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*Antonello A. Barresi[1] , Roberto Pisano[1], Davide Fissore[1,*], Valeria Rasetto[1],*

*Salvatore A. Velardi[1],  Alberto Vallan[2], Marco Parvis[2] , Miquel Galan[3]*

1. Dipartimento di Scienza dei Materiali ed Ingegneria Chimica, Politecnico di Torino,

corso Duca degli Abruzzi 24, 10129 Torino (Italy)

2. Dipartimento di Elettronica, Politecnico di Torino,

corso Duca degli Abruzzi 24, 10129 Torino (Italy)

3. Telstar Lyo,

Josep Tapiolas 120, 08226 Terrassa (Spain)

[*] Corresponding author:
Dr. Davide Fissore
e-mail: davide.fissore@polito.it
Tel.: +39-011-0904693
Fax.: +39-011-0904699

**Abstract**

An innovative and modular system (*LyoMonitor*) for monitoring the primary drying of a lyophilization process in vials is illustrated: it integrates some commercial devices (pressure gauges, moisture sensor, mass spectrometer), an innovative balance and a Manometric Temperature Measurement system based on an improved algorithm (DPE) to estimate sublimating interface temperature and position, product temperature profile, heat and mass transfer coefficients and parameters required for control purposes and recipe optimisation. A soft-sensor using a multipoint wireless thermometer can also estimate the previous parameters in a large number of vials. The performances of the previous devices for the determination of the end of the primary drying are compared. Finally, all these sensors can be used for control purposes and for the optimisation of the process recipe; the use of DPE in a control loop will be shown as an example.

**Topical heading**

Process systems engineering

**Keywords**

Freeze-drying; monitoring; primary drying; pressure rise test; observer.

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**Introduction**

Freeze-drying (FD) is the process where water (or another solvent) is removed from a frozen solution by sublimation, thus obtaining a porous and friable structure that can be easily re-hydrated. This process is used as an alternative to traditional drying processes because of the lower operating temperatures that make it particularly suitable for those heat-sensitive materials (e.g. pharmaceuticals) that could be damaged by the higher temperature required by traditional drying treatments. Anyway, due to the slow drying rate, to the very low temperature, to the use of vacuum and, generally, to the high investment and operating costs, FD is employed only for valuable goods: FD is in fact ubiquitous in the pharmaceutical field, where working at low temperature and in sterile conditions is mandatory (Snowman, 1991; Liapis, 1987).

After freezing the product most of the solvent is removed by sublimation during the primary drying stage (PD) by exposing the frozen solution to a very low solvent partial pressure. PD has to be carried out at an optimal temperature that minimises the duration of the process, beside maintaining the maximum temperature below the upper limit corresponding to the value that causes the collapse of the solid product; this requires to monitor the product temperature and the position of the moving front.

A limitation of the present technology is the impossibility of obtaining a direct measure of the parameters of interest without interfering with the process dynamics or impairing the sterile conditions needed by some products. A thin thermocouple (or a thermoresistence) inserted in a vial is a widespread, but invasive, system used to monitor the process. This method may alter the elementary phenomena of nucleation and ice crystal growth; it has been evidenced that the freezing bias is small in the semi-clean laboratory environment, but may be much more relevant, posing scale-up problems, in the clean environment of the sterile production operation (Roy and Pikal, 1989). Measurable freezing bias may not occur for every

product, but, in any case, the insertion of thin thermocouples affects the heat transfer to the product: as a consequence, the drying kinetics is faster in the monitored vial and the results are not representative of the whole system. Nevertheless, this method has been proposed to monitor the PD and to detect the end-point of the PD stage. Finally, the probe insertion itself compromises the sterility of the product.

Moisture sensors, as well as mass spectrometry and thermal conductivity gauges, have been proposed in the past to monitor the PD (Oetjen and Haseley, 2004): they can be very useful to detect the end of the PD, but they do not provide any information about the status of the product during the operation. A technical comparison of these and other recently proposed devices is given in Mayeresse et al. (2007).

Non-invasive monitoring techniques have been recently proposed as valuable alternatives to the use of thermocouples, as they can monitor the state of the whole system. These techniques are based on the Pressure Rise Test (PRT): they use the in-line measure of the pressure rise due to the shut-off of the valve placed between the drying chamber and the condenser for a short time interval (e.g. 30 seconds): the plateau value of the chamber pressure is related to the temperature of the sublimating interface by means of a mathematical model. Various algorithms were proposed in the past to this purpose, namely the Manometric Temperature Measurement (MTM) of Milton et al. (1997), the Dynamic Pressure Rise of Liapis and Sadikoglu (1997), the Pressure Rise Analysis (PRA) of Chouvenc et al. (2004, 2005). Moreover, performing some Pressure Rise Tests (PRTs) throughout all the PD, it is possible to monitor the evolution of the product temperature and, since the slope of the curve at the beginning of the test gives the sublimation flux of the solvent, it can be used to detect the end-point of the PD.

In this paper an innovative and modular monitoring system is illustrated: it uses several commercial and proprietary sensors to monitor both single vials and the whole batch and a special balance to weigh groups of vials. In-line measures of temperature and of pressure are also used with a mathematical model of the process

to estimate those variables of interest that cannot be directly measured (e.g. interface temperature and position, full temperature profile in the product, heat and mass transfer coefficients). To this purpose, a soft-sensor (observer), that uses the measurement of the temperature in a vial, and an innovative algorithm (Dynamic Parameters Estimation, DPE) that uses the measure of the pressure during the PRT, are discussed: the former provides information about the monitored vial, while the latter gives estimations about the whole batch.

All these devices have been tested in a prototype freeze-dryer (Lyobeta 25 by Telstar) with a chamber volume of 0.2 m$^3$ and equipped with thermocouples, capacitance and thermal conductivity gauges, moisture analyser and Quadrupole Mass Spectrometer (QMS); the pressure in the chamber has been generally regulated by controlled leakage, but runs using only the valve on the vacuum pump have been also carried out. The performances of these sensors and their reliability in determining the end of the PD will be compared and discussed. Finally it will be shown how the information about the state of the system can be used in a control loop designed to minimise the drying time, beside ensuring product quality, and an example using the DPE algorithm will be presented.

**Monitoring of the primary drying stage**

As it has been stated in the Introduction, monitoring of PD stage is needed to carry out the process at a controlled temperature, thus avoiding irreversible product damages and minimising the time required by this step. Moreover, the monitoring system has to be able to detect the end-point of the PD, beyond which secondary drying has to be started. In recent years, some devices have been realised and patented by our research group in order to monitor the PD of a lyophilization process in vials. Figure 1 gives a sketch of the monitoring system that will be discussed in the following; a special software, called *LyoMonitor* (see the user

interface in Figure 2), has been realised in order to manage the various sensors and to collect their measurements; currently, the following systems are included in the prototype:

i.    a multi-point wired thermometer (see (1) in Figure 1): this instrument is composed of a set of nine copper-constantan thermocouples, a conditioning circuit and a commercial multimeter equipped with a multiplexer. The multimeter is connected to the PC #1 by means of the IEEE-488 interface and it is able to perform an auto-diagnosis test in order to detect the damaged thermocouples (see the "Test" button in Figure 2).

ii.   a new wireless thermometer (Vallan et al., 2005a): this instrument (see (8b) in Figure 1) is a modular thermometer that has been specifically designed in order to provide a large number of sensors. This can be useful, for example, for temperature mapping. No wires are required to connect the sensors placed inside the vacuum chamber to the external acquisition system. The thermometer can manage one or more measurement modules, equipped with 14 thermocouples each, and it sends the results to a "reader" placed outside the vacuum chamber and connected by means of a serial interface to a PC that schedules, acquires and collects the measurements. The reader powers the thermometer and the modules through the same radio-frequency link that is employed for the data communication, so that the modules can work without batteries. Up to 20 modules can be connected at the same time thus extending the measurement capability up to 280 temperature sensors. The modules also embed an auto-diagnosis system in order to detect damaged thermocouples.

iii.  a weighing device, proprietary and patented (Vallan et al., 2005b; Vallan, 2007) working inside the vacuum chamber and able to measure contemporaneously weigh and temperature of a group of vials during the drying process. This device is composed of a motorised balance (see (2) in Figure 1), which is able to rise and weigh up to 15 vials, and a miniaturized radio-controlled thermometer (7) that is located near the group of vials and transmits their temperatures to the

balance. In this way both mass and temperature of the same vials can be measured contemporaneously. The balance is connected to PC #1 by means of a serial interface and can be controlled and calibrated through the LyoMonitor control panel. The balance, that has been characterized by comparison with a commercial analytical balance both in vacuum and in air, has a resolution of about 10 mg and a total uncertainty of about 100 mg from –40°C to +40°C.

iv.  a valve control (6b) and an acquisition system (6a) for the PRT. The PC #1 is equipped with a Digital Acquisition Board that collects the pressure measurements during the PRT: the sampling frequency used is 10 Hz, but other values, ranging from few Hz to some kHz, can be set.

v.  pressure (5) and moisture (4) sensors: the system is able to acquire the output signal of a thermal conductivity gauge (Pirani PSG-101-S: full scale pressure = 2 bar), of a capacitance manometer (MKS Type 626A Baratron: full scale pressure = 100 Pa, accuracy = 0.001% of full scale, resolution = 0.25% of reading) and of a moisture analyser (Panametrics MMS35-131-1-100: accuracy of the M series probe in the measure of the dew/frost point temperature = ±2 °C from 60°C to  -65 °C and ±3 °C from -66°C to -110 °C; repeatability = ±0.5 °C from 60°C to -65 °C and ±1.0 °C from -66°C to -110 °C) thanks to an external multimeter that is interfaced to the PC through the IEEE-488 interface; the ratio between the signals of the Pirani and Baratron sensors can be calculated as it can be useful for monitoring purposes.

*LyoMonitor* continuously acquires and stores the measures obtained by the devices listed above: moreover some other process variables that are measured in the freeze-dryer using embedded devices (i.e. shelf and fluid temperatures, controlled-leakage valve opening and inert mass flow rate for pressure control) are acquired by *LyoMonitor* through a dedicated RS485 interface, thus providing a complete evaluation of the status of the system. *LyoMonitor* can be controlled by means of a remote PC through an Internet connection. The measuring cycle is set by the user, but also spot measurements are allowed. As it has been designed as a modular

system, other measuring devices can obviously be added to *LyoMonitor*; in particular the implementation of a new device for monitoring mixed solvent lyophilization is ongoing.

A Quadrupole Mass Spectrometer GeneSys 300 by European Spectrometry Systems (upper mass detection limit = 300 amu, detector = Faraday cup) has also been used to monitor the FD (see (9) in Figure 1); the instrument is managed by a dedicated computer (PC #2 in Figure 1).

*Monitoring of single vials*

Through a simple temperature measurement it is possible to monitor the dynamics of a single vial by means of a soft-sensor (or observer, that we called *smart vial*, see (8a) in Figure 1): it is a device that combines the *a priori* knowledge of the physical system (i.e. a mathematical model of the process) with some experimental data (i.e. in-line measurements, like temperature of the product or of the vial) to provide a real-time estimation of some parameters or state variables (Barresi et al., 2008). The smart vial consists of a special vial equipped with a thermocouple: the whole product temperature profile and the mass/heat transfer coefficients are estimated by the soft-sensor using the temperature measurement and a simplified mathematical model of the process. The main drawback of this approach is that the state estimation is limited to a single vial, but, on the other hand, the temperature estimation concerns the entire temperature profile of the product in the vial and not only the temperature in a particular point, as obtained using a thermocouple. Moreover, the results obtained for a particular vial can be compared to those obtained for other vials placed in different positions into the drying chamber, thus allowing to evaluate the heterogeneity of the batch. With this respect, the use of the multiple wireless thermometer allows easily and economically the monitoring even of a large number of vials.

The synthesis of an observer is a complex task and a lot of different approaches have been proposed in the Literature. The Extended Kalman Filter (KF) is one of the

most common techniques (Becerra et al., 2001) and it has been applied to the FD process using a simplified model: the heat transfer in the dried and frozen layer is accounted for, but heat transfer by radiation is not considered and pseudo-stationary conditions are assumed (Velardi and Barresi, 2008). The observer uses the measurement of the product temperature at the bottom of the vial to estimate the temperature and position of the moving front (and thus it can be used to detect the end-point of the PD) as well as the heat and mass transfer coefficients; Appendix 1 gives the equations of this observer. As an alternative, a High Gain (HG) observer has been designed and tested as this approach allows for a simpler mathematical formulation of the problem and the computational time required for the estimation is lower; moreover, the HG observer exhibits less sensitivity towards noisy measurements.

Both soft-sensors have been validated by numerical simulations using firstly a detailed mono-dimensional model as a source of experimental data (Barresi et al., 2008; Velardi et al., 2008a); preliminary experimental results confirm that in-line estimations are very good, as it is shown in Figure 3, which shows the prediction of the interface temperature and the calculated position of the frozen interface obtained using the KF. The quality of the estimations given by both observers has been verified to be roughly the same, but the computational effort requested by the KF is higher and its tuning is quite tricky, while the estimations of the HG observer are provided faster and the tuning is simpler.

These observers can be used to monitor the behaviour of several vials located in different positions on the shelf. Figures 3b and 3c show an example of the estimations of the time evolution of the interface temperature and of the temperature profile at a certain instant in four different vials. In Figure 3b the front temperature of several vials estimated by the KF is compared with the interface temperature calculated using the DPE algorithm (that will be described in the following): it can be remarked that DPE gives an average product temperature of the whole batch while the observer estimates the temperature in the single vial monitored. In Figure 3d the

moving front position estimated by the KF for the vial #2 is compared to that estimated by DPE: the agreement between the two profiles is good, as it can be seen also for the interface temperatures given in Figure 3b. This is due to the fact that DPE estimates a mean value of the interface temperature and position for the whole batch and most of the vials have a behaviour similar to that of vial #3, as this vial is placed in the central part of the tray.

Since the insertion of a probe, although extremely tiny, in contact with the product should be avoided because of the various troubles mentioned in the Introduction, another observer has been designed, exploiting the measurement of the external temperature at the bottom of the vial (Barresi et al., 2007; Barresi et al., 2008; Galan et al., 2007) and using a different simplified mathematical model that takes into account also the heat transfer along the glass vial (Velardi and Barresi, 2008).

Other indirect methods that have been proposed in the past to monitor single vials are briefly reviewed in the following: these are alternatives to the collection of samples inside the freeze-drier chamber and direct weighing, a technique that is generally applicable only in small laboratory apparatuses, and that has several drawbacks.

NMR technique was proposed by Monteiro Marques et al. (1991) to detect the end-point of PD by observing an abrupt increase in the longitudinal and transverse relaxation times.

XRD photography was proposed by Schelenz et al. (1994) to check the estimations of the temperature profile inside a vial.

Jennings and Duan (1995) proposed a different technique to calculate the duration of the PD: it is required to know the total energy necessary to carry out the PD process and to make a calorimetric measurement to calculate the heat transfer coefficient in the vial, and thus the rate of heat transport. To this purpose a differential method, called Drying Process Monitoring (DPM), is used: two thermocouples are fixed to the bottom of an empty vial and of a vial filled with the product, thus allowing to calculate the heat transfer to the filled vial used for the

sublimation of ice; a drop of the heat transfer rate at the end of the main drying can be observed (Jennings, 1999). This method requires to introduce two vials with thermocouple connections in the production charge; thus, even if more sophisticated and probably more reliable than the simple Product Temperature Response method, the DPM maintains most of its drawbacks, including obviously the fact that measuring the situation in the two special vials may not represent an average of all the batch vials.

Remmele et al. (1997), Brülls et al. (2003) and Presser (2003) investigated the use of near-infrared spectroscopy to monitor the FD in-situ exploiting the spectral changes generated by freezing, sublimation and desorption; the rate of desorption can also be determined.

Dielectric measurements, using remote electrodes for in-situ determination of water content in vials have also been proposed and tested (Suhermann et al., 2002). The evolution of electric properties has been proposed for the automatic control of the whole operation (Rey, 1961; Bouldoires, 1969; Nail and Gatlin, 1985).

*Monitoring of a group of vials*

While the sensors described in the previous paragraph allow to monitor only one vial, a balance placed directly into the vacuum chamber of the freeze dryer allows to monitor a group of vials. The direct weight measurement of a single or of a group of vials is very important as it allows to monitor the progress of PD and, thus, to detect with good accuracy the end-point of this stage and to evaluate (by differentiation) the average sublimation rate in the time interval between two successive measurements. Remarkable improvements can be obtained with respect to sample-extractors (Nail and Gatlin, 1993; Tang and Pikal, 2004), as the vacuum conditions are not modified and the weighing procedure is performed in an automatic way.

Other weighing devices previously proposed in the Literature for monitoring FD processes were affected by several problems. Bruttini et al. (1986, 1991) proposed a balance which supported the heating plate and the tray: freezing was carried out as

a separate step because when it was carried out *in situ*, the vibrations induced by fluid pulsation from the cryostat severely disturbed the measurement.

Another relevant drawback about weighing devices is that the measurement could not be representative of the process: in the capacitive balance proposed by Rovero et al. (2001) the heat transfer to the product is limited by the volumetric gap that acts as an additional resistance when heat is transferred through the shelf by conduction, while it works efficiently in case of radiative heating. Moreover, most of the balances so far proposed requires glass vials with a specific geometry that not always corresponds to that of the vials of the batch; the measure is limited only to that special vial and thus the measurement can be not fully representative of the whole system (Christ, 1994; Roth et al. 2001).

On the contrary, the balance managed by *LyoMonitor* is able to monitor vials that have the same geometry of those of the batch; moreover, since these vials are almost always in contact with the shelf and they are lifted just during the measurement, the thermal exchange between the vials and the surface is not significantly affected and, therefore, the measurement is representative of the whole batch. The balance is currently equipped with a mobile tray where up to 15 small vials can be placed, but can be adapted easily to larger vials; mass measurements are stored on PC through a standard serial interface. Finally, a radio controlled thermometer, connected to the balance tray, has been designed in order to measure also the temperature of these vials, without altering the mass measurement because of the force transmitted by the thermocouple wires (see (7) in Figure 2).

A series of tests has shown that the weighing frequency can be chosen in a wide range without affecting the process, but both the monitored vials and the balance case must be properly shielded to avoid systematic errors due to radiation effects from the walls (Pisano et al., 2008); if this is not done, the vials lifted by the balance are in a condition similar to that of the vials at the sides of the batch, where radiation effects are much more important, and the balance response can be considered representative of this fraction of vials. This device has been tested with various FD

cycles performed in different working conditions: a good agreement between the time evolution of the values of mass and temperature has been evidenced during these runs. Examples of the results obtained using this balance are given in Figures 2, 4 and 5; in Figure 4 the measure of the temperature obtained by means of thermocouples placed at the bottom of some vials is also shown for comparison, together with the calculated average sublimation rate. When the mass of the vials weighed by the balance becomes almost constant it means that the end-point of PD has been reached; of course, the calculation of the sublimation rate from the derivative of the mass measurement increases the sensitivity and allows to determine the end of PD with good accuracy, generally better than other methods, as shown in the upper graph of Figure 5 (that will be discussed in the following). The end of the sublimation phase in Figure 4 is confirmed by the strong increase of the product temperature occurring at the same time: it is well known, in fact, that in correspondence of the end-point of the PD there is a relevant increase of the product temperature measured at the bottom of the vials due to the loss of the thermal contact between the sensor and the ice; moreover, the product temperature increases as there is no more an endothermic sublimation process that uses the heat supplied by the heating shelf.

It must be noticed that the conditions of the three runs are very different: in the runs shown in Figures 2 and 5, in fact, the contribution of radiation is quite limited, and in particular is very low in the first, as the vials are shielded, and moderate in the second one, with few vials loosely distributed on the shelf: in these conditions consistent results can be obtained using either the balance or a MTM approach, as will be shown in the following. In the case of Figure 4, on the contrary, the shelf temperature is very high and therefore the heat supplied by radiation from the upper shelf is also very high; as a consequence, all MTM-type approaches proposed up to now, included the DPE, fail because they are based on a model that assumes the main heat contribution from the shelf, while in this case it is expected that the product temperature increases toward the top. The balance can works effectively also

in these conditions, determining both the sublimation rate and the end of PD, even if in conditions of strong radiation the uniformity of the batch can be more problematic. In the case shown in Figure 4, analysing the solvent flux curve obtained from mass weighing, it can be noticed that there is a fast increase at the beginning as a consequence of the product temperature rise and, after 4 h from vacuum, it slowly decreases: this could be related to collapse phenomena that occur when the product temperature increases above the glass transition temperature (equals to 254 K for the formulation considered). Then, the sublimation rate remains constant until nearby the end-point of PD, when it suddenly starts decreasing. In the case shown in Figure 5 the shelf temperature is much lower, and this strongly reduces the sublimation rate: it can be noted that after the pressure reduction it increases up to a maximum, following the increases in the shelf temperature, then continuously decreases.

Finally it can be pointed out that if the bottom temperature of the vial is also recorded, and the contribution of radiation from the walls and the upper shelf is limited, from the calculated average sublimation rate it is possible to evaluate the heat transfer coefficient.


*Monitoring of the whole batch*

*LyoMonitor* manages also some commercial devices that can be used to monitor the dynamics of the whole batch, namely two pressure sensors, a moisture detector and a Quadrupole Mass Spectrometer.

Chamber pressure can be measured using either a capacitance manometer (MKS Type 626A Baratron) or a Pirani gauge (PSG-101-S); the latter is one of the various types of thermal conductivity gauges, that have typically an accuracy of 2% of full scale, using the working principle of the Wheatstone bridge. It is much cheaper than the capacitance one, but the accuracy is generally lower and its signal depends on the gas type and, in case of mixtures, like water and inert, by the composition. Thus, the use of Pirani (and generally of all the thermal conductivity gauges) should be discouraged for monitoring FD because the chamber gas

composition continuously changes during each run and is generally different in different cycles as it depends on set up, loading and product features (Armstrong, 1980). On the other hand, taking into account the known dependence of the Pirani response on the water vapour fraction, it is possible to evaluate the partial pressure of water into the drying chamber elaborating the different signals obtained from Baratron and Pirani sensors. Moreover, as suggested by Nail (Armstrong, 1980), it is possible to detect the end of the PD as at that point the concentration of water into the drying chamber becomes very low and the pressure measured by Pirani (that is generally calibrated for air) approaches that of the capacitive gauge. The use of the ratio of the pressure signal given by the two gauges, that approaches unity at the end of the PD, instead of the simple measure by the Pirani, is more reliable to this purpose, because eliminates the possible effect of a variation of the total pressure. One possible limitation to the application of this simple method is the restriction in the use of the thermal conductivity sensors in equipment where steam sterilisation is required, even if producers claim that new models using different filament materials (nickel, platinum) rather than the standard tungsten can cope with sterilisation. Recently, new sensors based on the same principle of the Pirani gauge have been proposed: a stainless steel shield is used to protect against condense and a pulsed mode of operation allows for a higher signal resolution, an extended range of measure and a higher long-term stability (Salzberg, 2007).

Other methods are available to monitor the time evolution of the water concentration in the chamber, e.g. the use of moisture sensors or mass spectrometers. Dew point sensors can detect the gas composition or the relative humidity owing to a change in the dielectric constant of a gold sputtered foil material: they indicates a sharp decrease in the dew point when the water vapour decreases to almost 0% (Bardat et al., 1993) and they can have a greater sensitivity with respect to other known methods such as thermal conductivity gauge. Roy and Pikal (1989) used a moisture sensor (Ondyne, by Endress+Hauser HydroGuard 2250, Greenwood, IN) that exploits the variation of the capacity of a thin film of aluminum oxide due to

moisture: according to the authors, the sensor has the sensitivity to determine the presence of ice in less than 1% of the vials. This device, that had been firstly proposed by Bouldoires (1969), was successively used by Genin et al. (1996) and Rambhatla et al. (2004) to monitor the process and to detect the sublimation end-point. Genin et al. (1996) developed also a procedure based on the standard law of mass transfer that lead to a patented method (René et al., 1995) to estimate the residual water content of the product at any time during the process; this method was initially applied to apparatus with internal condenser and then proposed also for freeze-driers with external condenser. Trelea et al. (2007) and Chouvenc et al. (2004) used a similar moisture sensor, developed by Panametrics; as said before, our freeze-drier is also equipped with a Panametrics Moisture Analyser (Panametrics MMS35-131-1-100) and its performance will be compared with those of the other systems previously described.

The use of a QMS to monitor the PD has been proposed in the past (Jennings, 1980, 1999; Connelly and Welch, 1993). The working principle of a QMS is simple: the gas is sampled to the instrument, where the molecules are fragmented, ionised and accelerated by an electric field and the ions are driven to the detector which gives a signal proportional to the concentration and to the type of the impacting fragment. The resulting mass spectrum, constituted by the intensity of the current corresponding to each ion as a function of time, has thus to be manipulated to get the gas composition, but this operation can be quite tricky, mainly due to the difficulties in the calibration. The signal measured by the QMS is in fact the sum of:

i.  a base signal, varying with time over a long time interval, which is the response of the instrument when the species is not present in the feeding;

ii. a false signal (named "interaction") generated by the fact that each molecular species entering in the QMS chamber originates a wide variety of ions and, as there is no chromatographic separation of the different species, an ion with the same mass/charge ratio may belong to different compounds. It is therefore necessary to select, for each species, the ion that should be monitored in order to

avoid, or at least to minimise, these interferences. In our application, the only specie that we desire to monitor is water, whose spectrum is constituted mainly by the fragment of mass 18; nitrogen (or air) is present, but none of the fragments originated by $O_2$ and $N_2$ can give interference with the fragment of mass 18;

iii. the true signal generated by the molecule identified by the selected mass fragment.

Even if the base signal can be easily computed and the interactions can be minimised, the calculation of the response factor of the instrument is difficult as it is not obvious at all how to "create" a calibration mixture with water; moreover, it is necessary to repeat this calibration before each run as the response factor of the instrument can be variable. Jennings (1980) suggested to use a capacitance manometer in conjunction with the QMS to make the calibration; anyway, significant information can be obtained from the QMS even if only the ionic currents are investigated: the time evolution of the ionic current corresponding to mass 18 divided by the total pressure reading made by the QMS was proposed by Jennings (1980) to detect the end of the PD as this signal was almost constant during all the PD and decreased in correspondence of the ending point. Our freeze-drier is equipped with a QMS GeneSys 300 by European Spectrometry Systems: the results that we obtained will be compared in the following with those of the other systems previously described.

Recent developments and proposals in the monitoring of the whole batch comprise the use of a mass flow controller to measure the gas flow necessary for pressure control (Chase, 1998), a Tuneable Diode Laser Absorption Spectroscopy sensor, that was shown to be effective for real-time determination of vapour concentration and mass flux (Kessler et al., 2004) and a cold plasma ionisation device for the monitoring of the moisture content in the freeze-drying chamber (Mayeresse et al., 2007). The measure of the inert mass flow for pressure control and the measure of the water vapour concentration in the chamber, using one of the previous devices, are closely related methods as they all are sensible to a strong variation of the sublimation rate and, thus, they can be used to detect the end of PD. Nevertheless, it

must be taken into account that these devices give only an indirect evaluation of this quantity as the partial pressure that is established in the chamber (at constant total pressure), and the controlled leakage required to maintain a constant pressure, also depend on the performance of condenser and of the vacuum pump, which in turn can be affected by the nature of the inert. The cold-plasma sensor seems anyway particularly promising: it is steam sterilisable, simple to integrate even in an industrial-scale freeze-drier, reproducible and sensitive; the limitations include suitable positioning in the lyophilization chamber, calibration and signal integration. Finally it can be remembered that heat balance at the condenser has also been proposed for sublimation monitoring.

A first example of the results obtained in our system is shown in Figure 5, where the output from the balance and the ratio of the pressure sensors are compared; the estimations given by DPE are also shown. In this cycle 28 vials were placed over the heating shelf in group of 7 vials (a central vial rounded by six vials) and 15 vials were placed on the balance: the radiating contribution is thus similar for the vials on the shelf and for those on the balance. From these data it can be observed that the end of the PD (estimated at about 11 hours from the beginning of the drying and evidenced with the vertical line) is more clearly defined if detected through the mass measurement than using other sensors, like for the example the pressure gauges, even if a very small and uniform batch is considered. In large batches the end of PD may be much more widespread, as will be shown later, as a consequence of the fact that some of the vials can experience conditions different from the average, and thus have a different drying rate, while the lot of vials weighed by the balance takes into account the normal variability between vials, but, if properly shielded, it is representative of the core of the batch. It must be remarked that the mass measurement is affected by an uncertainties of 0.1 g, hence the end-point estimation can vary in a range of 0.2 h as it is shown in Figure 5 by the grey bar.

All the devices discussed in this section (with the exception of the balance), even if useful, do not provide any information about the state of the whole system,

i.e. the temperature and the position of the sublimating interface: methods based on the PRT are able to provide such information as it has been discussed in the Introduction. *LyoMonitor* implements the Dynamic Parameters Estimation (DPE) algorithm that uses the measurement of the pressure rise which occurs when the valve between the drying chamber and the condenser is closed to estimate the product temperature, the moving front position and the heat and mass transfer coefficients. The DPE algorithm is based on a non-linear regression analysis that looks for the best fitting between the experimental data of pressure rise and the simulated ones. This approach is not new at all, but, in this algorithm, an unsteady-state mathematical model is used to describe the pressure rise in order to take into account the different dynamics of the temperature at the interface and at the vial bottom; moreover, this model can estimate the whole temperature profile in the vial. A summary of the model and some details about the algorithm are given in Appendix 2; a detailed description of the algorithm and its validation are given elsewhere (Velardi et al., 2008b).

The characteristics and the assumptions of the previous MTM approaches are briefly summarised here in order to evidence the differences with the DPE. In the original MTM method (Milton et al., 1997; Tang et al., 2006a) a simple addition of contributions from different mechanisms was considered. The algorithm proposed by Liapis and Sadikoglu (1998), that is based on the detailed model of Sadikoglu and Liapis (1997), requires many parameters to perform the analysis, e.g, diffusivity and permeability of the porous layer, vial bottom heat transfer coefficient, temperature and the partial pressure at the top of the vial: the quantity and the type of the parameters and of the measurements required to implement the model make its practical in-line application a complex task, even if feasible in theory. The PRA method proposed by Chouvenc et al. (2004) is based on a simple macroscopic heat balance for the frozen product, assuming that the temperature increase at the interface is the same as the mean product temperature rise: this implies a constant temperature gradient along the ice during the PRT, that can be reasonable towards

the end-point of the PD, but seems questionable when the frozen layer thickness is high and accumulation effects prevent the temperature gradient to be constant. Moreover, PRA assumes constant temperature at the vial bottom during the PRT and constant thickness of the frozen layer between two subsequent PRTs, but this is realistic only if a relatively high frequency of the PRT is adopted, in order to obtain a good accuracy. The model at the basis of our algorithm is an improvement of that of Chouvenc et al. (2004): instead of an equation describing the dynamics of the temperature at the interface, an energy balance for the whole frozen layer is written and solved, thus obtaining also the temperature profile in the product.

The radiation flux from the bottom is accounted for in the overall heat transfer coefficient that is estimated from numerical regression ($K_v$). Radiation from the upper tray has generally a negligible effect due to the presence of the stopper that, at least partially, shields the product, and of the dried layer; in fact, as we are writing a thermal balance for the frozen layer, this flux does not play an important role in normal operating conditions. Radiation from the side-walls affects the dynamics of a very low number of vials (only 6-7% of the vials of a batch in an industrial apparatus are affected by radiation as they are placed at the side of the tray) while in a small-scale apparatus, used for R&D purposes, the problem should be avoided by proper shielding; anyway, a small radiative contribution to total heat transfer is not a problem and, until the shape of the axial temperature profile is not significantly modified, the interface temperature is still predicted with good accuracy and the radiation has the only the effect of increasing the value of the estimated effective heat transfer coefficient.

With respect to the role of the vial wall in the thermal balance of the system, it can be relevant during the PD (see, among the others, Schelenz et al., 1994; Brülls and Rasmuson, 2002, Hottot et al., 2006, Velardi and Barresi, 2008). Actually, it has been proven that the effect of the vial wall, with respect to the heat conduction in the axial direction and to the radiative flux from the chamber wall, can be accounted for in a one-dimensional model by using an effective heat transfer coefficient (Velardi and

Barresi, 2008); the validity of the one-dimensional approach was verified even for the smallest vials investigated in this work in a previous paper (Velardi et al., 2005a). Moreover, the contribution of the vial wall to the dynamics of the system during the PRT has been shown, by means of numerical simulations, to be negligible and, for this reason, it has not been considered in our model, differently from Chouvenc et al. (2004) that included in the lumped model a fraction of the heat capacity of the glass to be determined by fitting.

It must be emphasised that the rate limiting mechanism that occurs is not relevant: the DPE works efficiently both under heat-transfer and mass transfer control (Galan et al., 2007) as it estimates both the heat transfer coefficient and the resistance to the mass flow. This is an advantage with respect to other approaches proposed in the Literature which required to asses if the system was under heat or mass transfer limitation (Liapis and Litchfield, 1979; Litchfield and Liapis, 1982).

In our apparatus the valve used to separate the drying chamber from the condenser during the PRT is fast closing (the time required to fully close it is lower than 0.5 s), as it is generally the case of medium-small equipment, and thus its dynamics has been neglected; anyway, it has been shown that a slow dynamics can be accounted for in the algorithms based on the PRT (Oetjen and Haseley, 2004; Chouvenc et al., 2005).

Finally it must be reminded that all the methods based on the PRT allow to estimate an average state of the system; for this reason it is thus generally assumed that these methods give accurate and coherent data only in the first half of the PD (see, for example, Hottot et al., 2005; Tang et al., 2005), as at the end of this step a fraction of the vials can have completed sublimation before the rest of the batch. This can occur when the heat flux is not homogeneous in the batch (and wall radiation for edge vials is usually the main reason). In these cases it is necessary either to include in the model the information about the local heat flux, or to estimate a correction coefficient that takes into account the heterogeneity of the batch (or simply the reduction of the sublimating surface), as explained in Appendix 2 for the DPE. As

concern the sensibility of the MTM approach, it depends on the chamber volume and on the sensibility of the pressure gauge, in addition to the operating conditions and the nature of the product, that influence the value of the sublimation rate. In fact, according to the Literature (Milton et al., 1977), MTM sensitivity depends on the ratio of the active sublimating surface to chamber volume and it decreases as the batch size decreases, but it is not specified any lower bound needed to get reliable data. Moreover, it may be noted that in production apparatus generally the most sensible capacitive gauge is used, with a full scale of only 100 Pa, while in lab scale freeze-dryers often a device with larger scale is used. As a matter of fact we were able to carry out PRTs even with a very small number of vials, or rather a chamber volume to sublimating surface area ratio equals to 58 m that corresponds, for the cases investigated, to a sublimation rate/chamber volume ratio equals to $1.4 \cdot 10^{-2}$ kg h$^{-1}$ m$^{-3}$.

DPE results have been validated firstly by means of the detailed mathematical model proposed by Velardi and Barresi (2008) and then by comparison with experimental data. An extended experimental campaign, whose results are reported elsewhere (Velardi et al. 2008b), has been carried out to validate the DPE tool on a large range of working conditions, varying process parameters, batch properties and vial characteristics.

Figure 6 shows an example of DPE outcomes, i.e. the values of the moving front ($T_i$) and bottom ($T_B$) temperature during the PRT; two experimental curves are shown, obtained for the same product, but at a different chamber pressure, namely 20 Pa in the upper graph and 5 Pa in the lower graph. In both cases the product temperature increases during the PRT as the vials are continuously heated, while heat removed by sublimation is reduced because the driving force for sublimation decreases. Since this can damage the product, it is very important to predict the temperature increase connected to PRT, taking it into account, for instance, in the heating policy defined by a process control system.

Figure 7 shows another example of a FD cycle where various sensors have been used to monitor the process; in this case a relatively large batch (more than 700 vials)

of lactose solution, with no empty vial as radiation shield, is considered. In the upper graph the temperature at the bottom of the product estimated by DPE is compared with the value measured by the thermocouple inserted into a vial (close to the bottom): measured and estimated values are in good agreement until the PD in the monitored vial is completed (but in the monitored vial ice sublimation seems to terminate much earlier than in the rest of the batch).

The water vapour concentration in the chamber, measured using the Panametrics moisture sensor, as well as the pressure ratio between the Pirani and Baratron sensors are shown in the middle and lower graph respectively; in the middle graph the results obtained for the same experiment using the QMS are also given: here the time evolution of the ionic current corresponding to mass 18 divided by the total pressure reading made by the QMS is shown. The response of the three devices is consistent, and taking as estimation of the end of PD the point where the signal reaches a minimum and constant value, a time of about 28-29 hours results in all cases. Actually all the devices measure the concentration, or partial pressure, of water, even if adopting a different principle; as the chamber gas composition is dependent on the sublimation rate, and this is generally strongly reduced at the end of PD, a large variation of its value can be easily captured and used as indication of end of sublimation step, but, as already discussed, it must be taken into account that this is an indirect measurement, affected by several variables, and problems can arise changing the scale of the apparatus, the size of the batch, the method of pressure control in the chamber, or even the nature of the product. The variation of the sublimation rate, estimated by the initial slope of the pressure rise curve, is shown in the upper graph; it can be noted that the sublimation rate reduces almost to zero in correspondence of the point where also the signal of the other devices reach a minimum, but the same signal starts to drop only when the sublimation rate is already very low.

The shape of the curves obtained with the different devices is anyway quite different. The moisture sensor shows quite a typical behaviour: after the total

pressure has been reduced to the operating value the signal sharply increases first, and after reaching a maximum slowly decreases for almost all the PD, to drop toward the end. This behaviour had been already described by Genin et al. (1999) who explained the slow decrease with the slight reduction in the drying rate due to increased thickness of the dry layer and, thus, of the mass transfer resistance, in the phase of almost linear variation of residual ice with time; the fast drop was explained with a large reduction in the sublimation rate. Comparison with the actual sublimation rate, shown in the upper graph, evidences that the explanation is probably more complicated. The moisture sensor was considered very sensible by the first authors that studied it, being able to sense as few as 0.3% of the vials with ice remaining (Roy and Pikal, 1989); in fact, variation in the signal are large enough to detect the end of PD, but is suffers for low accuracy, that strongly limits the possibility of accurately estimating the amount of sublimated water from the integration of the moisture sensor signal, and low response time, mainly due to water desorption from alumina, as noted by Genin et al. (1999) who reported that in the third phase the water desorption rate from the porous alumina could be an order of magnitude greater than desorption from the product at the beginning of secondary drying.

The QMS requires a relatively long initial time interval for the stabilisation of the pressure inside it; after that, the signal remains almost constant, until it drops, similarly to the moisture sensor. It can be concluded that, in case of water solvent, the performances may be comparable to that of the moisture sensor, but it must be reminded that calibration is extremely difficult for QMS, the drift of the baseline may be large, the cost is much higher and operation require caution.

The use of the two pressure gauges, in our opinion, offers the best ratio between cost and performance: the signal remain practically constant, until it starts to drop, and the use of the ratio of the two pressure signals, instead of a simple differential measure, allows a good sensitivity. The presence of a leakage valve for the chamber pressure control did not give significant trouble in our case, differently from what

reported by Mayeresse et al. (2007), even if it can cause negative spikes after each PRT, and the system can be applied also in case the total pressure is controlled manipulating the valve on the vacuum pump, as shown in Figure 5, even if in this case the typical saw-tooth behaviour appears. The only inconvenient that has been observed is the fact that sometimes the ratio of the two pressure gauges, at the end of PD, can show a baseline signal shift, mainly due to the relatively low accuracy of the Pirani instrument that can make uncertain the determination of the end of sublimation, but the decrease is initially very sharp and in very good agreement with the signal of the other devices.

The previous considerations may change if a third component is present in addition to inert and water (as in the case of use of mixed solvents). In that case the response of the Pirani will be affected by the presence of the new species, being unable to discriminate, even if the signal will get close to that of the capacitive gauges when the composition of the chamber becomes constituted only by inert. The moisture sensor response in principle is independent of the presence of hydrocarbons, freon and carbon dioxide, but can be affected by low molecular weight alcohols, even if Roy and Pikal (1989) report to have used it for lyophilization of solutions containing up to 10% ethanol without problems. The residual gas analyzer in this case can obviously work efficiently and monitor the different species simultaneously. In any case it must be taken into consideration that the response of all these devices can be biased by the fact that the atmosphere that they measure is not exactly the average one in the chamber and this depends on the positioning of the sensor, on the use of controlled leakage and on the hydrodynamics of the chamber: the moisture sensor can be positioned properly in the chamber, but the movements of the shelf for stoppering limit generally its use to a peripherical position, while the Pirani is connected to the chamber by a short duct and the QMS has to sample the gas from the chamber. This aspect will surely deserve an accurate investigation in the next future.

It can be remembered that the cold plasma ionization device also measures the

water concentration in the chamber and gives a response curve similar to the previous ones: the sensitivity seems to be very high in that case, but the uncertainty on the final point determination, the problem of calibration and the dependence of the response on the probe location must be considered also in that case (Mayeresse et al., 2007).

As concerns the detection of the end of sublimation, as discussed above, it is generally assumed in previous works that it corresponds to the decrease of the signal to a low constant value, but it has been already pointed out that it may be difficult to define a numerical criterion and an uncertainty of a few hours can derive: a significant difference in the value of the first derivative (end of plateau value), a zero value for the first derivative (end of the sharp signal decrease) or a zero value for the second derivative (inflection point in the middle of the sharp signal decrease) have been proposed, but without a link to a theoretical background, while in the case of the moisture sensor, on the base of an inspectional analysis, a special function called SEP(t) has been proposed which makes use of the values of the total and partial pressure in the chamber and of the partial pressure in the condenser (Genin et al., 1996). It must be also considered that the exact determination of the end point with the previous type of sensors may be made intrinsically difficult by the fact that the desorption rate from the fraction of dried material and from the chamber walls may be comparable with the sublimation rate at the end of the PD.

A last comment concerns the duration of the period in which the signal drops; this is surely related to the heterogeneity of the batch (compare for example the case shown in Figure 5, where also a different method for total pressure control is adopted), and can be quite long, e.g. about 4-5 hours in the case shown in Figure 7 which refers to a relatively large batch where the side vials can be affected by radiation; in any case, as shown, this corresponds only the very last period of sublimation rate decaying (see the upper graph). It must be stressed that the point where the signal start decreasing with a large slope does not correspond to the point where some vials have completed drying: in fact, Roy and Pikal (1989) and Tang et

al. (2005) have evidenced that the signal of the moisture sensor does not change slope even when a significant fraction of the vials have completed PD; these results have been confirmed using the different sensors by our experiments carried out on a batch in which the heat flow was different on purpose for a fraction of the vials, stopping the cycle and measuring the residual water in the different vials.

DPE temperature estimations are consistent almost up to the main drying end-point, as detected by Pirani-Baratron pressure ratio, QMS and Panametrics moisture sensor. One point to be evidenced is that generally the estimated product temperature decreases nearby the end-point, but this drop may be only an artefact because a fraction of vials, the edge-vials, has already finished sublimating while DPE continues interpreting pressure rise curves assuming batch uniformity, or rather a constant number of sublimating vials. Thus, a decrease in pressure rise, corresponding to a lower sublimation rate, may be interpreted by the DPE algorithm as a reduction of the front temperature, even if must be pointed out that generally the DPE predict quite accurately the interface temperature, while in these conditions it is the prediction of the mass transfer resistance that becomes less reliable, as it is modified for compensation. As a matter of fact it can be noticed in the upper graph that the estimated temperature actually start decreasing when the sublimation rate becomes significantly lower, and this can approximately correspond, as discussed in the previous paragraph, to the time where some vial have completed ice sublimation.

This decrease in the interface temperature estimated by a MTM method was already reported (Tang et al., 2005), and actually Oetjen and Haseley (2004) proposed to use it as an indication of end of sublimation; according to the results shown this is not correct, or at least should be interpreted as an indication of end of the fist vial, but in our opinion this criterion is not very robust. Anyway the end of PD could be reasonably estimated by extrapolating the predictions of the interface position obtained in the initial part of the run. A better way to detect the main drying end-point could be by assessing the solvent flux evolution estimated, for instance, through PRT or using a balance; anyway this information must be used with caution

and should be better coupled with information coming from other devices.

Beside the product state, DPE estimates the overall heat transfer coefficient (between the heating fluid and the frozen product) as well as the product resistance to mass transfer. To this purpose it must be remembered, as discussed before, that the mathematical model used in the DPE algorithm (see Appendix 2) does not account explicitly for the role played by the vial wall. Actually, the energy coming from the shelf is provided to the product mainly at the bottom of the sample, but to same extent is transferred to the product from the vial side too as a consequence of conduction through the glass (Velardi and Barresi, 2008). Thus, the coefficient $K_v$ is an effective heat transfer coefficient that also account for the additional heat input due to heat transfer from the vial sides. This must be taken into account when comparing the obtained values with those that can be estimated using correlations from the Literature (e.g. Pikal et al., 1984) or measured with direct methods. As an example for 4 mL tubing vials ($d_v = 14.2 \cdot 10^{-3}$ m) over a tray the maximum estimated heat transfer coefficient ranged from 11.5 to 14.1 W m$^{-2}$ K$^{-1}$ varying the chamber pressure from 5 to 15 Pa; in the case of 12 mL vials ($d_v = 20.8 \cdot 10^{-3}$ m) $K_v$ varied from 5.4 ($P_c = 5$ Pa) to 9.7 W m$^{-2}$ K$^{-1}$ ($P_c = 20$ Pa) (Velardi et al., 2008b). Figure 9, that will be discussed in the following paragraph about the design of a control system, gives an example of the in-line estimations of $K_v$, considering also a change of the set-point of the chamber pressure during the cycle. The previous values are consistent with those calculated using the correlations of Pikal et al. (1984) and in good agreement with the values reported by Hottot et al. (2005), taking into account the additional resistance due to the steel tray that approximately halved the value of the overall heat transfer coefficient, according to Oetjen and Haseley (2004).

As said before, when side-wall radiation gives a non-negligible (but limited) contribution to the energy balance, as in the case shown in Figure 5, DPE is able to take it into account: here $K_v = 40.0$ W m$^{-2}$ K$^{-1}$ is estimated by DPE, a significantly higher effective heat transfer coefficient than in the runs carried out at the same pressure ($P_c = 15$ Pa), but where the vials has been shielded with a row of empty vials

and were placed over a tray.

**Control of the primary drying stage**

Poor process control is a limitation of the current technology due to the difficulty of measuring the parameters of interest (product temperature and interface position). Currently, even the most advanced industrial freeze-dryers have control systems that are no more than data acquisition systems for certain key variables (Liapis et al., 1996). Control actions are often based on the monitored data and on empirical information obtained in previous experimental runs carried out with the product of interest.

A major limitation concerning the control of FD of pharmaceuticals at the manufacturing scale was the fact that regulatory guidance imposed to operate the process in open loop, so that only an activity of monitoring was allowed during production. Nevertheless, during the phase of cycle development, which is carried out at laboratory or pilot scale, it would be very useful to have an in-line control that minimises the drying time, taking into account the final quality of the product. Cycle development, in fact, can be expensive and highly time consuming, but no regulatory restrictions apply during this phase, where the use of an efficient control system can give significant advantages.

Guidance for Industry PAT (Process Analytical Technology) issued by the US Food and Drug Administration in September 2004 introduces some novelty, encouraging the design, the analysis and the control of the manufacturing process through timely measurements (i.e. during processing) of critical quality and performance attributes of raw and in-process materials and processes, with the goal of ensuring final product quality. The goal of PAT is to enhance understanding and control of the manufacturing process as quality should not be tested into products, but it has to be built-in or it should be by design. It has been recently shown that an

in-line adaptive control procedure aiming to minimise the drying time is feasible using some of the monitoring devices described in the previous section (Tang et al., 2005; Velardi et al. 2005b; Fissore et al., 2008).

In this section the application of DPE for the optimisation and control of the PD is presented: the goal is to show that it can be used to realise a control tool able to reduce the drying time using an optimal control strategy for the heating shelf, which continuously adjusts the heating fluid temperature throughout all the PD. By this way, it is possible to maintain the product temperature just below a limit value, selected by the user, thus avoiding to overcome the collapse temperature of the product. This tool allows also to determine with few tests the optimal FD cycle recipe. This novel control software, named *LyoDriver*, uses the estimates of the time varying temperature of the product, of the effective global heat transfer and of the diffusivity coefficient obtained by means of DPE, as well as some process variables (i.e. the temperature of the fluid, the pressure in the chamber and the cooling rate of the freeze dryier) and a simplified mathematical model for the PD validated by Velardi and Barresi (2008). The controlled variable is the maximum temperature of the product, while the manipulated variable is the temperature of the heating fluid. The goal is to maintain the temperature of the product as close as possible to the maximum allowable value.

If a model-based predictive approach is used, the optimal sequence of shelf temperature set-points, $T_{SP,i}$, is calculated as a piecewise-linear function imposing that the value of the product temperature at the bottom, $T_B$, is equal to its target value $T_{B,SP}$:

$$T_{SP}(t): \begin{cases} T_{SP,1} = T_{B,SP} - \left[ 1 - K_v \left( \dfrac{1}{K_v} + \dfrac{L_{frozen}(t_0)}{k_{frozen}} \right) \left( T_{B,SP} - T_i(t_0) \right) \right]^{-1} & t_0 \le t < t_1 \\[3ex] T_{SP,2} = T_{B,SP} - \left[ 1 - K_v \left( \dfrac{1}{K_v} + \dfrac{L_{frozen}(t_1)}{k_{frozen}} \right) \left( T_{B,SP} - T_i(t_1) \right) \right]^{-1} & t_1 \le t < t_2 \\[3ex] \vdots \\[1ex] T_{SP,N} = T_{B,SP} - \left[ 1 - K_v \left( \dfrac{1}{K_v} + \dfrac{L_{frozen}(t_{N-1})}{k_{frozen}} \right) \left( T_{B,SP} - T_i(t_{N-1}) \right) \right]^{-1} & t_{N-1} \le t < t_N \end{cases} \tag{1}$$

where $t_0$ is the starting time of the control action and ($t_N$ - $t_0$) defines the prediction horizon that corresponds to the time interval along which the controlled process is simulated in order to determine the optimal control policy. The target value of the product temperature ($T_{SP,i}$) is calculated iteratively in such a way that the product temperature ($T_B$) never overcomes the maximum allowable value ($T_{MAX}$) even during the PRT. Finally, it must be pointed out that the control action is calculated taking into account the actual dynamics of the freeze-dryer and is based on the product temperature evolution estimated by a mathematical model and where the heating fluid temperature changes according to the actual cooling rate.

Tang et al. (2005) proposed a control tool for the PD based on a different logic and an extremely safe strategy; this system, implemented in the "*Smart Freeze Dryer*", uses the input from the MTM (Milton et al., 1997; Tang et al., 2006a) and, at every step, it calculates a new shelf temperature that guarantees, at steady state, the maximum sublimation rate, beside maintaining the product at the target temperature. Differently from *LyoDriver*, it does not take into account neither the thermal inertia of the apparatus, nor the product temperature rise during the PRT. Moreover, *LyoDriver* takes also into account the overshoot that can be exhibited by the temperature due to the control actions undertaken. By this way, this novel control tool can predict potentially damaging temperature overshoot and, at the same time, anticipate control actions to avoid it. Summarising, *LyoDriver* is a control tool able to choose in real-time an optimal heating strategy for PD so as to realise the best cycle in terms of minimisation of the duration of the operation, without

impairing product quality.

In order to test *LyoDriver* some FD cycles were carried out, varying both the number of the vials and the total pressure in the chamber. A typical result is shown in Figure 8: the mean values of several temperatures (detected by thermocouples) are shown, together with the ice temperature at the bottom of the vial estimated by DPE. As it has been previously pointed out, this curve shows a good agreement with the experimental data until the thermocouples are in contact with the ice. Finally, it can be observed that, thanks to the policy of the controller, the maximum product temperature, both estimated and measured experimentally, never overcomes the limit value fixed by the user (equals to 241 K in this run) and, thus, the maximum allowable heating rate is exploited throughout all PD, thus minimising the duration of this step.

Figure 9 shows some results obtained using *LyoDriver* to monitor and control the PD in case of larger vials. After 5 h from vacuum, chamber pressure has been reduced (see upper graph) in order to investigate how *LyoDriver* controller acts in case of imposed transients: according to Literature and as shown in Figure 10 (middle graph), both product temperature and heat transfer coefficient, estimated through DPE, decrease when the pressure is reduced; as a consequence, *LyoDriver* rises fluid temperature in order to maximise the sublimation rate, beside maintaining the product temperature close to its limit as shown in the lower graph of Figure 9.

The progressive reduction of the estimated $K_v$ is not surprising, and is a consequence of the fact that the estimation of $K_v$ is strongly related to that of the mass transfer resistance, which is affected by a larger uncertainty at very low chamber pressure, as already evidenced also by Tang et al. (2006b); in any case the estimations of the two parameters compensate each other and the controller is able to estimate the evolution of the product temperature with good accuracy.

**Conclusions**

Monitoring of PD is a subject of remarkable importance and several studies have been focused on this topic in the past, most of them based on either invasive methods or even non-intrusive techniques that can be surely improved.

The use of thermocouples, pressure gauges, mass spectrometer and moisture sensor for in-line monitoring of the PD has been addressed, pointing out and comparing the results that can be obtained. The couple of capacitance and thermal conductivity gauges can be very useful to detect the end-point of the PD and its measurement seems even more sensitive than that of the moisture sensor investigated in this work (especially if the ratio of their output is considered). In any case all the previous systems suffer a strong limitation for application in industrial plants as they are not steam sterilisable. An alternative method for monitoring the PD, based on mass measurements, has been proposed and validated to determine in-line the progress state of the process. Other sensors have been recently proposed, and have been reviewed, but they are still in the developing stage, are applicable only in lab-scale equipment, or are quite expensive. The most important point is that all these systems do not give any information about the state of the product and are useful only for the detection of the end of PD; thus, some new instruments that combine the measure of the temperature in the product (observer) or the measure of the pressure during the PRT (DPE) with a mathematical model have been presented and experimentally compared with the "traditional" sensors. The proposed devices were tested in a wide range of conditions, with a number of vials ranging from about 40 to 700, different vial sizes and products, chamber pressure varying from 5 to 20 Pa. They demonstrated to give a quick and reliable estimations not only of the temperature of the product, but also of the position of the moving front and of the transfer coefficients, which must be known a priori in most approaches presented in the Literature. Reliable estimations of heat and mass transfer coefficients as well as of sublimation flow rate can be obtained by this way. On the other hand, the DPE does not give a clear estimation of the end-point, at list at the current state of

development, even if the information obtained evaluating the sublimation rates can be used to this purpose, as shown also by Tang et al. (2005). Moreover, its use, like all the MTM-type approaches, towards the end of the PD can be problematic, and can cause an excessive increase in the product temperature. Nevertheless, using the information coming from DPE and Baratron-Pirani pressure ratio (or from a moisture sensor) we get a tool able to both monitor some product-quality affecting parameters as well as the evolution of the process itself.

Moreover, these devices may be used for controlling and optimising the PD. An example of the application of DPE in a very simple control system has been given and it has been shown how it can actually optimise the heating policy for the PD and, consequently, minimise the process costs. Moreover, this tool allows to determine the optimal processing conditions for the PD by carrying out a small number of experimental tests, thus avoiding a large number of time consuming and expensive runs required to determine the optimal recipe in a FD cycle by trial and error.

**Acknowledgements**

**Notation**

| | |
|---|---|
| $A$ | cross surface of a sample, m² |
| $c_p$ | specific heat at constant pressure, J kg⁻¹ K⁻¹ |
| $d_v$ | vial diameter, m |
| **f** | vectorial function giving the derivatives of the state |
| $F_{leak}$ | leakage rate, Pa s⁻¹ |
| **h** | vector of equations giving the state space equations of the measures |
| $\Delta H_s$ | heat of sublimation, J kg⁻¹ |
| $k$ | thermal conductivity, J m⁻¹ s⁻¹ K⁻¹ |
| $k_1$ | effective diffusivity coefficient, m² s⁻¹ |
| $\mathbf{K}(t)$ | observer gain |
| $K_v$ | overall heat transfer coefficient, J m⁻² s⁻¹ K⁻¹ |
| $i$ | ionic current measured by the QMS, A |
| $L$ | total product thickness, m |
| $L_{frozen}$ | frozen layer thickness, m |
| $M$ | molecular weight, kg kmol⁻¹ |
| $N_v$ | number of vials in the batch |
| $P$ | total pressure (or pressure measured by gauges) |
| $p$ | partial pressure, Pa |
| R | ideal gas constant, J kmol⁻¹ K⁻¹ |
| $R_p$ | mass transfer resistance in the dried layer, m s⁻¹ |
| $R_s$ | mass transfer resistance in stopper, m s⁻¹ |
| $r_{sub}$ | sublimation rate, kg m⁻² s⁻¹ |
| $\mathbf{S}(t)$ | matrix giving the solution of the dynamic Riccati equation |
| $t$ | time, s |
| $T$ | temperature, K |
| $T_g'$ | glass transition temperature for the product in equilibrium with ice |

| | |
|---|---|
| **u** | vector of the control variables |
| $V$ | volume, m$^3$ |
| **x** | state space vector |
| **y** | vector of the measured outputs of the system |
| $z$ | axial coordinate in the frozen layer, m |

*Greek letters*

| | |
|---|---|
| $\alpha,\ \beta,\ \delta,\ \varepsilon$ | variables defined by eq. (A.8) |
| $\Lambda$ | matrix of tuning parameters for the Kalman filter |
| $\gamma$ | correction coefficient to take into account the heterogeneity of the batch |
| $\rho$ | mass density, kg m$^{-3}$ |

*Subscripts and superscripts*

| | |
|---|---|
| ^ | observer estimate |
| 0 | value at $t = 0$ |
| (-1) | PRT before the actual one |
| $B$ | sample bottom, corresponding to $z = L_{frozen}$ |
| $c$ | chamber |
| *dried* | dried product |
| $f$ | end of a PRT |
| *fluid* | heating fluid |
| *frozen* | frozen product |
| $i$ | sublimating interface, corresponding to $z = 0$ |
| *in* | inert gas |
| *MAX* | maximum value |
| *meas* | measured |
| $N$ | pressure rise test number |
| *shelf* | heating shelf |

| *SP* | set point |
|------|-----------|
| *w* | water vapour |

*Abbreviations*

| DPE | Dynamic Parameters Estimation |
|-----|-------------------------------|
| DPM | Drying Process Monitoring |
| FD | Freeze-drying |
| HG | High Gain |
| KF | Kalman Filter |
| MTM | Manometric Temperature Measurement |
| PC | Personal Computer |
| PD | Primary drying |
| PRA | Pressure Rise Analysis |
| PRT | Pressure Rise Test |
| QMS | Quadrupole Mass Spectrometer |

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**Appendix 1**

The mathematical model used to design the observer is the simplified model of Velardi and Barresi (2008):

$$\frac{dL_{frozen}}{dt} = \frac{1}{\rho_{frozen} - \rho_{dried}} \frac{M_w}{RT_i} \frac{k_1}{L - L_{frozen}} \left( p_{w,i}(T_i) - p_{w,c} \right) \tag{A.1}$$

$$\left( \frac{1}{K_v} + \frac{L_{frozen}}{k_{frozen}} \right)^{-1} \left( T_{shelf} - T_i \right) = \frac{\Delta H_s M_w}{RT_i} \frac{k_1}{L - L_{frozen}} \left( p_{w,i}(T_i) - p_{w,c} \right) \tag{A.2}$$

$$T_{frozen}(z) = T_{shelf} - \frac{1}{K_v} \left( \frac{1}{K_v} + \frac{z}{k_{frozen}} \right)^{-1} \left( T_{shelf} - T_i \right) \text{ for } 0 \leq z \leq L_{frozen} \tag{A.3}$$

where $z = 0$ corresponds to the position of the sublimating interface, and $z = L_{frozen}$ to the vial bottom. The state of the process that has to be estimated is given by the following vector:

$$\mathbf{x} = \begin{pmatrix} T_i & K_v & k_1 \end{pmatrix}^T \tag{A.4}$$

while the measure is the bottom temperature of the product:

$$\mathbf{y} = T_B \left( T_i, K_v, k_1, T_{shelf} \right) \tag{A.5}$$

where the shelf temperature is taken as process input, thus $u = T_{shelf}$. The equations describing the dynamics of the system are therefore:

$$\dot{x}_1 = \frac{dT_i}{dt} = f \left( T_i, K_v, k_1, T_{shelf} \right)$$
$$\mathbf{y} = h \left( T_i, K_v, k_1, T_{shelf} \right) \tag{A.6}$$

obtained setting the derivatives of $K_v$ and of $k_1$ equal to zero because they are assumed to be constant parameters of the process. With simple mathematical passages (Velardi et al., 2008a) the dynamic evolution of the interface temperature $T_i$ can be explicitated:

$$\frac{dT_i}{dt} = \left[ \varepsilon \frac{(\alpha + \beta)^3}{\delta^2} + \alpha \frac{d\beta}{dT_{shelf}} \frac{dT_{shelf}}{dt} \right] \frac{1}{\beta \frac{\partial \alpha}{\partial T_i} - \frac{d\beta}{dT_i} \alpha} \tag{A.7}$$

where:

$$\begin{cases} \alpha = \dfrac{\Delta H_s M_w}{R} \dfrac{k_1}{T_i} \left( p_{w,i}\left(T_i\right) - p_{w,c} \right), \ \beta = k_{frozen}\left(T_{shelf} - T_i\right) \\ \delta = \dfrac{k_{frozen}}{K_v} + L, \ \varepsilon = \dfrac{\Delta H_s}{\rho_{frozen} - \rho_{dried}} \end{cases} \tag{A.8}$$

while the equation of the measure (A.5) can be reformulated as:

$$y = T_{shelf} - \frac{\alpha + \beta}{K_v \delta} \tag{A.9}$$

Thus, with the previous notation, the final form of the dynamic system is:

$$\dot{\mathbf{x}} = \begin{pmatrix} \left[ \varepsilon \dfrac{\left(\alpha + \beta\right)^3}{\delta^2} + \alpha \dfrac{d\beta}{du}\dfrac{du}{dt} \right] \dfrac{1}{\beta \frac{\partial \alpha}{\partial x_1} - \frac{d\beta}{dx_1}\alpha} \\ 0 \\ 0 \end{pmatrix} = \mathbf{f}\left(\mathbf{x}, u\right) \tag{A.10}$$

$$y = u - \frac{\alpha + \beta}{K_v \delta} = h\left(\mathbf{x}, u\right)$$

The structure of the extended Kalman-type observer for the estimation of the state of a non-linear system with the general form given by eq. (A.6), is as follows:

$$\dot{\hat{\mathbf{x}}} = \mathbf{f}\left(\hat{\mathbf{x}}, \mathbf{u}\right) + \mathbf{K}(t)\left(\hat{\mathbf{y}} - \mathbf{y}\right) \tag{A.11}$$

$$\hat{\mathbf{y}} = \mathbf{h}\left(\hat{\mathbf{x}}, \mathbf{u}\right) \tag{A.12}$$

$$\mathbf{K}(t) = \mathbf{S}^{-1}(t)\left(\left.\frac{\partial \mathbf{h}}{\partial \mathbf{x}}\right|_{\hat{\mathbf{x}}}\right)^T \tag{A.13}$$

$$\dot{\mathbf{S}}(t) = -\left(\left.\frac{\partial \mathbf{f}}{\partial \mathbf{x}}\right|_{\hat{\mathbf{x}}}\right)^T \mathbf{S}(t) - \mathbf{S}(t)\left.\frac{\partial \mathbf{f}}{\partial \mathbf{x}}\right|_{\hat{\mathbf{x}}} + \left(\left.\frac{\partial \mathbf{h}}{\partial \mathbf{x}}\right|_{\hat{\mathbf{x}}}\right)^T \left.\frac{\partial \mathbf{h}}{\partial \mathbf{x}}\right|_{\hat{\mathbf{x}}} - \mathbf{S}(t)\Lambda\mathbf{S}(t) \tag{A.14}$$

where $\Lambda \in \mathbb{R}^{n \times n}$ is a semi-positive definite matrix and the Jacobian matrix $\partial \mathbf{f}/\partial \mathbf{x}$ and the gradient vector $\partial \mathbf{h}/\partial \mathbf{x}$ can be calculated analytically.

The proposed observer can be coupled with the DPE tool thus allowing to get the first estimates about a single vial using those about the whole batch obtained through the PRT. In order to improve the convergence of the observer it is in fact very useful to start the calculations at $t = 0$ from the values of the state $\mathbf{x}$ calculated using DPE, if these are available.

**Appendix 2**

The energy balance in the frozen layer during the PRT can be described by:

$$\frac{\partial T}{\partial t} = \frac{k_{frozen}}{\rho_{frozen} c_{p,frozen}} \frac{\partial^2 T}{\partial z^2} \qquad \text{for } t > t_0, \ 0 \le z \le L_{frozen} \qquad \text{(A.15)}$$

$$T|_{t=0} = T_{i0} + \frac{z}{k_{frozen}} \Delta H_s \left( \frac{k_1 M_w}{R T_{i0}} \right) \left( \frac{p_{w,i0} - p_{w,c0}}{L - L_{frozen}} \right) \qquad \text{for } 0 \le z \le L_{frozen} \qquad \text{(A.16)}$$

$$k_{frozen} \frac{\partial T}{\partial z}\bigg|_{z=0} = \Delta H_s \left( \frac{k_1 M_w}{R T_i} \right) \left( \frac{p_{w,i} - p_{w,c}}{L - L_{frozen}} \right) \qquad \text{for } t \ge t_0 \qquad \text{(A.17)}$$

$$k_{frozen} \frac{\partial T}{\partial z}\bigg|_{z=L_{frozen}} = K_v \left( T_{shelf} - T_B \right) \qquad \text{for } t \ge t_0 \qquad \text{(A.18)}$$

where $T = T(z,t)$, $T_i = T(t)|_{z=0}$, $T_B = T(t)|_{z=L_{frozen}}$. Thermodynamic equilibrium is assumed at the sublimating front, corresponding to the axial position $z = 0$; moreover, at the beginning of the PRT the heat fluxes at $z = 0$ and at $z = L_{frozen}$ are the same as pseudo-stationary conditions are assumed, similarly to Chouvenc et al. (2004), thus $K_v$ can be derived by equating the boundary conditions (A.17) and (A.18), both taken at $t = t_0$:

$$K_v = \left[ \frac{T_{shelf} - T_{i0}}{\Delta H_s \left( \frac{k_1 M_w}{R T_{i0}} \right) \left( \frac{p_{w,i0} - p_{w,c0}}{L - L_{frozen}} \right)} - \frac{L_{frozen}}{k_{frozen}} \right]^{-1} \qquad \text{(A.19)}$$

Due to the contribution of gas conduction, $K_v$ is dependent on the pressure, that is varying during the PRT, but, as it has been evidenced by Chouvenc et al. (2004) and confirmed by our simulations, due to the thermal inertia of the system this dependence is not relevant during the PRT and the constant value given by eq. (A.19) can be used.

The total pressure is calculated through eq. (A.20)-(A.21), taking into account a constant leakage in the chamber:

$$P_c = p_w + p_{in} = p_w + F_{leak} t + p_{in0} \qquad \text{for } t \ge t_0 \qquad \text{(A.20)}$$

$$p_w\big|_{t=0} = P_{c0} - p_{in0} \qquad \text{for } t = t_0 \tag{A.21}$$

Applying the ideal gas law and rewriting the mass flow rates as functions of the pressure driving force between the interface and the chamber, it follows that:

$$\left(\frac{M_w V_c}{RT_c}\right)\frac{dp_{w,c}}{dt} = N_v A \left(\frac{k_1 M_w}{RT_i}\right)\left(\frac{p_{w,i}(T_i) - p_{w,c}}{L - L_{frozen}}\right) \qquad \text{for } t \geq t_0 \tag{A.22}$$

that is valid if the contribution of all the vials is the same, i.e. all the vials have the same values of $L$, $T_i$, $k_1$. Nevertheless, recent studies about water vapour hydrodynamics and radiation effects have evidenced that the batch can be not homogeneous: as an example, vials located at the edge of the plate sublimate faster due to radiation from the wall. As a consequence, when in some vials the PD is completed, in other the main drying is still taking place and thus the number of vials contributing to the pressure rise during the PRT is different from $N_v$. To take into account this effect, the term $N_v A$ in eq. (A.22) can be multiplied by a $\gamma$ parameter that is equal to one at the beginning of PD and that decreases with the progress of drying (Rasetto et al., 2008); the value of this additional parameter can also be estimated by the DPE tool (as an option).

If the value of the gas temperature in the chamber, $T_c$, is not available, it can be substituted with the product temperature at the interface with a negligible error.

The actual thickness of the frozen layer is calculated from a mass balance written across the moving interface, which is solved contemporaneously to the previous equations. This balance can be integrated in time, using for example the trapezoidal rule of integration, between the previous PRT and the actual one, thus obtaining:

$$L_{frozen} = L_{frozen}^{(-1)} - \frac{M_w}{R\Delta\rho}\left[\left(\frac{k_1}{T_{i0}}\frac{p_{w,i0} - p_{w,c0}}{L - L_{frozen}}\right) + \left(\frac{k_1^{(-1)}}{T_{i0}^{(-1)}}\frac{p_{w,i0}^{(-1)} - p_{w,c0}^{(-1)}}{L - L_{frozen}^{(-1)}}\right)\right]\frac{t_0 - t_0^{(-1)}}{2} \tag{A.23}$$

where $\Delta\rho = \rho_{frozen} - \rho_{dried}$, and the superscript "(-1)" refers to quantities calculated or measured in the previous PRT.

The steps of the DPE algorithm are thus the followings:

1) Initial guess of $T_{i0}$, $k_1$ (and $\gamma$)

2) Determination of $L_{frozen}$ using eq. (A.23); the values measured and computed in the PRT run at time $t = t_0^{(-1)}$ are required.

3) Determination of $K_v$ using eq. (A.19).

4) Determination of the initial temperature profile in the frozen product using eq. (A.16).

5) Integration of the discretised ODE system in the time interval ($t_0$, $t_f$), where $t_f$-$t_0$ is the time duration of the PRT.

6) Repetition of points 1)-5) and determination of the values of $T_{i0}$, $k_1$ (and $\gamma$) that best fit the calculated chamber pressure ($p_c$) to the measured data ($p_{c,meas}$), in order to solve the non-linear least square problem:

$$\min_{T_{i0},k_1,\gamma} \tfrac{1}{2}\| p_c - p_{c,meas} \|_2^2 = \min \tfrac{1}{2}\sum_k \left[ p_c - \left( p_{c,meas} \right)_k \right]^2 \tag{A.24}$$

In the previous equations, and in particular in eq. ((A.16)-(A.17)), the gas flow rate in the dried layer has been explicited giving the dependence on the cake thickness, and neglecting the resistance of the stopper; as an alternative the global mass transfer resistance can be used, $R_p$:

$$\frac{1}{R_p} = \frac{M_w}{RT_i} \frac{k_1}{L - L_{frozen}} \tag{A.25}$$

and, in any case, in previous equations and in all those derived from them, the stopper resistance can be added explicitly, if relevant, substituting to the terms given in eq. (A.25) the following one:

$$\frac{1}{R_p + R_s} = \left( \frac{RT_i}{M_w} \frac{L - L_{frozen}}{k_1} + R_s \right)^{-1} \tag{A.26}$$

**List of Figures**

(a): Product temperature at the moving front (solid line) and at the bottom (dashed line) estimated using the Kalman filter; (o) temperature at the bottom of the product measured by a thermocouple.

(b): Estimated front temperature in vials placed in different positions on the shelf (solid lines); the average value estimated using DPE (•) is shown for comparison.

(c): Estimated product temperature profiles of some monitored vials (the same shown in graph b) at $t = 5000$ s after the beginning of the PD.

(d): Interface position estimated by the Kalman filter (solid line) from the temperature measurement (given in graph a) and through DPE (•).

*Figure 4*    Example of results obtained during a FD cycle run using the special balance with the embedded wireless temperature measurement in order to monitor the PD stage of a mannitol-dextran solution (6-14% by weight, $T_g' = 254$ K) in a pilot-scale freeze-drier. The freezing phase has been run at 223 K for about 5 h, while the main drying has been carried out setting the fluid temperature at 293 K and the chamber pressure at 10 Pa ($N_v = 98$ on tray, $d_v = 14.2 \cdot 10^{-3}$ m, $L = 7.2 \cdot 10^{-3}$ m).

Upper graph: time evolution of the product mass (line: the gross mass of 15 vials containing 1 cm³ of solution each, including glass vials and rising tray, is shown) and average sublimation rate calculated from this measure (symbols).

Lower graph: time evolution of the heating fluid temperature (solid line) and of the product temperature measured by thermocouples inserted close to the bottom into three of the vials weighed by the balance.

*Figure 5*    Example of the results obtained during a FD cycle run using *LyoMonitor* to follow the primary drying stage of a 10% by weight sucrose solution

in a pilot-scale freeze-dryer. The freezing phase (not shown) has been run at 228 K for about 5 h, with an initial cooling rate of 1 °C min$^{-1}$, while the PD has been carried out at 258 K, with $P_c$ = 15 Pa ($N_v$ = 43, 15 of which weighed by the balance, $d_v$ = 14.2·10$^{-3}$ m, $L$ = 7.2·10$^{-3}$ m, not shielded). The grey bar evidences the end of PD as estimated by the balance .

*Upper Graph*: product mass evolution (normalised with respect to the initial product mass) measured by the balance (dashed line) and Pirani-Baratron pressure ratio (solid line); the total pressure in the chamber in this case has been controlled by the valve on the vacuum pump.

*Middle Graph*: solvent flux calculated from the mass evolution of the 15 monitored vials.

*Lower Graph*: heating fluid temperature (solid line) and front temperature estimated by DPE algorithm (symbol).

*Figure 6*    Product temperature at the moving front ($T_i$) and at the vial bottom ($T_B$) estimated by DPE during a PRT; experimental (symbols) and calculated (solid line) values of the chamber pressure are shown. Two PRT carried out at different chamber pressure are shown (the whole cycle is shown in Fig. 10); freeze-drying of a 10% by weight sucrose solution ($N_v$ = 155 on tray, $L$ = 9.9·10$^{-3}$ m, $d_v$ = 20.8·10$^{-3}$ m).

Upper graph: PRT after 2 h from vacuum time, $P_c$ = 20 Pa, $T_{fluid}$ = -21.5 °C; estimated parameters: $K_v$ = 9.7 W m$^{-2}$ K$^{-1}$, $R_p$ = 7.3·10$^4$ m s$^{-1}$.

Lower graph: PRT after 6 h from vacuum time, $P_c$ = 5 Pa, $T_{fluid}$ = -23.1 °C; estimated parameters: $K_v$ = 5.4 W m$^{-2}$ K$^{-1}$, $R_p$ = 1.04·10$^5$ m s$^{-1}$.

*Figure 7*    Example of the results obtained during a FD cycle run using *LyoMonitor* to follow the primary drying stage of a 5.5% by weight lactose solution in a pilot-scale freeze-dryer. The freezing phase has been run at 223 K

for about 5 h, with an initial cooling rate of 1 °C min$^{-1}$, while the PD has been carried out at 263 K, with $P_c = 10$ Pa ($N_v = 713$ on tray, $L = 7.2 \cdot 10^{-3}$ m, $d_v = 14.2 \cdot 10^{-3}$ m). The grey bar evidences the end of PD.

*Upper graph*: Comparison between bottom product temperature estimated by DPE (○) and the values measured by thermocouples (dashed line). The heating fluid temperature (solid line) and the solvent flux estimated by DPE algorithm, and referred to the product surface, (symbol) are also shown.

*Middle graph*: Moisture content in the chamber measured by Panametrics Moisture Analyzer (solid line) and ratio between the ionic current of mass 18 and the pressure measured by the QMS (dotted line).

*Lower graph*: Pirani to Baratron pressure ratio.

*Figure 8*    Example of the results obtained in a FD cycle using *LyoDriver* to monitor and control the PD stage: bottom product temperature estimated by DPE (symbols), temperatures measured in some vials by thermocouples (solid line), set-point fluid temperature calculated by LD (dashed line). The batch is composed of glass vials on tray ($N_v = 205$, $d_v = 14.2 \cdot 10^{-3}$ m, shielded) filled with 1 mL of a 10% by weight sucrose solution. The freezing phase has been run using a fluid temperature of 223 K for about 5 hours; then, the primary drying stage has been run at 10 Pa and the heating fluid temperature was increased up to 263 K for about 30 minutes after which it has been automatically manipulated by LD controller.

*Figure 9*    Example of the results obtained during a FD cycle run using *LyoDriver* to monitor and control PD stage. The batch is composed of larger glass vials on tray ($N_v = 155$, $L = 9.9 \cdot 10^{-3}$ m, $d_v = 20.8 \cdot 10^{-3}$ m, shielded) filled with 3 mL of a 10% by weight sucrose solution. After freezing, the

chamber pressure has been set at 20 Pa and then lowered to 5 Pa after about 5 h. The fluid temperature is set by *LyoDriver* to maximise the sublimation rate; the maximum allowable product temperature has been set to 240 K, corresponding to $T_g'$ of the freeze-dried solution.

*Upper graph*: Chamber pressure (solid line) and sublimation rate (▲) evolution estimated through PRT.

*Middle graph*: Moving front temperature (●) and heat transfer coefficient (□) estimated by DPE.

*Lower graph*: comparison between temperature at vial bottom estimated by DPE (○) and measured through thermocouples (short-dashed line). The maximum product temperature allowable (equals to 240 K) and the set-point fluid temperature sequence calculated through *LyoDriver* (solid line) have been also reported.
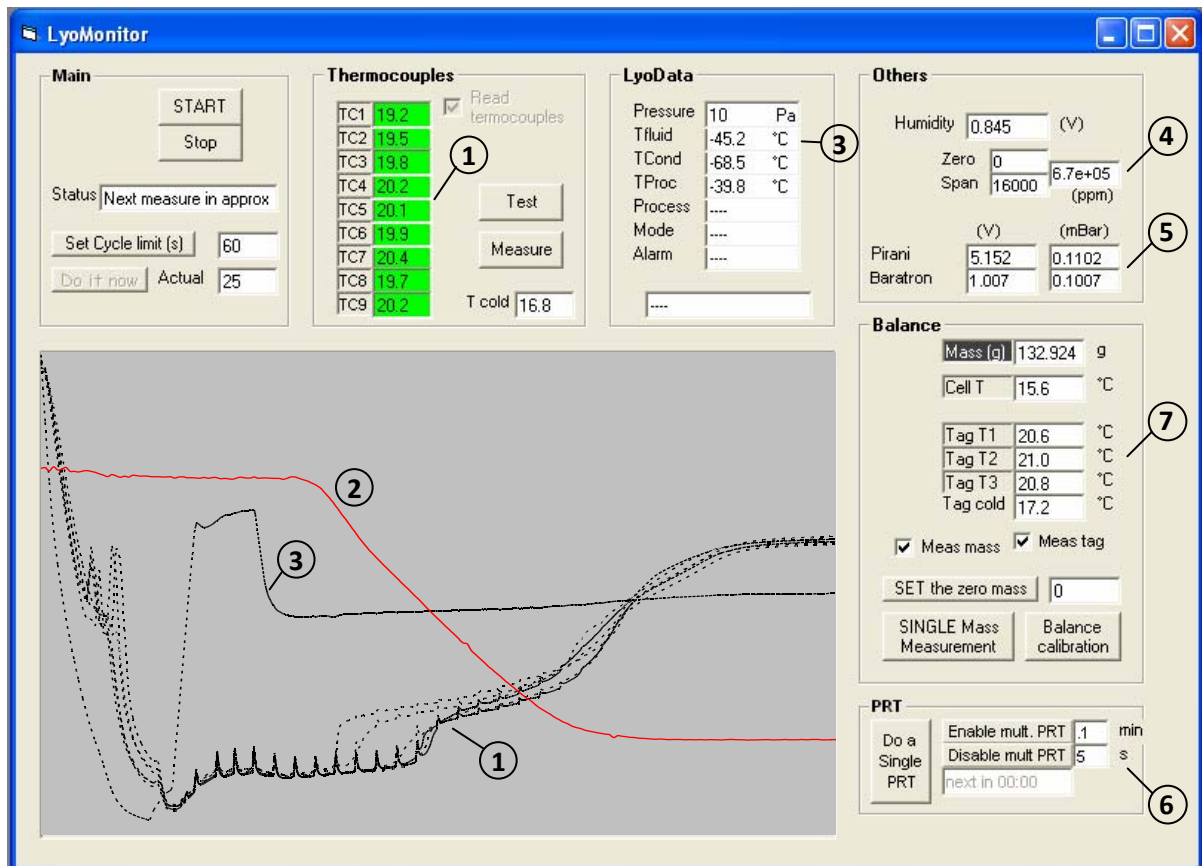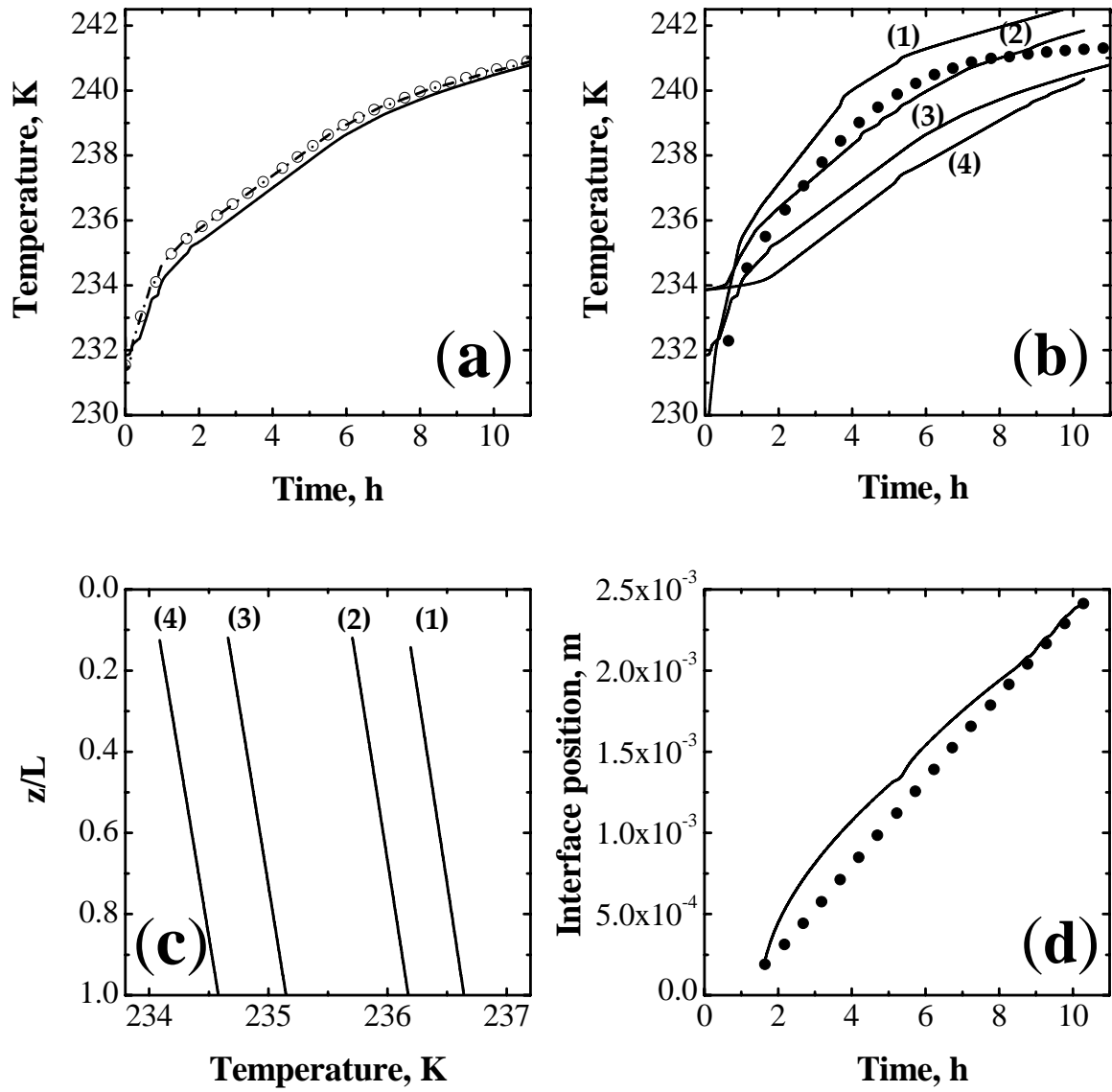
*Figure 1*



- Multimeter + Multiplexer
- Panametrics
- RS232 to PC #1
- IEEE488
- Pirani ⑤
- Baratron
- Vacuum Chamber
- Wired Thermometer ①
- ④
- Measurement Module #1
- ⑨
- Quadrupole Mass Spectrometer
- ⑦
- ②
- Balance
- Heating/Cooling Shelf
- RF link
- Reader
- Wireless Thermometer ⑧b
- RS232
- From FD embedded devices ③
- RS485
- ⑥b
- Condenser
- PC #1 LyoDriver Monitoring System
- Smart Vial ⑧a
- ACQ ⑥a
- DPE ⑥
- Internet
- PC #2 Mass Spectrometer

*Figure 2*

*Figure 3*

*Figure 4*
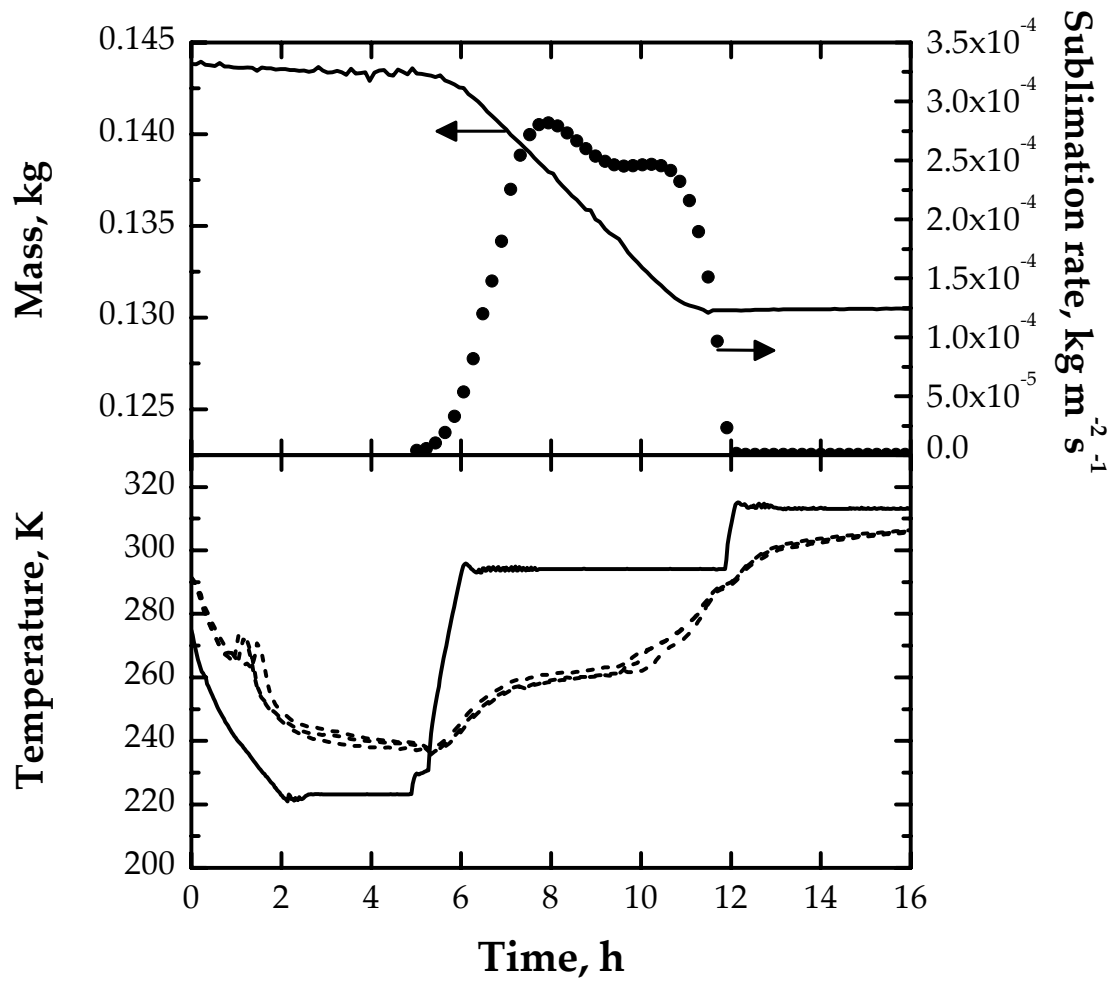
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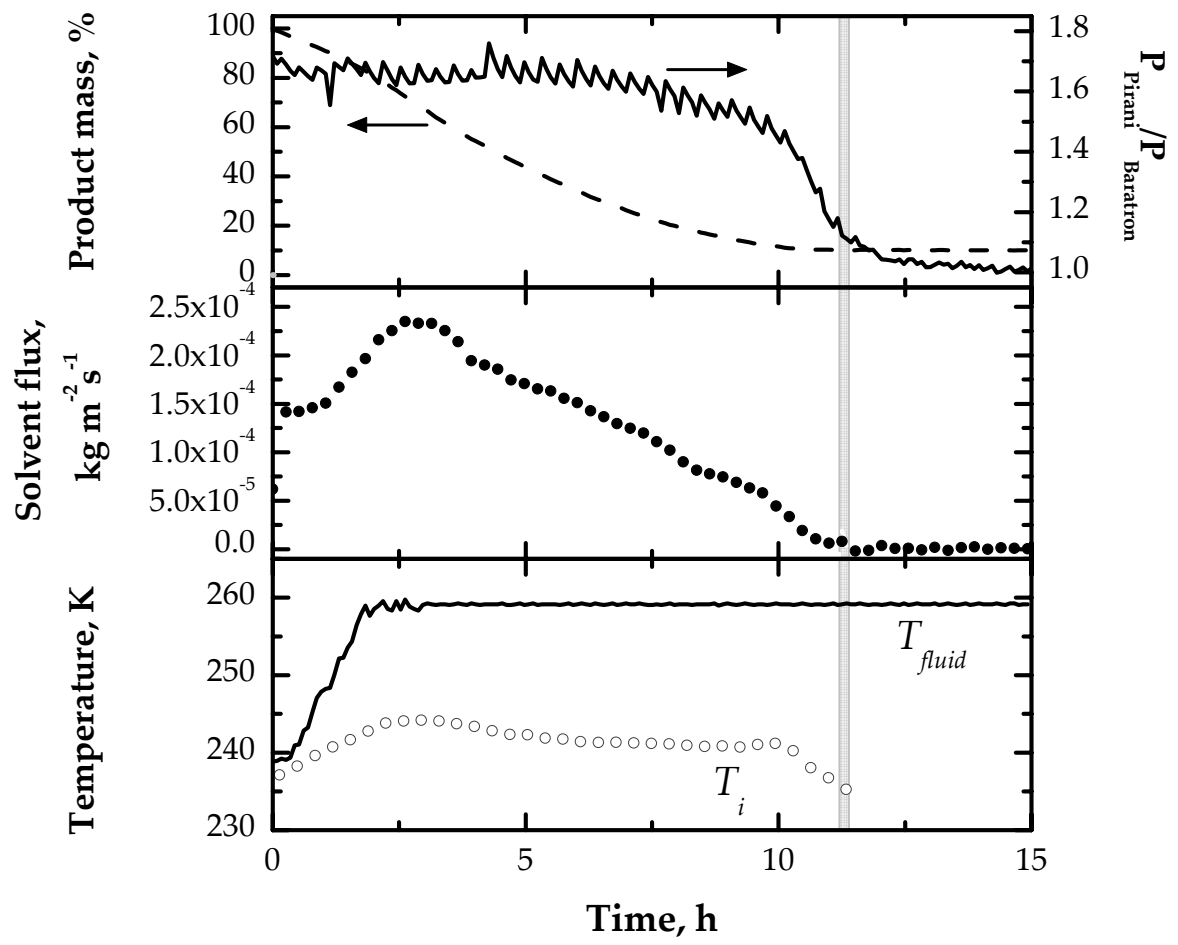
*Figure 6*
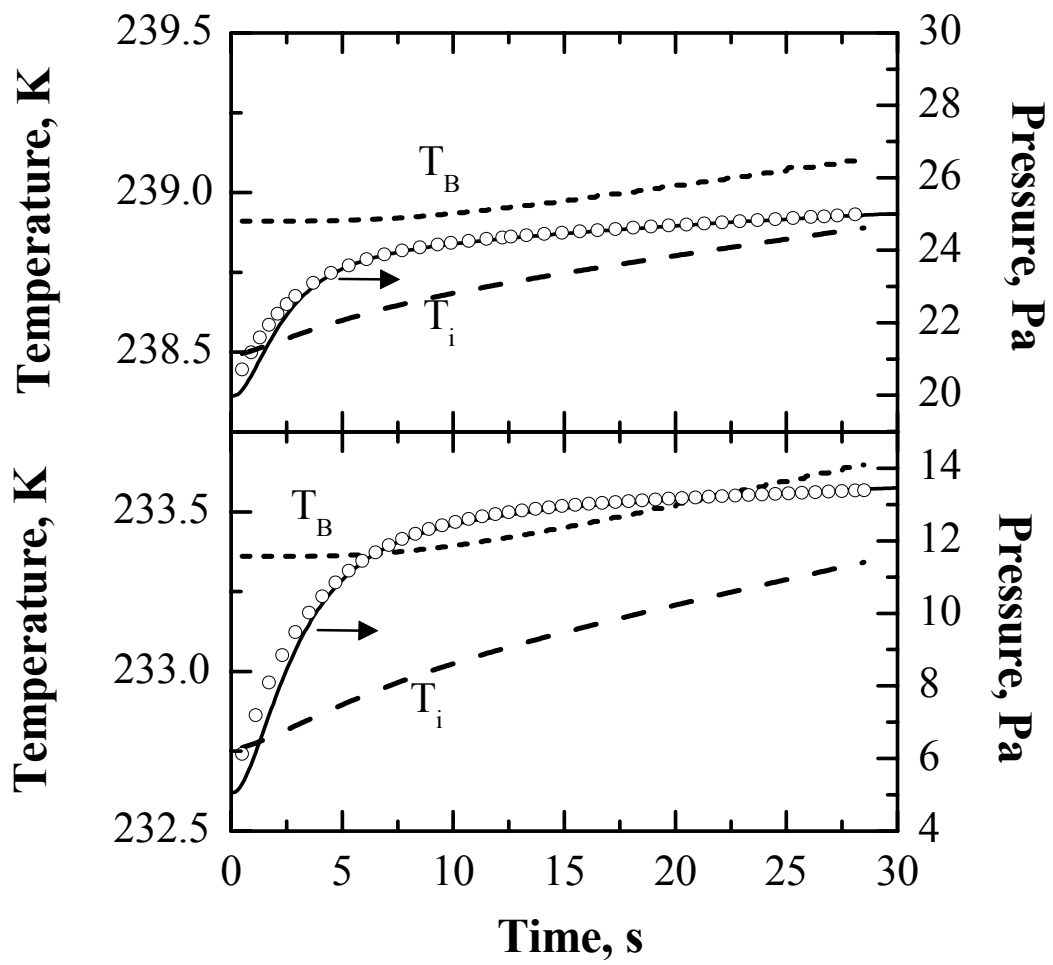
*Figure 7*

*Figure 8*

*Figure 9*