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NEW APPARATUS FOR THE IN-SITU OBSERVATION OF A FREEZE DRYING PROCESS

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Abstract

This work presents the performances of a new apparatus developed for the *in-situ* observation of a freeze drying (lyophilization) phenomenon. The results obtained on the lyophilization kinetics performed on a standard mixture (aqueous saturated NaCl solution) are presented.

Keywords: DSC, freeze drying, instrumentation, lyophilization, simulation

Introduction

Lyophilization also called freeze drying is now commonly used in the pharmaceutical, biological [1] and food industries. This technique consists in removing water or other solvents from a frozen substance by sublimation and mass transfer at low temperature and under vacuum. The bringing into play of a freeze-drying cycle requires the knowledge of an important number of physical parameters (Fig. 1): the initial temperature (T_{init}), the final temperature (T_f) and the cooling rate (r_c) used during the freezing stage; the temperatures (T_{iso}), the durations (D_{iso}) of the isothermal plateaus and the vacuum performances used during the sublimation and mass transfer period; the heating rate (r_h) between each plateau and for going back to room temperature. During a freeze drying process, important structural changes appear. The characterization of the freezing solid states, because of their complexity [2] (crystalline, amorphous, etc.) requires, in addition to the classical experimental methods of investigation (X-ray, IR, UV, spectrometries, DSC ...) the microscopic observation of the events as soon as they occur [3].

In the past, different apparatuses were proposed [4, 5]. Nevertheless, their complex technologies and their expensiveness have limited their use. Because of the electronic and computer science progress (miniaturization, performances, cost, ...), envisaging the realization of a new apparatus for the *in-situ* optical analysis of the freeze drying processes appears to be now reasonable. This is one of the goals of our recent works.

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This work deals with the presentation of a new apparatus devoted to the microscopic observations, the analysis and the optimization of freeze drying cycles. The first results obtained on a standard mixture are presented.

Experimental

The apparatus used for the *in-situ* observation of a freeze drying process (Developed by Normalab S. A and called O.T.A.) exhibits the following performances. A volume of some µl is analysed. The range of temperature scanned is 200 to −150°C. Controlled cooling rates of 0.01 to 100 deg·min^{-1} and controlled heating rates of 0.1 to 100 deg·min^{-1} may be used. The accuracy of the temperature measurement is ±0.4°C in the whole range of temperature scanned during the experiment. A vacuum of 10^{-3} Torr can be obtained inside the freeze drying cell. The observations can be made with the reflection and/or the transmission mode with any commercial microscopic instrument. In our case, a Nikon (Optiphot) microscopy equipped of lens X 5, X 10, X 20 is used. The recordings of the observations are made during the experiments with a video tape recorder via a camera (SONY XC-999). Finally the measurements (sizes, distances, numbers) are made with an image processor (Argus 10, HAMAMATSU).

Two chains of programs were written, the first one computes the sequences of the thermal cycles, commands the different instruments of measure, the second one analyses the results (recording) and allows the determination of the ki-

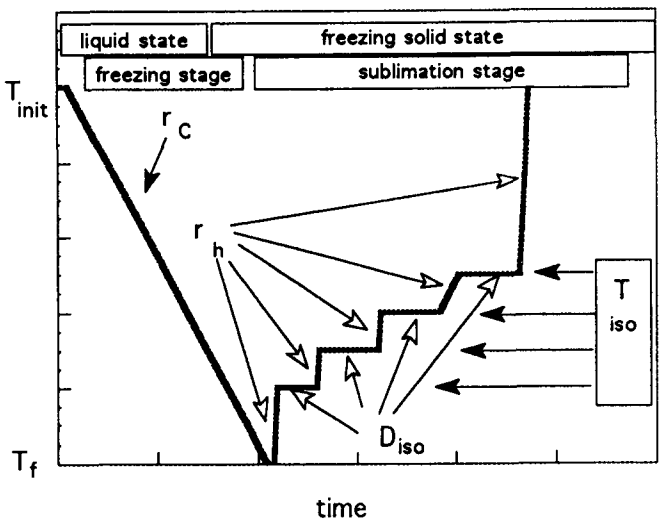

Fig. 1 Diagram showing the thermal cycles performed for bringing into play a freeze drying process. r_c, r_h are the cooling and heating rates respectively, T_{init} is the initial temperature, T_f is the lower temperature reached during the freezing stage, D_{iso} and T_{iso} are the isothermal durations and temperatures of the plateau where the sublimation must appear

netics of transformations liquid/solid, solid/gas and eventually solid/liquid at each stage of the process. On the Fig. 2 the synoptic diagram of the OTA apparatus is displayed.

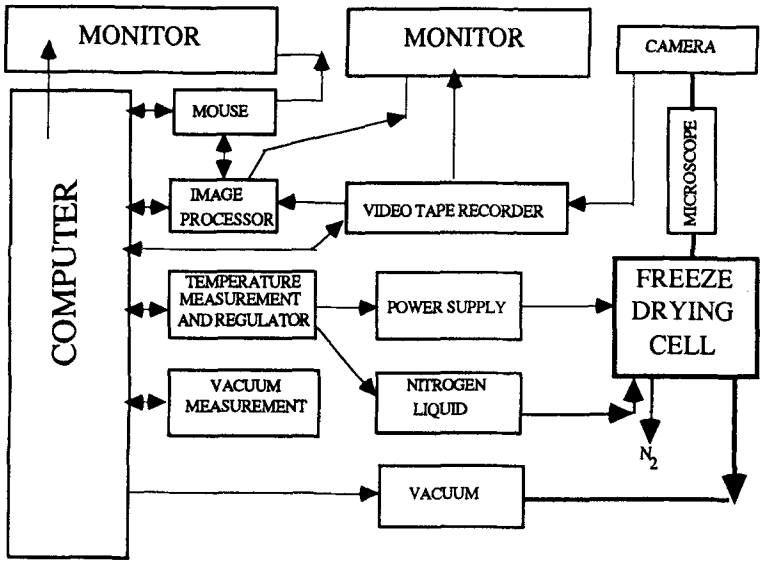

Fig. 2 Schematic diagram of the Micro Freeze Drying Apparatus used in this study

Calorimetric studies were performed with a differential scanning calorimeter (DSC4 Perkin Elmer). Calibration of the calorimeter is obtained from the measurement at different heating rates of the enthalpy and the temperature of fusion of different standards. The analysed sample is an aqueous saturated solution of NaCl.

Results and discussion

An aqueous saturated NaCl solution (>370 g/l) is used to perform the calibration in temperature of the OTA apparatus. At first, we have determined from DSC investigations the variations of the crystallization temperatures with the cooling rate. The enthalpic curves displayed on the Fig. 3 show that the crystallization reactions appear as two consecutive exothermic peaks. During the first crystallization (called primary crystallization), the solvent freezes out and the solute becomes more concentrated. During the second one (called secondary crystallization), the components of concentrated solution solidify. In Table 1, the temperatures of each minimum of the exothermic peaks (T_{m1} and T_{m2}) are reported. We have to take into account that our DSC measurements can be performed only in a range of controlled cooling rates lower than -20 deg·min^{-1}. For

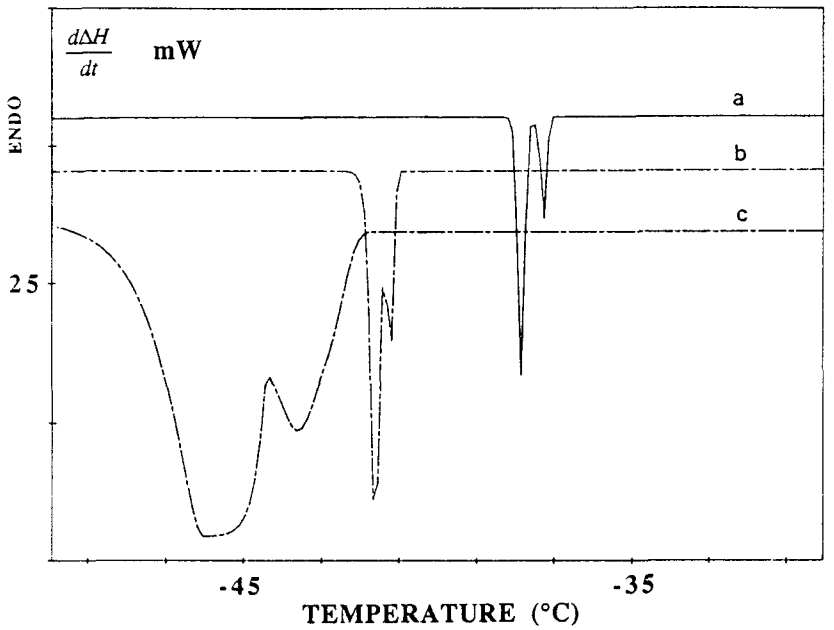

Fig. 3 Enthalpic curves obtained with the aqueous NaCl solution. Curve a for -0.5 deg·min^{-1}, curve b for -1 deg·min^{-1} and curve c for -10 deg·min^{-1}

greater values of the cooling rate, the temperature is not controlled during all the stage of the freezing process. On the other hand, the crystallization reaction depends drastically on the cooling rate (supercooling phenomenon). For the NaCl solution the value of the crystallization temperature is lower than -50°C for cooling rate greater than -10 deg·min^{-1} and so this reaction cannot be observed by our DSC means.

During the same thermal cycles performed on the OTA apparatus the two crystallization reactions are also observed. The characteristic temperatures of the crystallizations (T_{c1} and T_{c2}) determined from $T_c=(T_2+T_1)/2$ (where T_1 corresponds to the temperature of the emergence of the first crystallites, T_2 corresponds to the temperature of the end of the crystallization) are reported in

Table 1 Crystallization temperatures determined from DSC and OTA measurements. The accuracy of the temperature values is estimated at ±1°C

	DSC		OTA	
T_c/deg·min^{-1}	T_{m1}/°C	T_{m2}/°C	T_{m1}/°C	T_{m2}/°C
−0.5	−37.2	−37.8	−38.9	−39.1
−1	−41.2	−41.6	−43.4	−43.6
−10	−43.6	−45.9	−46	−46.2

Table 1. Both methods (DSC and OTA) lead to similar results, that shows that the calibration of the OTA apparatus is performed.

The determination of the crystallization and sublimation kinetics are performed following the thermal cycles described in Fig. 1, with the following experimental conditions: $T_{init}=20°C$, $T_f=-80°C$, $r_c=-0.5$ deg·min^{-1}; for $T_{iso}=$ −60°C, −50°C, −40°C, −30°C, $r_h=10$ deg·min^{-1} and $D_{iso}=20$ min. The Fig. 4 shows an example of images recorded at different steps of the crystallization phenomena. The primary crystallization (Figs 4a, 4b, 4c and 4d) at −38.8°C is

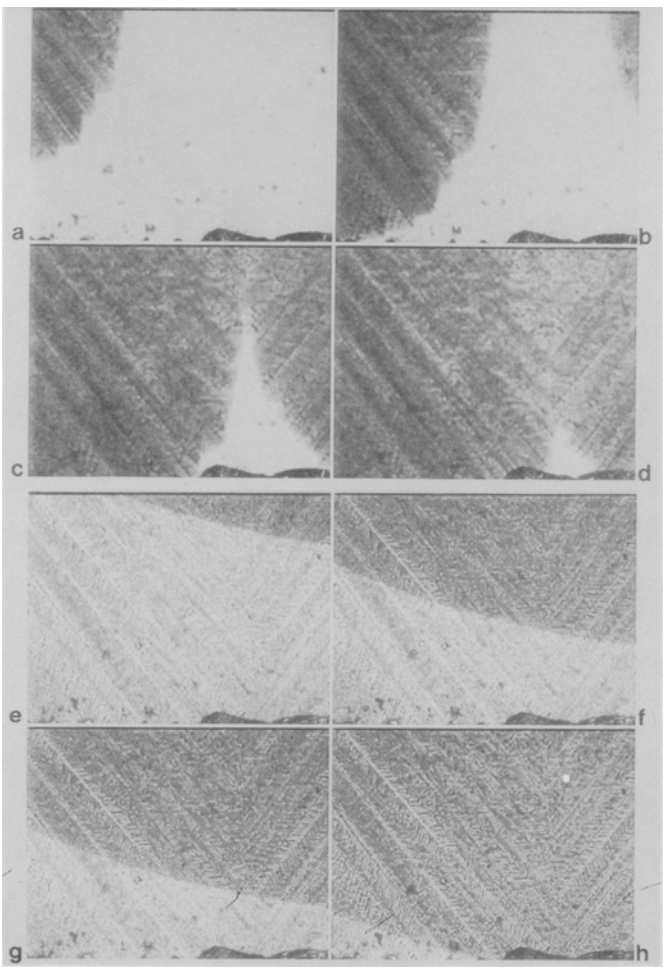

Fig. 4 Primary crystallization at −38.8°C: images 4a, 4b, 4c and 4d are recorded at t_o, t_o+2s, t_o+4s, t_o+5s respectively. Secondary crystallization at −39.1: images 4e, 4f, 4g and 4h are recorded at t_1, t_1+3s, t_1+6s, t_1+7s respectively. The scale is 1 cm for 184 μm

developing with a velocity of 177 μm/s while the secondary crystallization (Figs 4e, 4f, 4g and 4h) occurs at −39.1°C with a velocity of 112.2 μm/s.

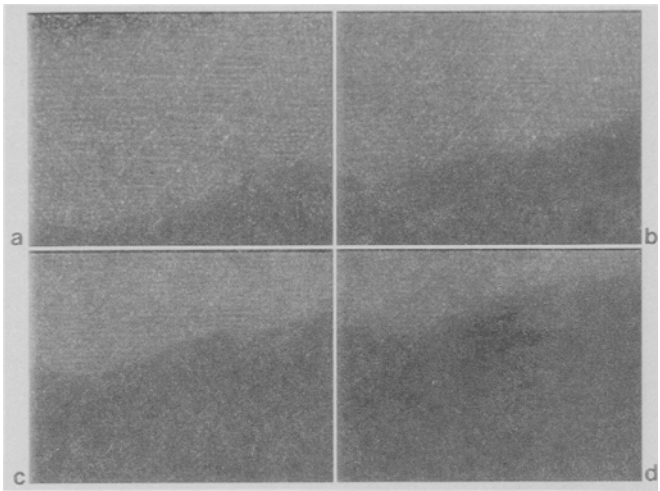

Fig. 5 Freeze drying phenomena at $T_{iso} = -40°C$: images 5a, 5b, 5c and 5d are recorded at t_2, t_2+588s, t_2+657s, $t_2+1333s$ respectively. The scale is 1 cm for 46 μm

The same methods of investigations are performed for the sublimation stage. The Figs 5 (a, b, c, d) show an example of images recorded at different steps of the sublimation phenomena ($T_{iso}=-40°C$). The sublimation rate at each plateau and during each heating period between two plateaus are determined. The results are presented by the means of an histogram on Fig. 6.

In this experiment, only the solvent which contributes to the primary crystallization undergoes the sublimation process. The sublimation rates are found greater during a temperature ramp than during a plateau. No sublimation appears for temperatures lower than −50°C, and excluding the transitory periods between two plateaus, the higher value of the sublimation rate (220 nm/s) appears during the isothermal plateau at −40°C.

Conclusion

In this first study, we have observed the crystallization and the freeze drying processes undergone by a saturated aqueous solution of NaCl. For each reaction, the velocity of the phase transformations was determined. Our first goal was the verification of the real performances of this new apparatus developed by Normalab and devoted for the *in-situ* observations and quantifications of the freeze drying processes. Our first results show that its performances are sufficiently good to allow the computation of the freeze drying kinetics. The originality of this new apparatus is the possibility to make observations in

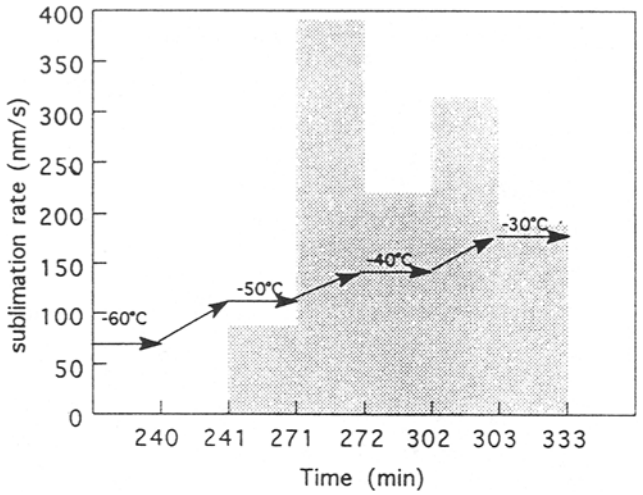

Fig. 6 Histogram showing the values of the sublimation rate at each step of the freeze drying cycle

transmission mode in a large range of temperature, cooling rates and heating rates variation. Our next goal will be the observation of the freeze drying process and the measurement of its kinetics for more elaborated systems.

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Zusammenfassung — Vorliegend wird die Funktion einer neuen Apparatur beschrieben, der für die in situ Beobachtung einer Lyophilisierungserscheinung entwickelt wurde. Die Ergebnisse der Lyophilisierungskinetik bei einem Standardgemisch (wäßrige gesättigte NaCl-Lösung) werden dargelegt.