Virtual Thermocouple: A Non-Invasive Multipoint Product Temperature Measurement for Lyophilization

Xiaofan Jiang

Purdue University

Petr Kazarin

Purdue University

Michael Sinanis

Purdue University

Nithin Raghunathan

Purdue University

Alina Alexeenko (✉ alexeenk@purdue.edu)

Purdue University

Dimitrios Peroulis

Purdue University

Research Article

Keywords: lyophilization, Temperature

Posted Date: December 7th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-1093493/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Virtual Thermocouple: A Non-Invasive Multipoint Product Temperature Measurement for Lyophilization

Xiaofan Jiang^{1,+}, Petr Kazarin^{2,4+}, Michael Sinanis¹, Nithin Raghunathan⁴, Alina Alexeenko^{2,3,4,*}, and Dimitrios Peroulis^{1,4}

¹School of Electrical and Computer Engineering, Purdue University, West Lafayette, 47907, USA

²School of Aeronautics and Astronautics, Purdue University, West Lafayette, IN 47907, USA

³Davidson School of Chemical Engineering, Purdue University, West Lafayette, IN 47907, USA

⁴Birck Nanotechnology Center, Purdue University, West Lafayette, IN 47907, USA

*alexeenk@purdue.edu

+these authors contributed equally to this work

ABSTRACT

Monitoring product temperature during lyophilization is of critical importance, especially during the process development stage, as the final product may be jeopardized if its process temperature exceeds a threshold value. While conventional thermocouples can track product temperature, they are invasive and can significantly alter the freezing and drying behavior. In this work, a new methodology for non-invasive product temperature monitoring and drying behavior during the entire lyophilization process is proposed and experimentally validated. The method is based on a new flexible wireless multi-point temperature sensing probe that is attached to the outside of the vial. Combining the wirelessly-collected data with advanced multi-physics simulations allows the accurate extraction of the product temperature non-invasively.

Introduction

Lyophilization, or freeze-drying, is a commonly used and well-established process that is developed to preserve the original structure of heat-sensitive biological and/or pharmaceutical products (e.g., antibodies, peptides, vaccines, etc.) for drying and long-term storage (shelf life of pharmaceutical formulations). Freeze-drying involves ice removal from a frozen product at low pressure through a sublimation process. According to "Markets and Markets" report (<https://perma.cc/Z34R-6WX2>), the global freeze-drying market is expected to reach \$7.3 billion by 2025 - from \$4.9 billion in 2020 - at Compound Annual Growth Rate (CAGR) of 8.2%. According to the Food and Drug Administration (FDA), about 50% of over 300 FDA and EMA approved biopharmaceutical products are freeze-dried¹.

Typically, freeze-drying process is divided into three stages or steps: freezing, primary drying, and secondary drying. At the freezing stage, the solution is completely frozen. In the primary drying step, the chamber pressure is lowered and heat from the shelf is supplied to the material for the water to sublime. During this stage, most of the water content is sublimated. The secondary drying step aims to remove the bound water. In this phase, the shelf temperature is raised higher than in the primary drying phase to break any physicochemical interactions that have formed between the water molecules and the frozen material. To preserve product quality, it is necessary for the product temperature to not exceed a threshold value throughout the process and, in particular, during the primary drying stage. This threshold value is a characteristic of the specific product being freeze-dried. For amorphous products, it is often related to the glass transition temperature of the dried product. If the threshold temperature is exceeded, the final dried product may collapse, which could also result in higher moisture content, a longer reconstitution time, and an unacceptable appearance.

Accurate process condition monitoring is not only related to the threshold temperature, but is also needed to alleviate machine-to-machine and run-to-run process variations. For instance, a vial's heat transfer coefficient and resulting temperature profile are sensitive to variations across different freeze dryers as well as the spatial distribution of vials inside a given freeze dryer. Although such differences may be tolerable in laboratory-scale experiments, they can cause considerable complications in production-level machines.

Inserting miniature fine-gauge thermocouples (TCs) inside the solution to be freeze-dried is the common industry practice today². However, this technique has several issues. First, TCs inserted into the vial may affect the product during drying. This is due to the fact that the thermal distribution inside the product is altered by the relatively high thermal conductivity of the TCs'

Figure 1. Prototype flexible temperature multi-point sensing probe: (a) schematic, and (b) real prototype. The sensors are attached to 6R vials whose height and diameter are 40 mm and 22 mm respectively. Each sensing probe contains 5 sensing elements. The distance between two adjacent sensing elements is 2 mm.

metallic wires with respect to glass conductivity. Second, when a TC comes into direct contact with the drying material, it acts as a nucleation site, thus altering the nucleation process. This may lead to a different structure of the frozen cake. Indeed, while the presence of TCs does not significantly alter the cake structure under non-GMP conditions³, differences can still be observed in the drying behavior in the vials with and without TCs. Furthermore, it should be pointed out that conventional thermocouples measure temperature only in specific points, which do not necessarily represent the entire product volume. This results in correct product temperature measuring only for a part of the primary drying stage⁴. Also, a thermocouple's position inside a vial strongly affects temperature reading. Demichela et al. pointed out that operational errors in thermocouple positioning could cause non-trivial temperature measurement uncertainties⁵. Despite these problems, conventional TCs are commonly used to estimate parameters of interest that cannot be measured directly, such as position and temperature of the moving front^{6,7}.

More advanced approaches have been proposed to monitor product temperature of individual vials during the freeze drying process. A non-invasive temperature monitoring method with thin-film thermocouples (TFTCs) was proposed by Oddone et al.⁸. The proposed method measures vial temperature with TFTCs printed on the outside of the vials. However, this approach does not address two crucial problems. First, the measured temperature is only recorded on the outside vial wall. Hence, it does not represent the actual temperature of the product. Second, TFTCs still require metallic wires to operate, which could cause unintentional heating that may alter the drying process. In our previous work⁹ we proposed a wireless solution based on low-power sensing electronics to measure product temperature. This approach resolves the TC-induced heating concern while still allowing for direct product measurement. However, the sensing is invasive and may interfere with the freeze-drying behavior. Ravník et al. proposed a numerical model to simulate the lyophilization process in a vial¹⁰. The model demonstrated a reasonably good agreement with experimental results. However, such modeling is highly dependent on pre-calibration/tuning of parameters (e.g., the heat transfer coefficient(k_v)) that can vary significantly from vial-to-vial, run-to-run, and machine-to-machine. Consequently, although such a modeling-only approach may be helpful in lab-scale-sized experiments, it is not suitable for large-scale experiments with hundreds of thousands of vials.

In this article we present a new technology called "Virtual Thermocouple" that allows for a non-invasive and fully wireless measurement approach that overcomes the main above-mentioned limitations. This technology comprises three main parts: a) the flexible non-invasive multi-point sensing probes that are externally attached to the vials, b) the low-power wireless electronics that read and transmit data wirelessly, and c) the numerical model that translates the temperature profile measured from the vial wall to the actual product temperature. In this study, we demonstrate that the proposed method can effectively be used for non-invasive real-time monitoring of the drying dynamics and product temperature during the freeze-drying process.

Methods

The non-invasive wireless process tracking system has been designed to monitor a freeze-drying process across the entire batch with near-zero interaction with the actual product. This is achieved by monitoring temperature at various locations and tracking the sublimation front of the lyophilization process. This method relies on a) attaching flexible temperature sensing probes to the outside of the vial and b) using multiphysics simulation to extract the temperature of the product inside the vial.

Figure 2. Fabrication process of the flexible Kapton sensors. a) manufacturing steps, b) manufactured sensor substrate.

Flexible Sensing Probe Design

A flexible multi-point sensing probe is designed and fabricated using photosensitive lithography and chemical etching. The manufactured device is capable of extracting information concerning the temperature of the product during the lyophilization process. Figure 1 shows a concept and several realized prototypes of the proposed sensor. Parvis et al.¹¹ explored sputtered thermocouples on vial walls, but this approach requires a reasonably complex manufacturing method of the sensor on the vial structure. An established, large-scale manufacturing method for standard electronic components is considered to produce the proposed flexible sensor. The proposed sensing device will not require any vial modifications. This approach provides the ability to use the sensing element multiple times and with different vial sizes. In addition, multiple NTC (Negative Temperature Coefficient) thermistors mounted to the flexible substrate allow measuring temperature at various heights across the vial. The end-user can revise the design accordingly to the vial dimensions used. In this paper we include 5 sensing elements in each sensing probe with the bottom element placed at the base of the vial. The distance between two adjacent sensing probes is 2 mm.

Figure 2 shows the employed fabrication process for creating the flexible temperature sensors. Sensors are fabricated on copper Kapton laminate Pyralux AP8555R by DuPont. The substrate thickness is 0.127 mm and the copper thickness is 0.018 mm. The copper is patterned using a photosensitive lithography microfabrication processes. Specifically, we used negative dry film photoresist TentMaster TM200i by DuPont hot rolled on the flexible substrate and exposed to 14 mW/cm² of UV light through a photomask using the MA6 Karl Suss aligner. We also used the Copper etchant CE-100 by Transene to form the desired copper traces at the end of the manufacturing step shown in Figure 2(b). The sensor assembly can be transferred on the outside or inside of the vial depending on the application as shown in Figure 1.

Temperature sensing element

The NTC thermistor is a small-footprint (0.4 mm × 0.2 mm) electronic component used to sense the product temperature. This thermistor is constructed of metal oxides, which when passed through a sintering process, give a negative electrical resistance (R) dependence versus temperature (T). Due to having a large negative slope, a small temperature change will causes a substantial change in electrical resistance at lower temperature. The disadvantage of such a thermistor is its nonlinear characteristic. Consequently, each thermistor has to be calibrated to ensure measurement accuracy. The Steinhart-Hart(S-H) equation is the most commonly used model to describe the nonlinear characteristic of the thermistor as shown below.

$$\frac{1}{T} = A + B \ln(R) + C (\ln(R))^3 \quad (1)$$

The symbols are as follows: T is the temperature in degrees Kelvin, $\ln(R)$ is the natural logarithm of the measured resistance of the thermistor, and A , B , and C are constants.

Figure 3. Example of measured and fitted resistance response vs. temperature of the 10K NTC thermocouple.

To obtain the values of these constants each thermistor is used at three different temperatures: 20°C, 0°C, and -40°C. Subsequently, we fit the coefficients of a third-order polynomial in the log-resistance values to best match the inverse-temperature values (Figure 3).

Low-power Wireless Sensing electronics

Figure 4 shows the block diagram of the low power wireless sensing electronics. Similar to previous work^{9,12,13}, the nRF52832 system-on-chip from Nordic semiconductor¹⁴ is employed to process and transmit the measurements via a 2.4 GHz radio link¹⁵. The sensing electronics are powered by the P2110B RF harvester from Powercast¹⁶ which stores the harvested RF energy into a supercapacitor. Temperature sensing also utilizes the built-in 12-bit successive-approximation analog-to-digital converter (SAADC). The temperature sensing thermistors are connected to a 97kΩ load resistor. Each voltage dividing circuit is independent for each thermistor and is independently powered by the general-purpose input/output (GPIOs) pins from the micro-controller. The bridge voltage from each voltage-dividing circuit is connected to an 8-to-1 multiplexer, a pre-gain amplifier, and then measured by the built-in 12-bit ADC (0.6 V reference voltage). During operation, the micro-controller dynamically adjusts the pre-gain amplifier for each temperature sensor to counter the nonlinear characteristic of the thermistor and avoid voltage saturation.

Modeling and Simulation

To understand the temperature profile measured by the multi-point flexible sensing elements, we create a numerical model for the primary drying stage of the solution in a vial using the COMSOL multiphysics¹⁷ software. The model allows to obtain the temperature distribution on the vial surface as well as inside the vial (product temperature). The simulation results are validated against the actual measurements and further investigated.

In the proposed model we numerically solve the transient (time-domain) heat and mass transfer problem during the primary drying phase of the product in a glass vial. In addition, the height variation of the product and vial temperatures as well as the position of the sublimation front are predicted. The geometry and the boundary conditions for the 2D axisymmetric problem statement are shown in Figure 6. The vial is initially filled with frozen mannitol (5% v/v solution). When the simulation starts, this is split into the frozen zone (96% of total volume) and the dried zone (4% of total volume).

Several heat transfer mechanisms are accounted for in this model: convective heat fluxes from ambient, heat exchange between vial, dried/frozen product, and shelf. The heat transfer equations for the ice region without convection and for the dried region with convection are solved. The mass transfer is resolved using Darcy's law and the vapor density is calculated with the ideal gas law. The heat exchange with the surrounding air and the shelf where the vial resides is considered through the heat transfer coefficients. The dried and frozen regions are assumed to be homogeneous and the presence of the inert gas during the primary drying process is neglected. The chamber pressure is set at the top of the vial. The temperature at the sublimation

Figure 6. Heat transfer mechanisms between the vial, product, shelf and ambient during the primary drying stage of lyophilization process.

The Stefan condition is applied to get interface velocity:

$$v_S = \frac{Q_S}{\epsilon \rho_{ice} L_S} \quad (3)$$

where Q_S is the normal heat flux jump at the interface. This is evaluated using the Lagrange multiplier with enabled weak constraints. The transient analysis with the deformed geometry interface is performed to track the ice surface inside the vial (Figure 6).

Experimental Setup

Freeze-drying runs were performed in a laboratory-scale freeze-dryer (REVO, Millrock Technology, Kingston, NY) located at the LyoHub research lab, Purdue University as shown in Figure 5. The freeze-dryer is equipped with a vacuum capacitance manometer and a Pirani gauge pressure sensor. A 915-MHz monopole antenna is mounted on the side of the chamber for wirelessly powering the sensors. Also, to prevent leaks and protect the coaxial cable from the vacuum during freeze drying, a custom vacuum feed-through SMA connector is used to pass the RF coaxial cable inside the chamber to power the antenna. The data-collecting computer is also equipped with a 2.4-GHz ANT-connectivity USB stick for enabling the needed sensor connectivity.

With this setup, three sets of freeze drying experiments are performed to evaluate the flexible temperature sensor performance. Each set focuses on exploring a different scenario as described in the next paragraphs. In addition, experiments in each set are repeated at least three times to provide reliable data. Predefined freeze drying recipes (Table 1) are used in all three runs in 6R SCHOTT[®] pharmaceutical vials with 4 ml filled with 5% D-mannitol solution (Sigma Chemical Company, Germany). Type T conventional thermocouples from Omega were used to measure the shelf temperature, air temperature, and product temperatures for all three experiments.

The first set of experiments (Figure 7(a)) focuses on establishing proper sensor performance on two vial types. Specifically, we test the sensors on two different types of vials made of glass (6R SCHOTT[®] vials) and plastic (SiO_2 vials). In each vial type we also insert conventional thermocouples (TCs) at the bottom-center location to measure the product temperature. A Thermal IR camera (FLIR Lepton[®] 3.5) is used to monitor the freezing behavior of the product.

Figure 7. Experimental setups: (a) Two isolated vials (glass and plastic) with a thermal camera (b) 2 center vials equipped with virtual thermocouples placed in the center of a full tray. (c) experimental set-up for testing the thermocouple heating

Table 1. Freeze drying recipe for 5% w/v mannitol solution in 6R Schott vials

Freezing step	1	2	3	4
Shelf setpoint [$^{\circ}\text{C}$]	20	20	-45	-45
Time [min]	0	10	180	120
Primary drying				
Shelf setpoint [$^{\circ}\text{C}$]	-45	20	20	
Time [min]	5	60	1800	
Vaccum setpoint [mTorr]	60	60	60	

The second set of experiments (Figure 7(b)) focuses on evaluating the performance of the virtual thermocouple in realistic freeze drying conditions. In this set, two vials equipped with the virtual thermocouples, as well as with conventional TCs, are placed in the center of the tray. The tray includes a total of approximately 400 vials.

In the third set of experiments (Figure 7(c)), four vials equipped with the virtual thermocouples are placed next to each other in the center of the tray. Unlike the first and second sets, only the center vial (red circle in Figure 7(c)) is also equipped with a conventional TC. The purpose of this set is to evaluate the conventional TC heating effects with the help of the proposed virtual thermocouple.

Results

First set of experiments: Flexible Sensing Elements Measurements for Glass and Plastic Vials

Figures 8a, 8b show the temperature profile as measured by the five sensing elements of the virtual thermocouple during the freezing stage of the first set of experiments for the glass and plastic vials. In both cases, the bottom sensing element reads the lowest temperature, while the top element shows the highest. This is expected since the bottom sensing element is placed right at the bottom of the vial, which is closest to the shelf. The thermal camera shots for the glass and the plastic vials are also depicted (Figure 8).

In both vials, thermal image #1 shows the moment right before nucleation occurs. As can be seen in Figure 8a and 8b, uncontrolled nucleation starts right after #1 and results in a sharp rise in temperature (image #2). Both moments are captured by the thermal camera for both vials. However, due to the different thermal conductivity of glass and plastic, the two temperature profiles captured by the sensing elements are different. For the glass vial, all sensing elements quickly rise to -2°C , just slightly below the product temperature. On the other hand, for the plastic vial, the flexible sensing elements reach lower temperatures up to -5°C .

In addition, the post-nucleation temperature profiles of the two vials are different as well. As the sensing elements indicate

Figure 8. Temperature profile measured by the sensing elements and thermal camera shots (5 moments of time) for the glass and the plastic vials for freezing stage of 4ml 5% Mannitol solution in 6R Schott vials.

on point #4, the glass vial is cooled from the bottom. Temperature is gradually increasing from the bottom to the top of the vial. On the other hand, such a cooling profile was not observed in the case with the plastic vial. The product seems to freeze uniformly inside the plastic vial. These results show that flexible sensing elements successfully capture the freezing dynamics in both vials.

Second Set of Experiments: Virtual Thermocouple Performance

Multi-physics Simulation

We model the primary drying stage and compare the "virtual thermocouple" readings with actual experimental data. A full shelf of 6R vials (403 units) filled with 4ml 5% Mannitol solution is freeze-dried in the REVO Millrock lyophilizer. The chamber pressure is set to 60 mTorr and shelf temperature to 20°C. Figure 11 demonstrates the simulated sublimation front position with computational mesh and temperature fields of the vial and the product for three moments of time. The porous and solid domains are meshed with a structured mapped grid while the vial domain is meshed with an unstructured grid. The simulation starts with a uniform initial temperature of 228K for vial and product and then the front advances downwards. The automatic re-meshing of the whole geometry occurs when cells' distortion reaches a certain level. The sublimation stops when the front touches the bottom of the vial after 15.7hr. During the primary drying process, the vial heats the product making the front propagate faster in the vicinity of the vial wall and it becomes convex. The product and the vial temperatures increase as the simulation advances due to several heat transfer mechanisms described above.

Virtual Thermocouple Measurements

Figure 9 illustrates the recorded vial #7 (position in the tray is shown in Figure 7(b)) temperature profile during primary drying for a 5% w/v mannitol solution, monitored by two non-invasive flexible sensing elements and two 36 gauge conventional thermocouples placed in the same vials respectively. Also, process data including shelf temperature, air temperature, Pirani/capacitance manometer pressure measurement were recorded. During this run, the predefined freeze drying recipes (Table 1) are used with shelf temperature set at 20°C and chamber pressure of 60 mTorr. At the beginning of the primary drying, the shelf temperature rises from -20°C to 20°C. This causes a sharp increase in vial temperature, as observed in both the sensing elements and conventional thermocouple readings. At the beginning of primary drying (after 8 hours in Figure 9),

Figure 9. Primary drying stage process parameters for recipe described in Table 1. CM - Capacitance Manometer readings and Pirani - Pirani Gauge readings, T_{sh} - shelf temperature, T_{air} - air temperature in the chamber and measured product temperature: TC - thermocouple readings and color coded flexible sensor readings of 6R SCHOTT® vials filled with 4ml 5% Mannitol solution.

as the product temperature rises, the bottom sensor shows the highest reading and the top sensor shows the lowest. As the primary drying continues and the sublimation front progresses, this trend reverses (inflection point) and the top sensor reading overpasses the top-mid, mid, mid-bot, and bottom sensor ones. As shown in Figure 9 is clearly captured by sensing elements readings. The endpoint of primary drying can be determined based on the Pirani pressure and capacitance manometer pressure measurements¹⁸. The primary drying ends as the Pirani reading converges to capacitance manometer measurement. All temperature sensing elements showed very good agreement in the temperature readings over time profile with the data obtained from the thermocouples. It is interesting to note that both multi-points temperature sensing elements indicated an early increase in temperature at the end of primary drying relative to conventional thermocouple data which identify the vials' walls heating.

Virtual Thermocouple and the Tuning Process

The performance of virtual thermocouple was validated using data from the performed freeze-drying experiments as mentioned in previous sections. To obtain the product temperature inside the vial, the numerical model was tuned to match the sensing element data during the primary drying stage demonstrated in Figure 9. As a result, the numerical thermocouple reading should be close to the product temperature measured by conventional thermocouple in the experiment which would mean the good performance of virtual thermocouple. To simplify the tuning process, input parameters were divided into three groups: the first group is the fixed simulation parameters (Table 2). These are parameters that are not subject to change from run to run for the same product (such as glass vial properties, material properties (i.e. dried product properties), and ice/vapor characteristics). The second group are the process simulation parameters (Table 3). These parameters are the real process data including shelf/air temperatures (measured with conventional thermocouples) and chamber pressure (measured with capacitance manometer) which are directly used in the model. The last group are the tuned process parameters (Table 4) are the parameters that vary from vial to vial (i.e. heat transfer coefficients). They are tuned to match the virtual thermocouple output with the actual data

Figure 10. Temperature sensing elements readings vs. virtual thermocouple reading at the vials' walls and inside the vial during primary drying stage for three sensors at the center vial #7 (schematic position of the vial is shown in Figure 7 (b))

Figure 11. Simulated sublimation front position (purple curve) with computational mesh and temperature fields of the vial and the product for 0, 8 and 15 h.

from sensing elements. The vapor viscosity was calculated using the expression derived by Alexeenko et al.¹⁹ where the experimentally measured^{20–23} values as well as the data from The International Association for the Properties of Water and Steam Formulation²⁴ were utilized for water vapor viscosity in the range of -23°C to 227°C . The power-law curve fit based on Variable Hard Sphere (VHS) molecular model with an effective diameter of $5.78\text{\AA}$:

$$\mu = 8.9007 \times 10^{-6} \left(\frac{T}{273.15} \right) [\text{Pa} \times \text{s}] \quad (4)$$

The solid lines in Figure 10 show the temperature profiles measured by sensing elements. The simulation is performed for

(a) Temperature time history profiles measured by flexible sensors (solid) vs. simulated temperature readings (dashed).

(b) Temperature time history measured by the conventional thermocouple in experiment (solid) vs. numerical thermocouple reading (dashed)

Figure 12. Virtual thermocouple performance evaluation for the central vial #6 (schematic position of the vial is shown in Figure 7 (b)) during primary drying stage.

(a) Temperature time history profiles measured by sensing elements (solid) vs. numerical thermocouple readings (dashed).

(b) Temperature time history measured by the conventional thermocouple (solid) vs. numerical thermocouple reading (dashed).

Figure 13. Virtual thermocouple performance evaluation for the central vial #7 (schematic position of the vial is shown in Figure 7 (b)) during primary drying stage

two vials: vial # 6 and vial # 7 as indicated in Figure 7(b). Both vials are surrounded by 6 other vials and can be considered as center vials. In both cases, the simulation is within 1°C of the experiment. The experimental readings of the air temperature in the vicinity of the vial as well as actual shelf temperature are used in the simulation. Figure 10 shows the measurements from sensing elements versus the virtual thermocouple measurements for vial #6 and 3 sensors: top, middle and bottom sensors. The close agreement between these readings is demonstrated.

Figure 13b shows the temperature profile that the numerical thermocouple readings match the conventional thermocouple readings after the model is tuned to match the sensing elements data of vial #6. With the heat transfer coefficients tuned to 9 and 12 W/m²/K for the center and the edge of the vial bottom correspondingly. Also, 0.2W/m²/K heat transfer was applied to the top part of the vial above the product during the tuning process. The sensing elements temperature readings and simulations results are shown for both vials in Figures 12a,13a. The simulation (dashed lines) are within 1 – 2 ° from the experimental data during the whole period of primary drying. The deviations close to the end of primary drying are due to the artificial criteria of the end of the process in simulation. The process is assumed to be over when the minimum distance between the freezing front and the vial bottom is close to zero. Thus, when the edge of the sublimation front reaches the bottom of the vial, the simulation stops. As shown in Figure 13b, the numerical thermocouple temperature data shows a great agreement with the conventional thermocouple reading. The same tuning process was done to vial #7. Figure 12b shows the conventional thermocouple vs. numerical thermocouple readings for this vial. The heat transfer coefficients equal to 8 and 11 W/m²/K were calibrated for the center and the edge of the vial's #7 bottom in the simulation to match the experimental data. Thus, virtual thermocouple is shown to measure the actual product temperature accurately and non-invasively.

Parameter	Dimension	Value
Ice Heat Capacity	J/Kg/K	1967.8
Product Heat Capacity	J/kg/K	1715
Vapor Heat Capacity	J/kg/K	1674.7
Latent Heat of Sublimation	J/kg	2.838×10^{-6}
Ice Thermal Conductivity	W/m/K	2.1
Product Thermal Conductivity	W/m/K	0.028
Vapor Thermal Conductivity	W/m/K	0.025
Vapor Molar Mass	g/mol	18
Vapor Viscosity	Pa × s	Equation 4
Ice density	kg/m ³	913
Product density	kg/m ³	75
Silica Glass Heat Capacity	J/kg/K	830
Silica Glass Density	kg/m ³	2230
Silica Glass Thermal Conductivity	W/m/K	1.14

Table 2. Fixed Simulation Parameters

Parameter	Dimension	Value
Air Temperature	K	Exp.
Initial Temperature	K	228
Shelf Temperature	K	Exp.
Chamber Pressure	mTorr	70

Table 3. Process Simulation Parameters

Figure 14 shows the mass transfer resistance calculated for a dried cake of 5% mannitol solution and compared with empirically obtained expression by Pikal et al.²⁵ as a function of the dried thickness or cake thickness L_{ck} as:

$$R_p = A_0 + \frac{A_1 \times L_{ck}}{1 + A_2 \times L_{ck}} \quad (5)$$

where $A_0 = 1.4$, $A_1 = 16$, $A_2 = 0$.

Parameter	Dimension	Value
Product permeability	m^2	3×10^{-6}
Vial bottom heat transfer coefficient (in/out)	$W/m^2/K$	Variable
Porosity	—	95%

Table 4. Tuned Simulation Parameters

The cake resistance from the current simulation is calculated according to²⁶:

$$R_p = \frac{A_p \times (P_{sub} - P_{ch})}{\dot{m}_{ice}} \quad (6)$$

where A_p is a product area, P_{sub} and P_{ch} are sublimation front and chamber pressures, $\dot{m}_{ice}$ is an ice sublimation rate. The R_p is a measure of vapor flow impedance resulting from the dried layer structure. It is worth noting that in the current multiphysics simulation, the product permeability is the parameter analogous to R_p .

Figure 14. Product resistance of calculated based on the simulation of primary drying stage of 4ml 5% Mannitol solution in 6R Schott vials.

Third set of experiments: Conventional Thermocouple heating

With the ability to measure the product temperature close to the center of the vial bottom during primary drying, we utilize the power of virtual thermocouple to investigate the effects of conventional thermocouple heating. Figure 7(c) shows the setup of this experiment, where 3 vials equipped with a virtual thermocouple were placed at the center of a full tray (green dots in Figure 7(c)), surrounding a vial equipped with both virtual thermocouples as well as conventional thermocouples. This effect is demonstrated in Figure 15. The temperature at the walls of four vials in the center of the shelf was measured using sensing elements. For each of the vials, the simulation was performed and heat transfer coefficients were adjusted so that the best agreement between experimental sensing elements readings and simulation is achieved. From Figure 15, it can be seen that a perfect agreement between the vial 10 conventional thermocouple measurement and numerical thermocouple simulation is reached. For other vials, the heat transfer coefficient was tuned to get the experiment/simulation agreement. Figure 15 shows 4 numerical thermocouples readings in 4 vials as well as one conventional thermocouple reading in vial # 10. Taking into account the perfect match between conventional/numerical thermocouple readings in vial#10, one can conclude that the difference between the actual product temperature and the one registered by conventional thermocouple can be up to 3° and is caused by the presence of the conventional thermocouple in a vial. Thus, the use of flexible sensors allows performing actual temperature measurements.

Figure 15. Product temperature profiles during primary drying stage. Virtual thermocouple readings for vials without thermocouple (Vial 6,7,8), vial with thermocouple (Vial 10 with TC) and experimental thermocouple readings (TC Experiment).

Discussion

The development of optimal lyophilization procedures for different formulations in vials includes a combination of experimental tests and computational approaches for measuring product temperature. Tight temperature control is essential in both the freezing and primary drying steps because the structure of the dried product (cake) is determined by the freezing and primary drying protocols. To obtain the uniformly dried product across the batch, one needs to accurately control the temperature during these stages. Particularly, the nucleation of ice during the freezing stage should occur in a tight temperature interval. Most importantly, the product temperature during the primary drying stage must be kept safely below the collapse temperature. Due to the presence of bound water in the product after the primary drying stage, the collapse temperature can be relatively low. Moreover, in order to optimize the process and reduce the primary stage duration, the critical process parameters should be controlled accordingly. Along with the chamber pressure, shelf temperature is one of such parameters which defines the design space for the primary drying stage of the freeze-drying process. Traditionally, the shelf temperature depends on the temperature of heat transfer fluid (i.e. silicon oil, methylene chloride, etc.) inside the shelves which is tracked by the control system and is set to follow the pre-set profile. However, the heat transfer control obtained by the control and manipulation of the shelf temperature is quite slow, partly because of the thermal inertia of the system, due to which shelf heating and cooling may induce a huge lag in the response of the product temperature. Alternatively, the chamber pressure of the dryer can be controlled and manipulated. This is a very responsive way to control the drying process because the heat flux from shelf to product strongly depends on chamber pressure. However, this approach can be quite risky, because the product temperature practically follows the pressure variations, therefore changes of few pascals could easily jeopardize the product quality.

Since the critical part of any lyophilization procedure is the primary drying phase, special attention has to be paid to critical modeling parameters of drying of a porous cake-solid ice system. In this work, a new technology, virtual thermocouple, based on the use of flexible multi-point temperature sensor and advanced multi-physics simulation was proposed and investigated as a means for the monitoring of freezing and drying behavior and product temperature during freeze-drying process. The developed virtual thermocouple combining the one-dimensional model with surface sublimation sub-model can be used as a stand-alone, fast and accurate computational tool for the prediction of lyophilization dynamics, but can also be included into a general 3D CFD computational framework as a vital part of the final virtual lyophilizer model. The proposed virtual thermocouple was also found to give quantitatively accurate results for drying behavior. In particular, the flexible multi-point sensing elements can give information about both the temperature profile and the position of the sublimating interface. This information then combined with the advanced multi-physics simulation provides the actual product temperature and shows a great matching with conventional thermocouple measurement. For the first time, the ability to non-invasive monitoring product temperature of individual vials during primary drying was demonstrated. This proves that the proposed virtual thermocouple technology can effectively track the profile of temperature within the volume of the solution of an individual vial during the

295 freeze-drying process.

296 References

- 297 1. Corver, J., Bockstal, P.-J. V. & Beer, T. D. A continuous and controlled pharmaceutical freeze-drying technology for unit
298 doses. In *AIChE Annual Meeting* (2017).
- 299 2. Nail, S. *et al.* Recommended best practices for process monitoring instrumentation in pharmaceutical freeze drying—2017.
300 *Aaps Pharmscitech* **18**, 2379–2393 (2017).
- 301 3. Bosca, S., Barresi, A. A. & Fissore, D. Use of a soft sensor for the fast estimation of dried cake resistance during a
302 freeze-drying cycle. *Int. journal pharmaceutics* **451**, 23–33 (2013).
- 303 4. Patel, S. M., Doen, T. & Pikal, M. J. Determination of end point of primary drying in freeze-drying process control. *Aaps*
304 *Pharmscitech* **11**, 73–84 (2010).
- 305 5. Demichela, M., Barresi, A. A. & Baldissoni, G. The effect of human error on the temperature monitoring and control of
306 freeze drying processes by means of thermocouples. *Front. chemistry* **6**, 419 (2018).
- 307 6. Velardi, S. A., Hammouri, H. & Barresi, A. A. In-line monitoring of the primary drying phase of the freeze-drying process
308 in vial by means of a kalman filter based observer. *Chem. Eng. Res. Des.* **87**, 1409–1419 (2009).
- 309 7. Bosca, S., Barresi, A. A. & Fissore, D. Use of soft sensors to monitor a pharmaceuticals freeze-drying process in vials.
310 *Pharm. Dev. Technol.* **19**, 148–159 (2014).
- 311 8. Oddone, I., Fulginiti, D., Barresi, A. A., Grassini, S. & Pisano, R. Non-invasive temperature monitoring in freeze drying:
312 control of freezing as a case study. *Dry. Technol.* **33**, 1621–1630 (2015).
- 313 9. Jiang, X. *et al.* Multi-point wireless temperature sensing system for monitoring pharmaceutical lyophilization. *Front.*
314 *chemistry* **6**, 288 (2018).
- 315 10. Ravník, J. *et al.* Lyophilization model of mannitol water solution in a laboratory scale lyophilizer. *J. Drug Deliv. Sci.*
316 *Technol.* **45**, 28–38 (2018).
- 317 11. Parvis, M., Grassini, S., Fulginiti, D., Pisano, R. & Barresi, A. A. Sputtered thermocouple array for vial temperature
318 mapping. In *2014 IEEE International Instrumentation and Measurement Technology Conference (I2MTC) Proceedings*,
319 1465–1470 (IEEE, 2014).
- 320 12. Raghunathan, N., Jiang, X., Peroulis, D. & Ganguly, A. Wireless low-power temperature probes for food/pharmaceutical
321 process monitoring. In *2015 IEEE SENSORS*, 1–4 (IEEE, 2015).
- 322 13. Raghunathan, N., Jiang, X., Ganguly, A. & Peroulis, D. An ant-based low-power battery-free wireless cryogenic
323 temperature probes for industrial process monitoring. In *2016 IEEE SENSORS*, 1–3 (IEEE, 2016).
- 324 14. Nordic Semiconductor, Inc. *nRF52832 ANT multiprotocol SoC* (2020).
- 325 15. Garmin Canada Inc. *ANT Message Protocol and Usage - THIS IS ANT* (2020).
- 326 16. Powercast Co. *P2110B 915 MHz RF Powerharvester Receiver*.
- 327 17. COMSOL Inc. *Freeze Drying*.
- 328 18. Gieseler, H., Kramer, T. & Pikal, M. J. Use of manometric temperature measurement (mtm) and smart™ freeze dryer
329 technology for development of an optimized freeze-drying cycle. *J. Pharm. Sci.* **96**, 3402–3418 (2007).
- 330 19. Alexeenko, A. A., Ganguly, A. & Nail, S. L. Computational analysis of fluid dynamics in pharmaceutical freeze-drying. *J.*
331 *pharmaceutical sciences* **98**, 3483–3494 (2009).
- 332 20. Smith, C. An experimental study of the viscous properties of water vapour. *Proc. Royal Soc. London. Ser. A, Containing*
333 *Pap. a Math. Phys. Character* **106**, 83–96 (1924).
- 334 21. Teske, V., Vogel, E. & Bich, E. Viscosity measurements on water vapor and their evaluation. *J. Chem. & Eng. Data* **50**,
335 2082–2087 (2005).
- 336 22. Yasumoto, I. Viscosity of water vapor in the temperature range from 6° c to 29° c. *Bull. Chem. Soc. Jpn.* **43**, 3917–3919
337 (1970).
- 338 23. Braune, H. & Linke, R. Über die innere reibung einiger gase und dampfe. *Phys Chem* **148**, 195 (1930).
- 339 24. Cooper, J. & Dooley, R. International association for the properties of water and steam: Release on the iapws formulation
340 2008 for the viscosity of ordinary water substance. *IAPWS Release, IAPWS Secr.* (2008).

- 341 **25.** Pikal, M. Use of laboratory data in freeze drying process design: heat and mass transfer coefficients and the computer
342 simulation of freeze drying. *PDA J. Pharm. Sci. Technol.* **39**, 115–139 (1985).
- 343 **26.** Pikal, M. J., Roy, M. & Shah, S. Mass and heat transfer in vial freeze-drying of pharmaceuticals: Role of the vial. *J.*
344 *pharmaceutical sciences* **73**, 1224–1237 (1984).

345 **Acknowledgements**

346 This work was funded by the National Science Foundation Grant #1827717: Sensors, Computational Modeling, and Bioanalyti-
347 cal Technologies for Closed-Loop Lyophilization.

348 **Author contributions statement**

349 D.P., X.J. and N.R conceived the sensor; N.R., M.S. and X.J. developed the sensor manufacturing method; P.K. and A.A.
350 developed the modeling approach; P.K. conducted modeling; D.P., A.A., X.J., N.R. and P.K. designed the experiments; X.J.
351 conducted experimental measurements; All authors analyzed the results and reviewed the manuscript.

352 **Additional information**

353 To include, in this order: **Accession codes** (where applicable); **Competing interests** (mandatory statement).

354 The corresponding author is responsible for submitting a [competing interests statement](#) on behalf of all authors of the paper.

355 This statement must be included in the submitted article file.