on the system. Even when the temperature offset is not optimized in the investigated range of temperature offset (from −1°C to −5°C), the difference between the weight loss between the external and the internal vials falls in the range (5–10%) that is usually considered acceptable (and unavoidable) in the central vials of a batch processed in a large (pilot or industrial-scale) freeze-dryer. Several papers appeared in the literature pointed out, in fact, that even in the central vials of the batch, where the heat transfer mechanism to the product in the vials are the same, a certain non-uniformity of the drying rate arises, e.g. due to the non-uniform contact between the vials and the shelf, and/or to a non-uniform temperature of the shelf. This non-uniformity in the sublimation flux is in general given as non-uniformity of the overall heat transfer coefficient K_v, being the heat flux considered proportional to the sublimation flux (Eq. (1)) and the driving force of the heat transfer considered the same in all the vials [21,34,35]. As an example, one of the most recent contributions in this field is that by Pikal et al. [35], showing that for a 20 cc tubing vial in the laboratory dryer where the investigation was carried out the standard deviation of the K_v values ranges from 3.8% to 6.7% changing chamber pressure from 55 to 150 mTorr (while the edge vials exhibited a higher degree of non-uniformity, with standard deviations ranging from 6.6% to 10.7%). Finally, considering the whole process, namely the freezing stage, the primary drying, the preparation of the batch, and the condenser defrosting, 9–10 h are enough to assess the effect of single value of temperature offset and, thus, the optimization of this parameter is not time consuming, and also the amount of active pharmaceutical ingredient required is very small.

In addition to the sublimation rate, product temperature is also an important concern and, thus, the temperature history in external vials and in the internal ones has to be considered for different temperature offset. Fig. 4 shows the results obtained for the same operating conditions previously considered (-20 °C and 60 mTorr) and two values of temperature offset, namely −3°C (upper graph) and −5°C (lower graph). It appears that for a temperature offset of −5°C the temperature evolution in the two groups of vials is almost overlapping, while for the offset value equal to −3°C the temperature of the edge vials is slightly higher, about 1 °C, than that of the central vials. Results are shown also for a different product, namely a 10% w/w sucrose solution: also in this

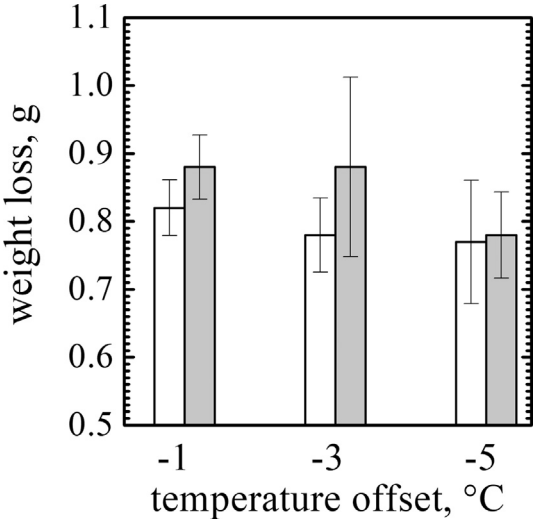

Fig. 3. Comparison between the weight loss in the edge (grey bars) and in the internal (white bars) vials for different values of temperature offset (difference between the temperature of the ring and that of the monitored vials). (Operating conditions: $T_s = -20$ °C, $P_c = 60$ mTorr, 5% w/w sucrose solution, thermocouples are placed in the whole batch).

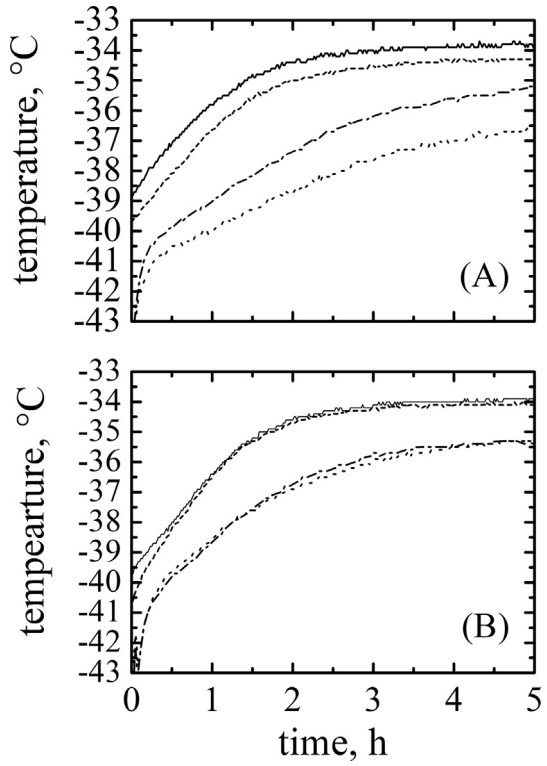

Fig. 4. Comparison between the temperature evolution in the edge and in the central vials for different values of temperature offset (graph A: −3°C, graph B: −5°C) and different products (5% w/w sucrose: dash-dotted line: edge vial, dotted line: central vial; 10% w/w sucrose: solid line: edge vial, dashed line: central vial).

case excellent results are obtained for a temperature offset of −5°C, although very good results are obtained also at −3°C (less than 0.5 °C temperature difference). As it can be expected, product temperature is higher when processing the 10% w/w solution since the porosity of the dried cake is lower, and this makes R_p higher and, being the overall heat transfer coefficient the same, this has the effect of increasing the temperature of the product.

The values of weight loss for the 10% w/w sucrose solution are shown in Table 1, evidencing that for this product excellent results are obtained for all the temperature offsets considered. In fact, considering the batch as a whole, the mean weight loss moves from 0.74 g ± 4.34%, to 0.74 g ± 7.53% and, finally, to 0.69 g ± 6.42% when the temperature offset is varied from −1°C to −3°C and, finally, to −5°C. Thus, in this case, the history of the product temperature can be the discriminating factor. ANOVA confirms the statistical independence of the weight loss measured in the external vials (and in the internal ones) at different offset temperatures.

At this point the experimental investigation was extended to verify the effectiveness of the ring system at different values of chamber pressure and shelf temperature. Results obtained for the 5% w/w sucrose solution at 0 °C and 60 mTorr are shown in Table 2. In this case it appears that the value of the temperature offset, in the investigated range, has less impact on the difference of the weight loss between internal and external vials, although a value of −5°C appears again to be more advisable as the standard deviation is lower, thus indicating a more uniform batch. Considering the mean values of weight loss for the whole batch, the values obtained as 1.58 g, 1.59 and 1.55 g, respectively, for a temperature offset of −1°C, −3°C and −5°C respectively, with a standard deviation of 5.13%, 4.52% and 3.57% respectively, thus pointing out that with an offset of −5°C the sublimation rate in more uniform in the batch, being the standard deviation lower.

Table 3 shows the results obtained at −20°C and 90 mTorr: if we focus on the difference between the mean values of weight loss in the two groups of vials it appears that the best choice is a temperature offset of −3°C or −5°C. If we look at the standard deviation of the whose batch, it appears that −3°C is the optimal value: considering the batch as a whole, the minimum of the standard deviation, i.e. 5.88% is obtained for this temperature offset (vs. 8.58% at −1°C and 9.52% for −5°C).

When considering a higher value of shelf temperature, namely 0°C (Table 4), the temperature offsets of −5°C appears again to be the best choice, resulting in the minimum difference between the mean weight loss in the external and in the internal vials, and in the minimum value of the standard deviation of the weight loss in the vials (3.46%).

Although the homogeneity of the batch, in particular with respect to the drying rate, appears to be quite satisfactory even without optimizing the ring temperature, the effect of using controlled nucleation was investigated. Results are shown in Fig. 5 for the 5% w/w sucrose solution processed at −20 °C and 60 mTorr, with a temperature offset of −3°C. It is possible to see that the introduction of a controlled nucleation step further increases the uniformity of the batch, as while in absence of controlled nucleation the mean measured weight loss was 0.79 g and 0.83 g in the internal and external vials respectively, while with controlled nucleation these mean values were, respectively, 0.88 and 0.89 g.

The second open issue related to the use of micro freeze-dryers is the possibility of obtaining the values of model parameters, namely K_v and R_p in such a way that mathematical modeling may be used for off-line process optimization and design space calculations (using one of the methods proposed in the literature [14–16]).

With respect to the heat transfer coefficient it has to be remembered that the heat flux sensor provides information about $K_{v,shelf}$: this value is not, evidently, modified by the temperature of the ring. If we consider, for example, the test carried out with the 5% sucrose solution processed at −20 °C and 60 mTorr, with temperature offset of the ring equals to −1°C, −3°C and −5°C the values of $K_{v,shelf}$ obtained are, respectively, 6.79, 6.73 and 6.53 $W m^{-2}K^{-1}$. Different is the case of K_v, that is an overall heat transfer coefficient that takes into account all the heat transfer mechanisms to the product. Using the algorithm described in the Materials and Methods section, for the test with the 5% sucrose solution processed at −20°C and 60 mTorr, with temperature offset of the ring equals to −1°C, −3°C and −5°C the values of K_v, obtained are, respectively, 15.8, 15.61 and 15.26 $W m^{-2}K^{-1}$. At a higher values of chamber pressure, namely 90 mTorr, as expected, higher values of $K_{v,shelf}$ and K_v, are obtained, respectively 9.70, 10.22 and 8.62 $W m^{-2}K^{-1}$ ($K_{v,shelf}$) and 20.01, 19.78 and 18.33 $W m^{-2}K^{-1}$ (K_v) for the three values of temperature offset considered in this study (−1°C, −3°C and −5°C). This issue is extremely important for process scale-up, as by manipulating the temperature of the ring it becomes possible to simulate in the small-scale system the dynamics of the target group of vials (central or edge) in the large-scale unit. This point was addressed in a recent paper [28] and, thus, it will not be further investigated here.

Beside K_v it is important to determine also the value of R_p to enable mathematical modeling for process development through mathematical simulation. For a given product, the resistance of the dried cake to vapor flux is a function of the freezing conditions (cooling rate,

Table 1
Results of the tests carried out processing the 10% w/w sucrose solution at −20 °C and 60 mTorr.

Temperature offset, °C	Δm Internal vials, g	Standard deviation	Δm External vials, g	Standard deviation
−1	0.74	4.09%	0.74	4.52%
−3	0.77	6.39%	0.73	7.58%
−5	0.69	8.37%	0.68	5.02%

Table 2
Results of the tests carried out processing the 5% w/w sucrose solution at 0 °C and 60 mTorr.

Temperature offset, °C	Δ*m* Internal vials, g	Standard deviation	Δ*m* external vials, g	Standard deviation
−1	1.55	6.24%	1.60	5.08%
−3	1.56	4.12%	1.60	4.49%
−5	1.58	2.86%	1.53	3.43%

Table 3
Results of the tests carried out processing the 5% w/w sucrose solution at −20 °C and 90 mTorr.

Temperature offset, °C	Δ*m* Internal vials, g	Standard deviation	Δ*m* External vials, g	Standard deviation
−1	0.85	7.83%	0.93	9.63%
−3	0.84	4.96%	0.87	6.22%
−5	0.88	6.24%	0.83	10.72%

Table 4
Results of the tests carried out processing the 5% w/w sucrose solution at 0 °C and 90 mTorr.

Temperature offset, °C	Δ*m* Internal vials, g	Standard deviation	Δ*m* External vials, g	Standard deviation
−1	1.76	2.92%	1.87	4.31%
−3	1.73	4.77%	1.69	6.77%
−5	1.89	3.87%	1.88	3.60%

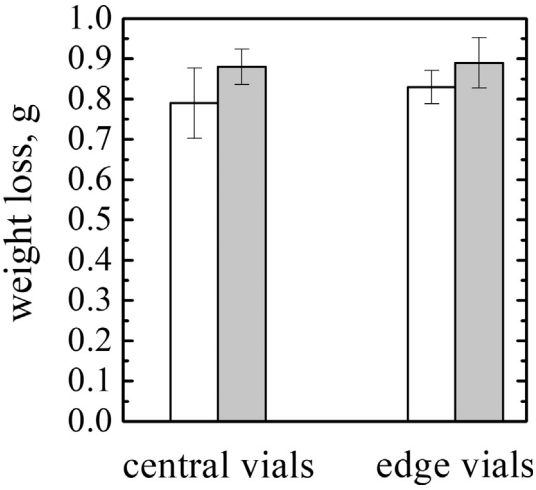

Fig. 5. Comparison between the weight loss in the edge and in the internal vials when using (grey bars) or not (empty bars) the controlled nucleation in the freezing step. (Operating conditions: $T_s = -20$ °C, $P_c = 60$ mTorr, 5% w/w sucrose solution, nucleation temperature: −5°C, thermocouples are placed in central vials; temperature offset: −3°C).

annealing and/or controlled nucleation). Thus, in case the freezing conditions and the product processed in the small-scale unit are the same that will be processed in the industrial-scale freeze-dryer it can be expected that the same values of R_p are obtained. Fig. 6 shows an example of the curves R_p vs. L_d obtained for the 5% w/w sucrose solution processed at −20 °C and 60 mTorr and considering three different temperature measurements, and the results obtained for similar operating conditions processing the 10% w/w sucrose solution. In both cases, three almost overlapping curves are obtained, as a consequence of the fact that the dynamics of ice sublimation in the three monitored vials (temperature and sublimation flux) are very close. For each formulation using the calculated curves it is possible to calculate the parameters ($R_{p,0}$, A and B) of the function generally used to express the dependence of R_p on L_{dried}:

$$R_p = R_{p,0} + \frac{AL_{dried}}{1 + BL_{dried}} \quad (8)$$

Through least-square minimization it is possible to get $R_{p,0} = 1{\cdot}10^4\ \mathrm{m\,s^{-1}}$, $A = 9.1{\cdot}10^7\ \mathrm{s^{-1}}$ and B $7.2{\cdot}10^2\ \mathrm{m^{-1}}$for the 5% sucrose solution and $R_{p,0} = 3{\cdot}10^4\ \mathrm{m\,s^{-1}}$, $A = 5.6{\cdot}10^8\ \mathrm{s^{-1}}$ and B $2.3{\cdot}10^3\ \mathrm{m^{-1}}$ for the 10% sucrose solution. The R_p vs L_{dried} curves obtained with these values are also shown in Fig. 6.

Once K_v and R_p are known it becomes possible to simulate *in silico* the dynamics of the system, namely the evolution of the temperature and of the sublimation flux vs. time, and to identify the time interval required to complete the primary drying. The operating conditions can also be considered in the study to verify whether or not the threshold temperature is exceeded. Fig. 7 shows an example of these calculations, comparing the values obtained through mathematical modeling and the experimentally measured values in two of the previous tests (where ice sublimation was not complete as the test was stopped well before the ending point). The one-dimensional model used to describe the dynamics of the product in the system was extensively validated in the past (and, actually, it is the reference model for freeze-drying practitioners using mathematical modeling for process optimization): the point is to check the adequacy of model parameters calculated independently (K_v using the gravimetric test and R_p using the temperature measurements). The good agreement between measured and calculated values of product temperatures indicates the adequacy of the model parameters calculated using the previously described approach.

4. Discussion and lessons learned

This Section of the paper has the goal of summarizing the main

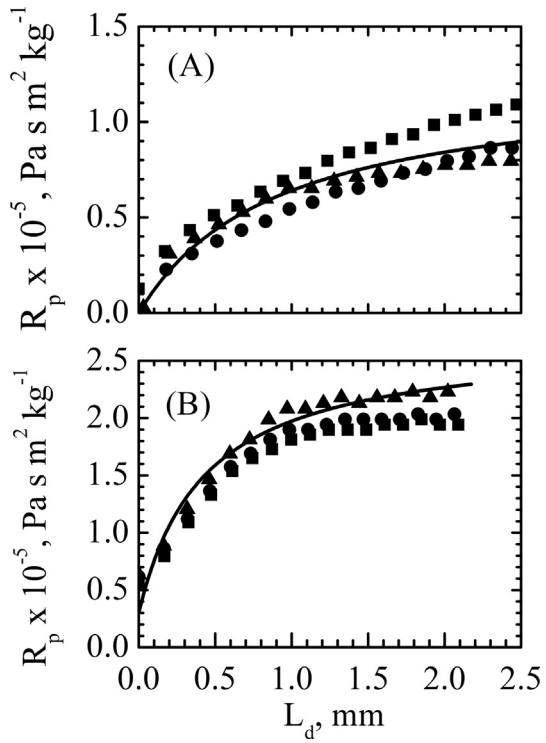

Fig. 6. Comparison between the values of R_p as a function of the thickness of the dried product calculated for the test with the 5% w/w sucrose solution (graph A) and for the 10% w/w sucrose solution (graph B) using three temperature measurements in each run (symbols), and the calculated curve (line). (Operating conditions: $T_s = -20$ °C, $P_c = 60$ mTorr, thermocouples are placed in central vials, temperature offset: −3°C).

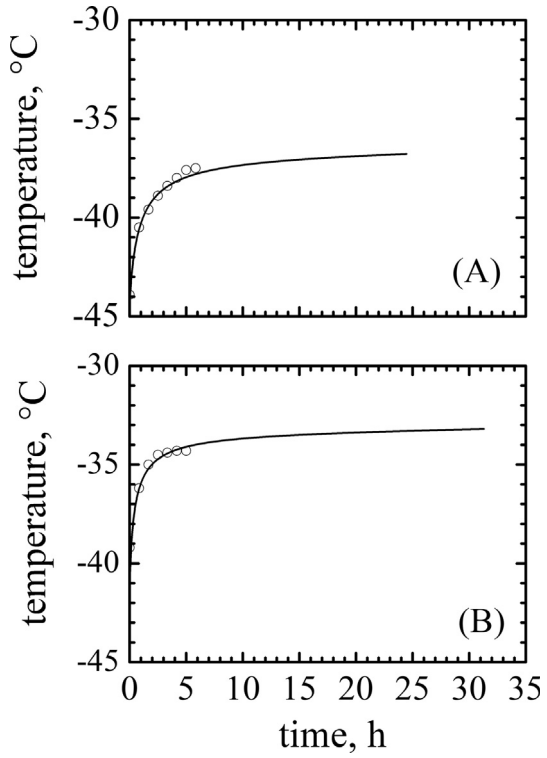

Fig. 7. Comparison between calculated (lines) and the measured (symbols) values of product temperature for the test with the 5% w/w sucrose solution (graph A) and for the 10% w/w sucrose solution (graph B). (Operating conditions: $T_s = -20$ °C, $P_c = 60$ mTorr, thermocouples are placed in central vials, temperature offset: −3°C).

lessons learned from the experimental investigation carried out using the MicroFD previously presented, and also to give some general operating instructions and advices about the use of this system.

(1) *Batch homogeneity*: At first, it should be clearly stated when a batch can be defined "homogeneous". In this paper we focused on drying rate, considering both the mean value of drying rate and its standard deviation, as well as distinguishing the mean values (and the standard deviations) of the external and internal vials of the batch. Besides, also product temperature, both in the internal and external vials, has to be considered. The temperature of the ring plays a crucial role in this framework, as its role is to mimic additional rows of vials, where ice sublimation occurs, thus acting as "heat sink" with respect to the vials in contact with it. Even without using an annealing step (which can be effective, but extending the duration of the process) or a controlled nucleation step, the standard deviation of the sublimation rate can be (if the temperature of the ring has been properly selected) lower than 5–6%, even better than the value usually expected for the central vials in a large-scale dryer.

(2) *Ring temperature optimization*: Few tests have to be carried out to identify the optimal value of the ring temperature. Considering that the time required for running a test is less than 1 day (due to the small size of the batch, the short duration of the drying step, the short time required for condenser defrosting), three tests may be carried out to assess which is the optimal value of the offset temperature, considering values of −1°C, −3°C and −5°C as a starting piint. Both sublimation rate and product temperature in external and internal vials have to be measured. The effect of the ring temperature in this range may be relevant or not, depending on the formulation processed, on the operating conditions, and also on the target desired, i.e. on the degree of homogeneity that the batch is expected to show. In the case studies shown in this paper, the optimal value appears to be around −5°C, but for some operating conditions it appears that both −3°C and −5°C provide satisfactory results.

(3) *Heat exchange coefficient*: Thanks to AccuFlux® it is possible to monitor in-line the heat flux from the shelf to the product in the vials, thus obtaining the value of the heat transfer coefficient $K_{v,shelf}$, one of the parameters of the mathematical model that may be used for off-line optimization and scale-up.

(4) *Mass transfer resistance*: From the tests carried out in this system it is also possible to get the values of R_p as a function of the thickness of the dried layer. To get these values it has to be considered that the parameter R_p is estimated from Eq. (4), being the sublimation flux known, as well as $p_{w,c}$ (corresponding to chamber pressure) and $p_{w,i}$ (requiring the measurement of product temperature). The sublimation flux is calculated from Eq. (1), once the (total) heat flux to the product is known. It is therefore required to get the overall value of K_v as indicated in the Materials and Methods section. From the measurement of weight loss and of product temperature it is straightforward obtaining this coefficient (see Eq. (5)).

The sequence of operations required to use the MicroFD for designing a freeze-drying process can then be summarized:

- Select a range of values of temperature offset for the ring temperature, e.g. −1°C, −3°C and −5°C.
- Select a couple of values of shelf temperature and chamber pressure to carry out the investigation. With respect to chamber pressure, a rule of thumb frequently used by freeze-drying practitioners is to use 1/3 of the value of ice vapor pressure at the limit temperature for the formulation being processed; with respect to shelf temperature, the value may be selected according to previous experiments with similar products.
- Carry out a sublimation test, with a duration of 4–6 h (or higher, it is just required to avoid the completion of the drying in the vials), setting the offset temperature of the ring to one of the possible values.
- Check the weight loss and the product temperature in the vials of the batch. If results are not satisfactory, then the offset temperature has to be modified, and an additional experiment has to be carried out. In case product collapse is observed, then shelf temperature has to be decreased in the following runs.
- Determination of the values of $K_{v,shelf}$ and K_v.
- Calculation of the curve of R_p vs. the thickness of the dried product.
- Once the values of K_v and R_p are known, then the one-dimensional model may be used for *in silico* process simulation, optimization, scale-up.

With respect to the calculation of model parameters it has to be highlighted that in normal use the primary drying stage is completed and, thus, their values are provided by the control system of the freeze-dryer. In the test carried out in this study, as they are gravimetric in nature, meaning that the process was interrupted prior to completing the primary drying process, the critical process parameters needed to be calculated.

5. Conclusions

One of the most recent innovations in the field of freeze-drying processes is the possibility of using small-scale units for process investigation and development as they allow significant savings of both raw materials (that can be expensive, or not available in enough amount) and time. The main concern is related to the fact that the drying conditions in the vials processed have to be uniform in order to get useful information from the investigation carried out in these small-scale units. The system considered in this study encompasses a metallic ring surrounding the external row of vials, being in contact with them,

and whose temperature can be adjusted on the basis of the mean temperature of the batch processed. Results evidenced that the presence of the ring affects the heat flux to the vials and, when its temperature is properly selected, homogeneous drying conditions are obtained. By this way it becomes possible to replicate in this small-scale unit the evolution of the vials in a large-scale freeze-dryer, where most of the vials, i.e. the central ones, have a quite homogeneous behaviour, and to use the system to evaluate model parameters, in such a way that mathematical modeling may be used for process design and optimization.

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Declaration of Competing Interest

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