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Measurement Uncertainty Issues in Freeze-Drying Processes

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Abstract

This paper deals with problems that have to be faced when performing mass and temperature measurements of substances subjected to freeze-drying processes. A brief description of a lyophilization process is initially presented and a deep investigation is performed in order to identify the main uncertainty contributions that affect mass and temperature measurements. A measurement system is then described that has been specifically conceived to work inside a freeze-dryer. Experimental results are reported that refer to the metrological characterization of the proposed measurement system and to its use for the monitoring of real freeze-drying processes. Experimental tests are also described that have been conceived to estimate the uncertainty contributions strictly related to this specific application.

Keywords: Mass measurement, temperature measurement, lyophilization, freeze-drying, uncertainty

1. Introduction

Lyophilization is a process commonly used in industrial field to prevent the deterioration of foods and drugs that are sensitive with respect to the heat. The substance under lyophilization, which is usually a water solution, is placed inside glass vials that are kept in tight contact with the shelf of a freeze-dryer, as shown in Fig. 1. Such a shelf is cooled by means of the circulation of a suitable fluid, thus causing a fast freezing of the substance inside the vials. Then, the pressure is decreased down to few pascal, thus allowing the sublimation of the ice from the substance (**primary drying**).

The energy that supports the sublimation process is provided through the heat transfer from the cooling shelf (conduction and irradiation) and from the walls of the freeze-dryer (irradiation) [1], as highlighted in Fig. 1. During this step, the temperature of the substance could reach -60 °C. In a second step, the temperature is increased in order to remove the water tightly linked to the dried product (**secondary drying**). A rubber cap, which is placed above the vial that contains the substance under lyophilization, allows the vial to be sealed at the end of the process thanks to a mechanical system embedded into the freeze-dryer.

The quality of the final product mainly depends on the sublimation rate during the primary drying: an optimum lyophilization requires the identification of the process parameters that allow a predefined sublimation profile to be performed [2]-[3]. This process optimization is commonly obtained in an empirical way using small-size pilot plants by measuring the substance mass before and after the primary drying and checking the characteristics of the dried product, e.g. through a scanning electron microscope [4]. A more convenient approach could be carried out by continuously measuring the mass of the substance during the lyophilization process, whose changes are related to the sublimated water vapor [5]. Unfortunately, this mass measurement is subjected to a series of constraints that make unsuitable the available commercial scales: the large temperature change that occurs during a lyophilization process enormously affects the calibration constants of the sensing devices; in addition, the very low pressure could cause an overheating of the scale, due to the absence of convection flows. Furthermore, the presence of a scale inside a freeze-dryer significantly affects the conditions of the system under measurement. This is important during a lyophilization process because the heat exchanges in vacuum conditions, which are mainly due to conduction and irradiation, have a very low extent. For this reason, the effects related to the presence of the scale on these heat-transfer processes have to be minimized. During the design of a scale for freeze-drying application, other two constraints have therefore to be considered: 1) the substance container has to remain in contact with the cooling shelf; 2) the heat generated inside the scale has to affect in a negligible way the lyophilization process.

Taking the identified constraints into account, a device has been developed [6] that has been specifically conceived to measure mass and temperature of substances subjected to lyophilization processes. In this paper, after a brief description of this device, a deep investigation of the main uncer-

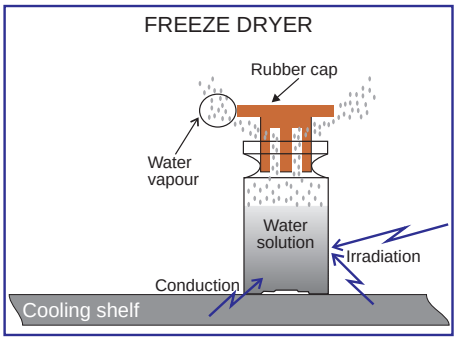

Figure 1: Schematic representation of the sublimation process.

tainty contributions is performed. Results are reported that refer both to the metrological characterization of the proposed device and the monitoring of lyophilization processes.

2. Definition of the parameter under measurement

The definition of the parameter under measurement fixes a lower bound to the uncertainty that can be assigned to the measured parameter, because of the limited amount of information the definition provides. Such an uncertainty contribution, which is called definitional uncertainty [7], can only be reduced if more information is provided, i.e. further complicating the parameter definition, which also requires a more complex method to be implemented. In the investigated situation, the parameter of interest can be defined as "*the mass of a substance contained in a glass vial during a lyophilization process*". **This definition suggests the use of a measuring system that is able to weigh a single vial, like the microbalance used in [8]. If this solution is adopted, the main contribution to the definitional uncertainty is due to the different behavior of the monitored vial with respect to the other vials of the batch, which mainly depends on the different thermal processes the vials are subjected to. These thermal processes depend** on the vial dimension, the characteristics of the freeze-dryer, such as the size of the cooling shelf and its temperature uniformity, and the distance between the shelf and the walls. **In addition,** the presence of other vials close to the vial under measurement

affect these heat-transfer processes and, in turn, the energy available for the ice sublimation. The estimation of the definitional uncertainty is therefore not an easy task and it **can be performed** only for a specific freeze dryer and for a specific vial.

An experimental approach has been followed by the authors in order to estimate the definitional uncertainty of the defined parameter. A double-shelf freeze-dryer has been used (TELSTAR model Lyobeta) to lyophilize 900 vials that contain about 1 g of a 10% mannitol water solution. The vials have been placed on the shelves of the freeze-dryer, as shown in the pictures of Figure 2. The mass of each vial has been weighted before starting the lyophilization process by means of a commercial analytical balance, whose standard uncertainty is of 1 mg. The lyophilization process has been stopped at almost the middle of the primary-drying process, then the vials have been sealed and eventually weighted again.

The obtained results have been analyzed in order to estimate the water-loss distribution among the different vials, taking also into account the vial position on the shelves of the freeze-dryer. Figure 3 shows the histogram of the water loss of the vials placed on the lower shelf of the freeze-dryer. The upper trace refers to the water-loss distribution of all the vials, whose mean value and standard deviation are 323 mg and 40 mg respectively. The lower trace instead shows the water-loss distribution of all the vials with the exception of those one that lie on the edges of the shelf. It should be noted that, as expected [9], the internal vials are subjected to a slower dry rate with respect to the peripheral vials, due to the lower energy that supports the sublimation process. For the internal vials, the mean water loss is 311 mg with a standard deviation of 26 mg, which highlights a lower spread with respect to the whole batch.

If the vials of the upper shelf are also taken into account, the mean water loss is of 344 mg, while the standard deviation is of about 50 mg, which is assumed as the estimation of the definitional uncertainty of the parameter under measurement.

The definitional uncertainty obtained for the investigated freeze-dryer represents an important contribution, since its relative value with respect to the mean water loss is of about 14%. Its reduction is possible if **only** the vials of one shelf is considered (relative value of about 12%) or if the vials on the edges of the shelf are excluded (relative value of about 8%)**, but it remains a large contribution**.

As suggested at the beginning of this section, the investigated

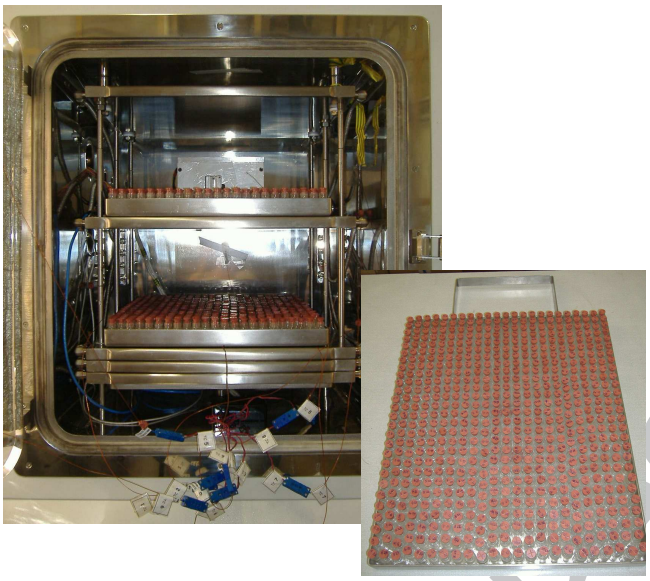

Figure 2: The two shelves inside the freeze dryer (on the left) and the vials inside a shelf (on the right).

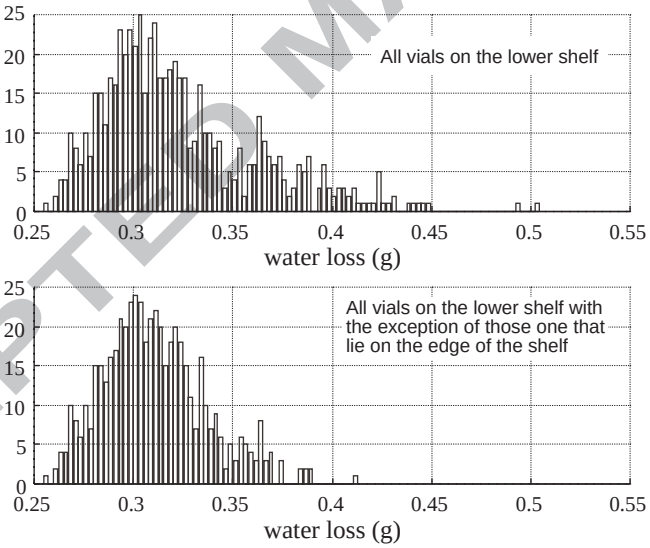

Figure 3: Water-loss distribution of all the vials on the lower shelf. The upper trace refers to all the vials, while the lower trace refers to the internal vials.

uncertainty contribution can be reduced by complicating the definition of the parameter under measurement, which can be expressed as "*the mass of a substance contained in each glass vial during a lyophilization process***". This new definition makes the effects of the uneven thermal processes along the shelf negligible, but it requires a system that is able to pick up and weigh any individual vial on the shelf during the process. A similar system would require a precise and very expensive mechanical arrangement, which should be designed taking into account the previously mentioned constraints.**

With the aim of maintaining the overall cost to a value that is suitable for industrial applications, the authors have developed a device that is able to weigh a preselected group of vials. In this way, the mechanical arrangement does not greatly affect the cost and the problems related to the definitional uncertainty should be mitigated, since the average mass of a group of vials will be available. However, the expected definitional uncertainty should be of the same order of magnitude of that one experimentally estimated, i.e. tens of milligram, since it is not possible to weigh a group of vials randomly distributed over the shelf.

An important indication the obtained result gives is the target instrumental-uncertainty of the device designed to measure the mass of **a group of vials**. This device has not to affect the overall uncertainty in a significant way, therefore its contribution has to be of the same order of magnitude of the definitional uncertainty. Specific efforts aimed to obtained a lower instrumental-uncertainty are not advisable, since they increase complexity and cost of the device without providing any advantage.

3. Measuring system architecture

The system the authors have arranged, whose block scheme is shown in Figure 4, has been conceived **to weigh a group of vials on the shelf of a freeze-dryer and to measure the temperature of the substance contained in some of these vials. The system has been designed** in order to make the monitored vials as representative as possible of the whole batch.

Apart from the definitional uncertainty, possible reasons that could make the monitored vials not representative of the whole batch are mainly related to the heat transfer processes. As far as the conduction is concerned, it is

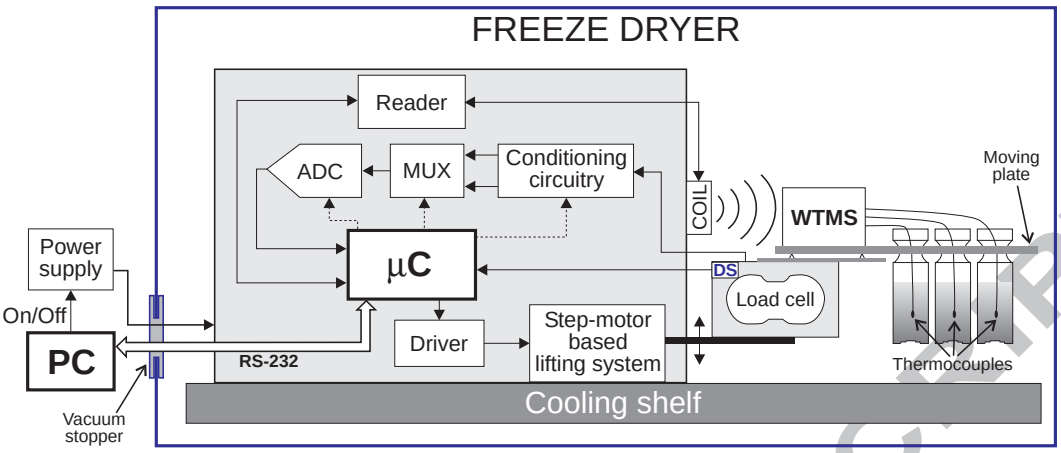

Figure 4: Block scheme of the mass and temperature measurement system.

necessary to maintain the monitored vials in contact with the cooling shelf, but this does not allow a mass measurement to be obtained. In order to meet these conflicting demands, a solution has been implemented by means of a step-motor based lifting system (see Fig. 4), which is driven in order to rise and release the monitored vials when a mass measurement has to be performed. During the measuring phase, the monitored vials are locked by their necks through a specifically designed comb-shaped moving plate [10]. Such a plate does not lock firmly the vials but leaves them a certain grade of freedom, so that they can return in close contact with the cooling shelf after each measurement session.

Other problems are related to the irradiation process, since the measuring system acts as an additional heat source for the monitored vials, thus making faster their lyophilization with respect to the other vials. Furthermore, the gap among the monitored vials is larger than the other vials of the batch, thus further accelerating the lyophilization process. With the aim to reduce these overheating effects on the monitored vials, the power supply of the whole system is turned on only during each measurement session, thus reducing the circuitry heating. This solution is implemented using a programmable power supply, which is controlled by means of the Personal Computer (PC) that triggers each measurement session, as shown in Fig. 4. In addition, the designed moving plate, which is made of a thin metallic sheet, acts as a shield with respect to the irradiated heat and also allows the monitored vials

to be placed not in close contact to the body of the measurement system, as can be observed in the picture of Figure 5.

One should note that the effects of the minor energy that the monitored vials receive through conduction during the measurement sessions is compensated by the major energy these vials receive through irradiation, which is the predominant effect. For this reason, it is expected that the monitored vials are subjected to a faster lyophilization, whose effect will be experimentally estimated (see section 4).

The temperature of the substance inside the vials is another quantity of interest in lyophilization processes, since this information is useful for controlling the freeze-dryer and avoiding substance melting due to overheating. However, also for this measurement it is necessary to minimize the effect of the temperature sensor, which could affect the substance nucleation during the freezing phase. Non invasive techniques have been proposed [11]-[12] that are based on the estimation of the substance temperature by means of complex models that takes also the vial temperature into account. In this work, the authors have employed small thermocouples, which are placed inside the vials in order to directly measure the substance temperature. The effects the thermocouple wires could exercise on the mass measurement have been eliminated thanks to a Wireless Temperature Measurement System (WTMS in Fig. 4) that is installed on the moving plate.

3.1. Mass measurement system

The mass measurement is carried out by means of a commercial load cell, whose lower surface is mechanically coupled to the lifting system, while the upper surface holds the moving plate. The load cell, which has a full range of 2 kg, embeds four strain gauges connected in a full-bridge configuration. The voltage output of the bridge is amplified and filtered through a conditioning circuitry that is based on a low-noise amplifier. The amplifier output-signal V_{out} and the voltage supply V_{s} of the bridge are multiplexed and acquired by means of a 24-bit $\Sigma\Delta$ Analogue-to-Digital Converter (ADC), whose acquisition time has been set to 0.1 s. A digital sensor (DS in Fig. 4) is attached to the load cell by means of a thermo-conductive rubber in order to measure its temperature.

A micro-controller (μC) manages the whole measurement system and also communicates with the PC through a serial RS-232 interface. After the PC turns the power supply on, a command is sent to the μC to start a measurement session, which can be subdivided into the following step:

1. the μC drives the step motor in order to rise the load-cell, which senses the mass of moving plate and vials;
2. the voltages V_{out} and V_{s} are obtained as the average values of the corresponding voltages measured reversing the cell voltage supply, then an estimation of the mass m_{T} of moving plate and vials is obtained as:

$$m_{\text{T}} = A \cdot \frac{V_{\text{out}}^{+} - V_{\text{out}}^{-}}{V_{\text{s}}^{+} - V_{\text{s}}^{-}} \tag{1}$$

   where the constant A is obtained during the calibration of the measuring system;
3. the vials are released on the shelf and the mass measurement procedure based on the equation (1) is repeated in order to obtain the zero mass m_{z};
4. the mass m_{v} of the monitored vials is obtained subtracting the zero mass from the first measurement:

$$m_{\text{v}} = m_{\text{T}} - m_{\text{z}} \tag{2}$$

5. the μC reads the output of the temperature sensor DS through an I2C interface and acquires the measurement of the substance temperature, as explained in the next section;
6. all the measurements are transferred to the PC, which eventually turns the power supply off.

Each measurement session lasts about 15 s, which are mainly required by the lifting system for rising and releasing the moving plate.

The proposed procedure allows the effects of offset and thermoelectric voltages to be minimized. In addition, the thermal effects on load-cell and conditioning circuitry have also to be taken into account, since during a lyophilization process temperature changes of more than 60 °C are expected. For this reason, the PC implements a compensation technique based on a linear model that takes the load-cell temperature into account, whose effectiveness will be showed in the section 3.3.

3.2. Temperature measurement system

The architecture of the temperature measurement system is similar to Radio Frequency IDentification (RFID) devices [13], since it is based on a circuit that is powered through a radio frequency signal sent by a coil, thus

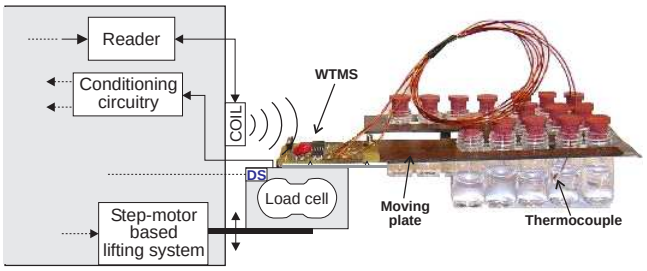

Figure 5: A picture of the moving plate.

avoiding the use of supply wires or battery. Furthermore, the same radio frequency signal is used to send back the measurement results.

The arranged Wireless Temperature Measurement System (WTMS) is located on the moving plate, as indicated in the Fig. 5. It embeds a conditioning circuitry that amplifies the voltage output of three thermocouples, which are placed inside three of the monitored vials. A micro-controller acquires the voltage signals related to the thermocouples and the output of a digital sensor, which provides the cold-junction temperature. The micro-controller is also responsible for the WTMS management and sends the results to the reader, which is placed into the body of the measurement system, as shown in Fig. 5.

3.3. System calibration

The mass measurement system has been calibrated against a standard analytical balance (Mettler AT200), which ensures a standard uncertainty of 1 mg in a measurement range of 200 g.

The calibration constant A of equation (1) has been obtained by weighing a mass with a value of about 200 g previously measured with the standard balance. Multiple readings have been performed during the calibration procedure in order to estimate the uncertainty contribution related to random effects, whose standard deviation was of about 10 mg. After the calibration constant has been obtained, the linearity of the measurement system has been characterized by weighing a set of vials with masses in the range from 100 g to 200 g, obtaining a maximum deviation from a straight line of 10 mg.

The behavior of the mass measurement system with respect to the temperature has been tested by employing a constant mass of about 190 g. During this test, the system has been placed inside a climatic chamber, whose

temperature has been changed in the range from −40 °C to 40 °C. Attention has been paid in order to avoid condensation on the vials, which could affect the measurement results. Figure 6 shows the total mass m_{T} of moving plate and vials, the zero mass m_{z} and the vial mass m_{v}, which is obtained according to equation (2). The behaviors of m_{T} and m_{z} highlight the thermal drift of the system under test, since mass changes of more than 25 g have been observed. The same figure shows that the vial mass m_{v} exhibits a residual mass change of about ±40 mg over the whole investigated temperature range after a linear model has been implemented for compensating temperature effects. Such an algorithm takes into account the load-cell temperature measured by the digital sensor DS.

The described tests have been performed over a time interval of about a week, then the obtained results also include reproducibility and short-term drift of the system under test. Accounting for the different uncertainty contributions and assuming a bimodal probability density function (pdf) for the non-linearity contribution and a uniform pdf for the contribution related to temperature effects, the estimated instrumental standard uncertainty of the mass measurement system is of about 27 mg**, which is of the same order of magnitude of the expected** definitional uncertainty.

The verification of the WTMS has been carried out in the temperature range from −40 °C to 20 °C against a standard thermometer that ensures a standard uncertainty of 0.3 °C, obtaining a maximum error of 1.5 °C and a reproducibility among the three thermocouples of about 0.02 °C.

4. Experimental results

Several experimental tests have been performed in order to characterize the described measurement system in operating conditions. Initially, the proposed system has been placed inside a freeze-dryer for monitoring a lyophilization process, then the load-effect of the system on the monitored vials has been estimated, in order to evaluate the representativeness of these vials with respect to the full batch.

4.1. Lyophilization-process monitoring

Once the system has been calibrated, it has been placed inside a freeze dryer and different lyophilization processes have been monitored. During these tests, the moving plate showed in Fig. 5 has been used, which holds 15 glass vials containing a 10% mannitol water solution.

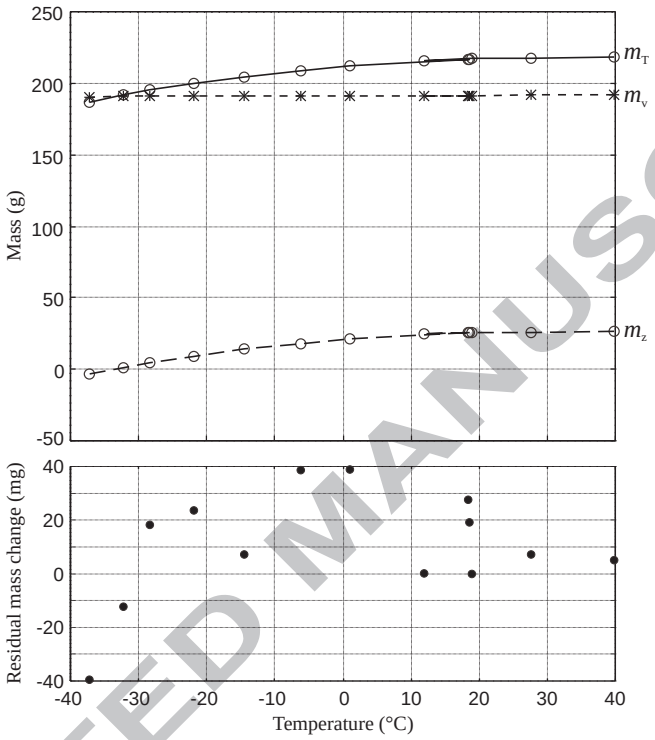

Figure 6: Results of the temperature test: behavior of the quantities m_{T}, m_{z} and m_{v} (top graph); residual change of the mass m_{v} after the temperature compensation has been implemented (bottom graph).

Figure 7 summarizes the results of one of these tests: the upper trace shows the measured mass m_{v}, while the lower trace reports the temperature of cooling shelf (black line) and of the solution inside three of the monitored vials (gray lines). Before placing the moving plate inside the freeze dryer, its mass has been measured with the same analytical balance used during the calibration, obtaining a value of 142.52 g. During the freezing phase, which lasted about 7 hours, the system under test showed a mean value of 142.56 g and a standard deviation of about 20 mg, which is mainly due to freeze-dryer vibrations. During this phase, the temperature of the cooling shelf reached -55 °C while the solution inside the vials reached a temperature of -40 °C. The pressure inside the freeze dryer was then decreased down to 8 Pa in order to enable the ice sublimation process, which caused the mass loss highlighted in the upper trace of Fig. 7. After about 15 hours, the measured mass was almost constant (128.26 g with a standard deviation of 20 mg), thus indicating the end of the primary drying. Eventually, the mass of the moving plate was measured by means of the analytical balance, obtaining a value of 128.20 g.

The obtained measurement error with respect to the reference analytical balance is in agreement with the expected uncertainty of the system under test, whose expanded value (coverage factor $k = 2$) is of about 0.065 g if the noise contribution due to the freeze-dryer vibration is taken into account.

4.2. Load effect estimation

In the lyophilization tests described in the previous section, the primary drying has been always completed, thus not allowing the effects of the measuring device on the drying rate to be estimated. For this reason, specific tests have been performed in order to estimate the effects of the heating transfer processes between the body of the proposed measurement system and the monitored vials, which have been already discussed in section 3. The measurement system has been placed on the cooling shelf of the freeze dryer and the monitored vials have been surrounded by other vials, as shown in Fig. 8. A gap of few centimeters was left between the moving plate and the surrounding vials.

The same procedure already used for the estimation of the definitional uncertainty has been performed (see section 2): measurement of the mass of each vial before the lyophilization process; interruption of the primary drying process; sealing of the vials and mass measurement.

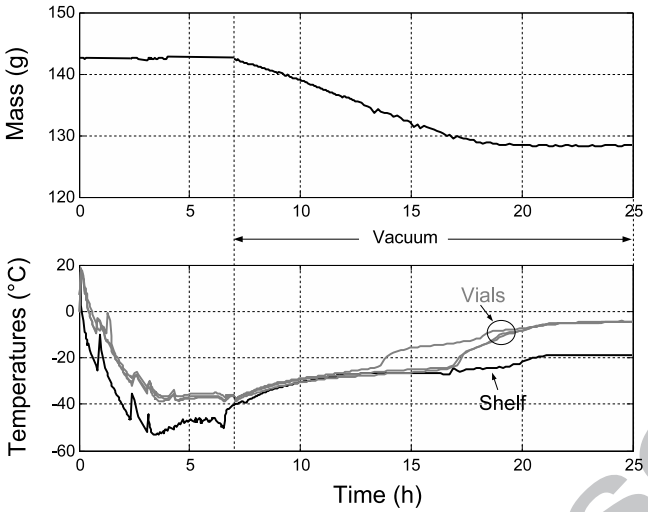

Figure 7: Results obtained during the primary drying of a lyophilization process.

Figure 9 shows the obtained results in terms of histogram of water loss of:

- 227 vials placed on the cooling shelf with the exception of the vials on the moving plate (upper trace);
- 15 vials placed on the moving plate (middle trace);
- 60 vials that lie on the edges of the shelf (lower trace).

It clearly appears that, in average, the monitored vials are subjected to a greater sublimation rate than the other vials of the batch. The mean water loss of the monitored vials is of about 490 mg (standard deviation 50 mg), while the other vials of the batch exhibit a mean water loss of 400 mg (standard deviation 55 mg). Further tests performed without turning on the measurement system have shown that this behavior is not related to the overheating such a system provides to the monitored vials, but it mainly depends on the gap around the monitored vials, which is necessary for allowing the lifting of the moving plate. This means that the vials on the moving plate almost behaves like the vials that lie on the edges of the shelf. The lower trace in Fig. 9 confirm this assumption: here the water-loss distribution of the edge vials is shown, whose mean value is of about 470 mg.

Figure 8: The measurement system on the cooling shelf.

Eventually, it is possible to state that the presence of the measurement system on the cooling shelf contributes in making worst the representativeness of the monitored vials with respect to the whole batch. It therefore can be assimilated to an added contribution to the definitional uncertainty and can be estimated as the difference between the mean water losses of the monitored vials and of the other vials, whose value is of about 90 mg. However, this contribution can be reduced by using suitable models that relate the vial packing density [14] to the quantity of interest, e.g. to the drying rate [4].

5. Conclusions

A system that is able to **weigh a group of vials and to measure the temperature of the substance contained in some of these vials** has been described in this paper. Such a system has been designed to operate in the harsh environment that is present inside a freeze dryer and to make negligible the effects of the system itself on the heating exchange flows that occur during lyophilization processes.

Particular attention has been paid in identifying and estimating the main uncertainty contributions, which can be essentially subdivided into three categories: definitional uncertainty, instrumental uncertainty, load effect. Definitional uncertainty and load effect, which take into account the represen-

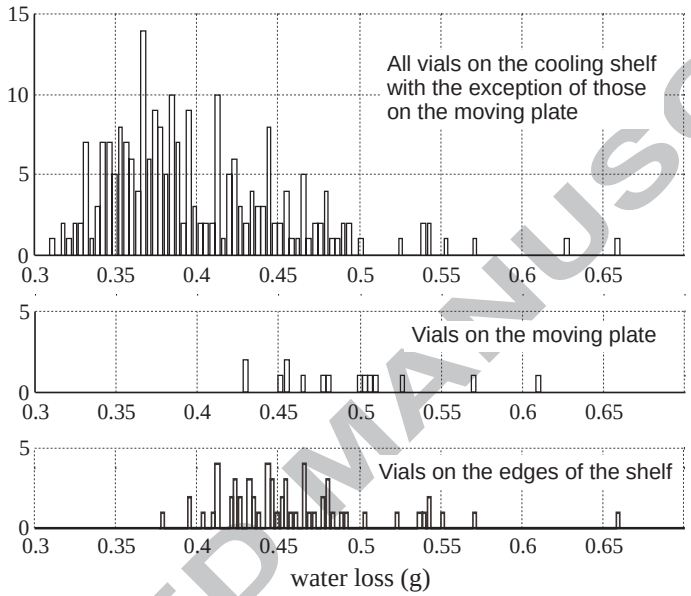

Figure 9: Water-loss distribution of: all the vials on the cooling shelf with the exception of the monitored ones (upper trace); vials on the moving plate (middle trace); vials on the edges of the shelf (lower trace).

tativeness of the monitored vials with respect to the whole batch, seem to be the major contributions, with an absolute value of 90 mg when a water loss of about 0.5 g is measured. This value can be decreased by means of an improved version of moving plate that is now under development, but it can not be reduced below 50 mg, which is the **definitional uncertainty** related to the different thermal processes the vials are subjected to inside the investigated freeze-dryer. **The solution that allows this uncertainty contribution to be drastically reduced consists in arranging a device that is able to pick up and weigh any individual vials on the shelf during a lyophilization process. Such a solution is very expensive and its implementation can only be justified for pilot freeze-dryers used in a laboratory.**

On the other hand, the instrumental uncertainty of the mass measurement system (about 30 mg in the whole ranges of temperature and pressure) gives a minor contribution to the overall uncertainty, thus indicating that further efforts for reducing its value are not useful. The temperature measurement system exhibited a standard uncertainty of less than 1 °C.

Despite the low representativeness of the monitored vials with respect to the batch under lyophilization, the proposed measurement system is a useful tool for monitoring lyophilization processes and obtaining information about the drying rate of the substance under lyophilization.

Acknowledgment

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