ELSEVIER

International Journal of Pharmaceutics 113 (1995) 133–140

international journal of pharmaceutics

Research Papers

# Sublimation during lyophilization detected by temperature profile and X-ray technique

Gerlinde Schelenz [a,*], J. Engel [a], H. Rupprecht [b]

[a] *ASTA Medica AG, Postfach 140129, 33621 Bielefeld, Germany*

[b] *Department of Pharmaceutical Technology, University of Regensburg, Regensburg, Germany*

Received 6 December 1993; modified version received 29 April 1994; accepted 26 May 1994

**Abstract**

The goal of the investigation was to obtain a more precise picture of the processes in a vial during lyophilization. The sublimation front during freeze-drying of a mannitol solution could be inferred from a temperature profile, according to which the ice sublimates mainly from top to bottom, and, to a lesser extent, from outside to inside. These findings contradict the previous assumption that the sublimation front proceeds symmetrically from all sides, in a spherical fashion. Apart from glass, other container materials (aluminium and plastic) were also tested. The process of sublimation is the same, except that aluminium speeds up the process of drying while the isolating plastic slows it down. In order to check these results, X-ray photographs were taken at different points of time during primary drying. They confirm the results obtained from the temperature profile.

*Keywords:* Freeze-drying; Lyophilization; Sublimation; Temperature profile; Heat transfer; Mass transfer

## 1. Introduction

Lyophilization or freeze-drying is a procedure which is widely used for the stabilization of substances that are thermolabile or unstable in aqueous solutions. The resulting porous lyophilizate has a long shelf-life and can easily be dissolved or suspended in a liquid. This procedure is used particularly in the pharmaceutical and food industry, in microbiology and biology.

The process of freeze-drying can be subdivided into three phases: freezing, primary drying and secondary drying. During primary drying, sublimation of the ice takes place until only mechanically or physico-chemically bound water remains which is removed during secondary drying (MacKenzie, 1977; Rey, 1977; Williams and Polli, 1984; Franks, 1990).

While the temperature profile and crystallization during freezing have been investigated in detail (Coger et al., 1990), the exact route of sublimation has not previously been the subject of an investigation. It has been assumed, e.g., that the ice core shrinks spherically and symmetrically

* Corresponding author.


*SSDI* 0378-5173(94)00174-4

from all sides (Leybold AG, 1979). In order to test this theory and to obtain a more precise picture of the route of sublimation, a temperature profile was taken in a vial during lyophilization. Container materials which differ considerably in their thermal conductivity, like glass, aluminium and plastic, have been considered.

## 2. Materials and methods

### 2.1. Materials

#### 2.1.1. Containers

50H/3 vials where the upper part of the glass had been removed (diameter, 4.2 cm; height, 4.4

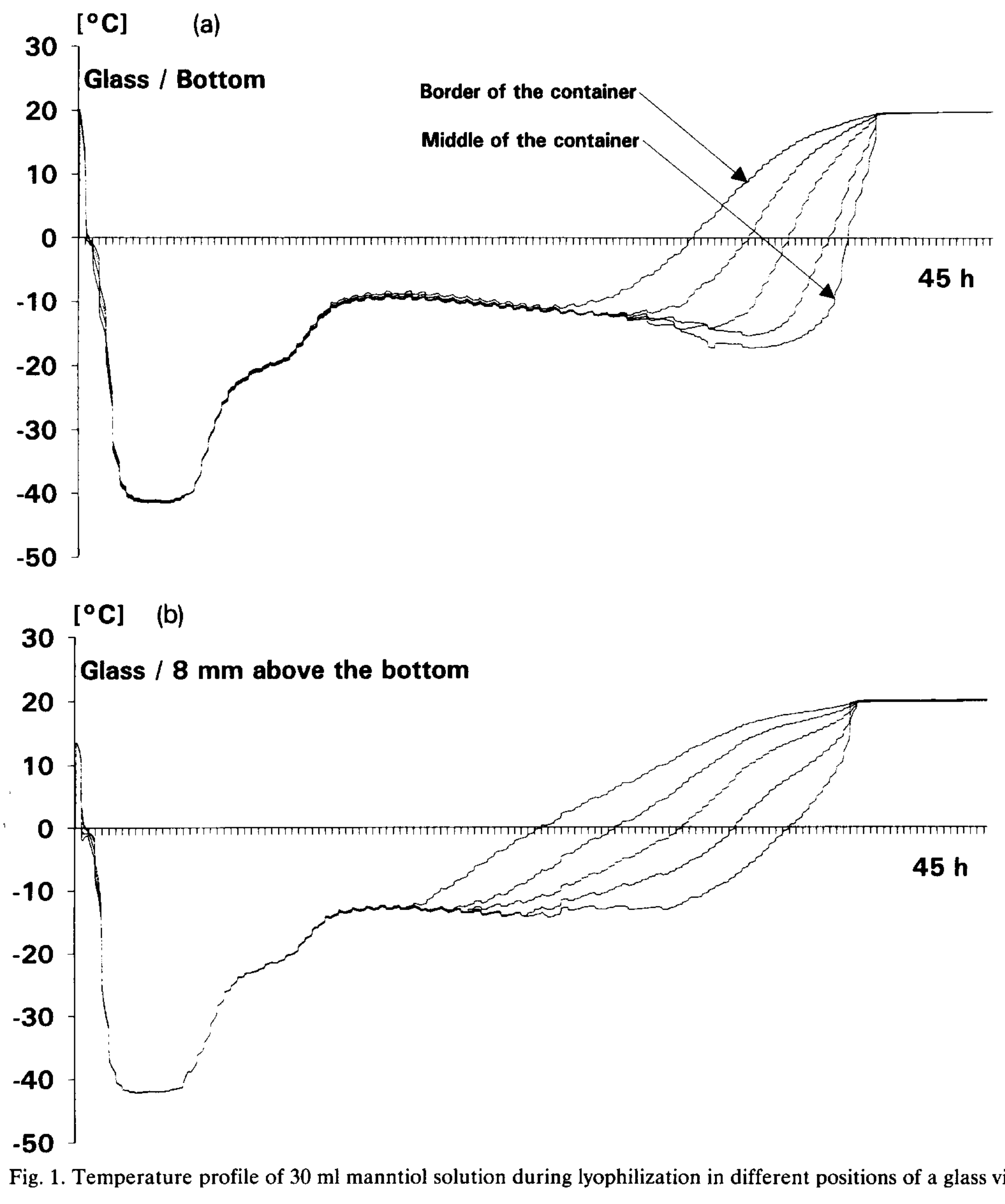


Fig. 1. Temperature profile of 30 ml manntiol solution during lyophilization in different positions of a glass vial.

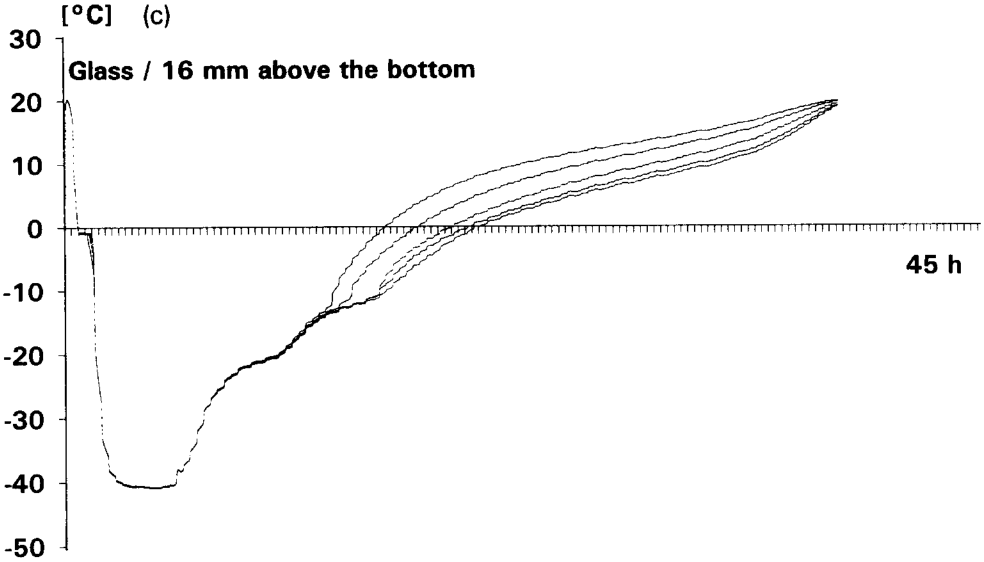


Fig. 1 (continued).

cm; wall thickness, 0.2 cm), and for the X-rays 4R/1 vials, Bündener Glas, Bünde, Germany. Aluminium and plastic containers (Alcan Aluminiumwerke, Göttingen, Germany and Dr Jaenice GmbH & Co. KG, Kehl, Germany, respectively) were chosen so that their geometrical properties were as similar as possible to those of the glass container (aluminium: diameter, 4.5 cm; height, 4.3 cm; wall thickness, 0.03 cm; plastic (polystyrol): diameter, 4.5 cm; height, 4.3 cm; wall thickness, 0.1 cm). Only the shape of the bottom surface was different, whereas the glass container had a curved surface so that the only contact points of this container with the shelf were with the circumference of the bottom.

Mannitol (no. K15344180, Merck, Frankfurt, Germany), GT6 automatic equipped with a sample thief (Leybold-Heraeus, Köln, Germany), thermocouples MT-EOS-KV05 (1 × Ni-Cr-Ni, isolated welded, Heraeus, Köln, Germany), digistrip 4 plus S (Kaye, U.S.A.), X-ray camera Oralix 65 (Philips, Germany) and photographic paper Ultra-speed (Dental Film DF-58, Kodak, Germany) were obtained from the indicated sources.

### 2.2. Methods

The glass (or aluminium or plastic) containers were filled with 30.0 ml of a 5% mannitol solution, and five thermocouples were distributed across half of the diameter of every container at a distance of 0.4 cm from each other. The temperature was measured at the bottom of the containers (without direct contact with the bottom surface) and at distances of 0.4 cm above this, up to a height of 2.0 cm.

Because of the curved surface of the glass container bottom, only the thermocouples in the middle of the vial were close to the bottom, the others slightly above the bottom depending on the degree of curvature. The product had a height of approx. 2.3 cm.

The samples were lyophilized according to the following program: 1.0 h freezing down to −40°C, 3.5 h at −40°C and 0.5 h pre-cooling of the condenser. At a pressure of 0.5 mbar the shelves were heated in 2.5 h to 0°C and left at this temperature for a further 2.5 h. Afterwards the shelves were heated in 2.0 h to their final temperature of 20°C, and these conditions were maintained until the end of the drying process. The duration of the whole process was 40–48 h. As the temperature value the average over 2 min measured at the thermocouple was taken.

The X-ray photographs were obtained in the following way: 4 ml vials were filled with 2.5 ml of a 2.5% mannitol solution and pre-sealed with a lyophilization plug. The drying program consisted of 1.0 h freezing down to −40°C, 12.5 h at −40°C, 0.5 h condensor pre-cooling, warming of the shelves in 4.0 h to 20°C at 0.5 mbar and keeping these conditions constant for a further 6.0 h. With the beginning of primary drying, samples were sealed six times every 2 h, channelled out with the help of a sample thief, and immediately stored deep-frozen. The X-ray photographs were taken with the Oralix 65 machine (Philips) with the adjustment 'upper jaw, incisor'.

## 3. Results and discussion

To evaluate the temperature profile, the average temperatures of the five thermocouples which had the same height in the vial were entered into a graph vs the time (Fig. 1 and 2). On inspection of the temperature profiles in a glass container (Fig. 1), one can see that the drying proceeds from top to bottom. The five thermocouples at the position of 2.0 cm from the bottom show a temperature adjustment earlier than the couples further below. The longest period for adjustment to shelf temperature, i.e., for drying, is required in the bottom region of the glass. This means that in this region there is ice left until the end of the drying process, which sublimates last. The drying process in a vial thus proceeds in vertical direction from the surface of the product to the bottom of the container.

Moreover, it is obvious that the thermocouples in the upper region of the product show almost identical temperatures, which can only be explained by symmetrical drying on one plane. The deeper one proceeds into the middle or bottom region of the container, the larger is the temperature difference between the five thermocouples. This distribution occurs always towards the end of the drying phase at the respective height in such a way that the thermocouple near the outside of the container shows a rise in temperature first and the thermocouple in the middle of the container, last.

The reason for this difference is the convex curvature of the sublimation front, increasing towards the bottom of the container. Thus, at the same height, the outer region can already be dry (e.g., 20°C) while in the middle ice is still left (e.g., −10°C). Eventually, this means that in addition to the drying process in a vertical direction from top to bottom there is a horizontal drying process, although to a much lesser extent.

These findings are comparable with previous investigations of the heat transfer in a glass vial during primary drying (Pikal, 1990a). Three possible routes exist for heat transfer, contact (shelf – container), convection (gas molecules – sides of container) and radiation (the shelf the vial is standing on and the one above). The energy transfer by radiation is negligibly small; energy transfer is mainly achieved by convection and, in particular, by direct contact of the containers with the shelves (Pikal, 1985, 1990). The heat transfer by contact and, to a lesser extent, by convection is strongly influenced by the shape of the bottom of the container (Pikal et al., 1984). While heat transfer by contact and radiation is independent of gas pressure, heat transfer by convection depends on gas pressure in such a way that with rising pressure, and consequently the greater number of molecules in the gas phase, more heat can be conducted to the product. This energy transfer, caused by the collision of gas molecules with the container surface, takes place at the bottom and the sides of the container. The heat transfer 'from the side' accelerates sublimation near the sides of the container, resulting in a curved boundary between ice and dried product.

It is rather unexpected at first that the ice does not melt at the bottom of the container in spite of massive heat transfer at this place by contact as well as by convection and radiation. This phenomenon is best explained by the differences in thermal conductivity between glass and ice (Table 1): ice conducts heat much better than glass. The heat input therefore is continually transferred to the sublimation front and used in sublimation.

Table 1
Thermal conductivities of some materials common to lyophilisation (Perry, 1963)

| Material | Temperature (°C) | Thermal conductivity (J cm$^{-1}$ h$^{-1}$ °C$^{-1}$) |
|---|---|---|
| Ice | – | 78.2 |
| Borosilicate glass | 0 | 39.3 |
| Aluminium | 0 | 10789.9 |

Thus, there never is an 'accumulation of heat' at the bottom of the container which would cause the ice to melt.

Returning to the experiment and the temperature profiles in Fig. 1, it will be noticed that during drying there is a fall in temperature down to approx. −20°C in the bottom region after there has been a rise to approx. −10°C. This

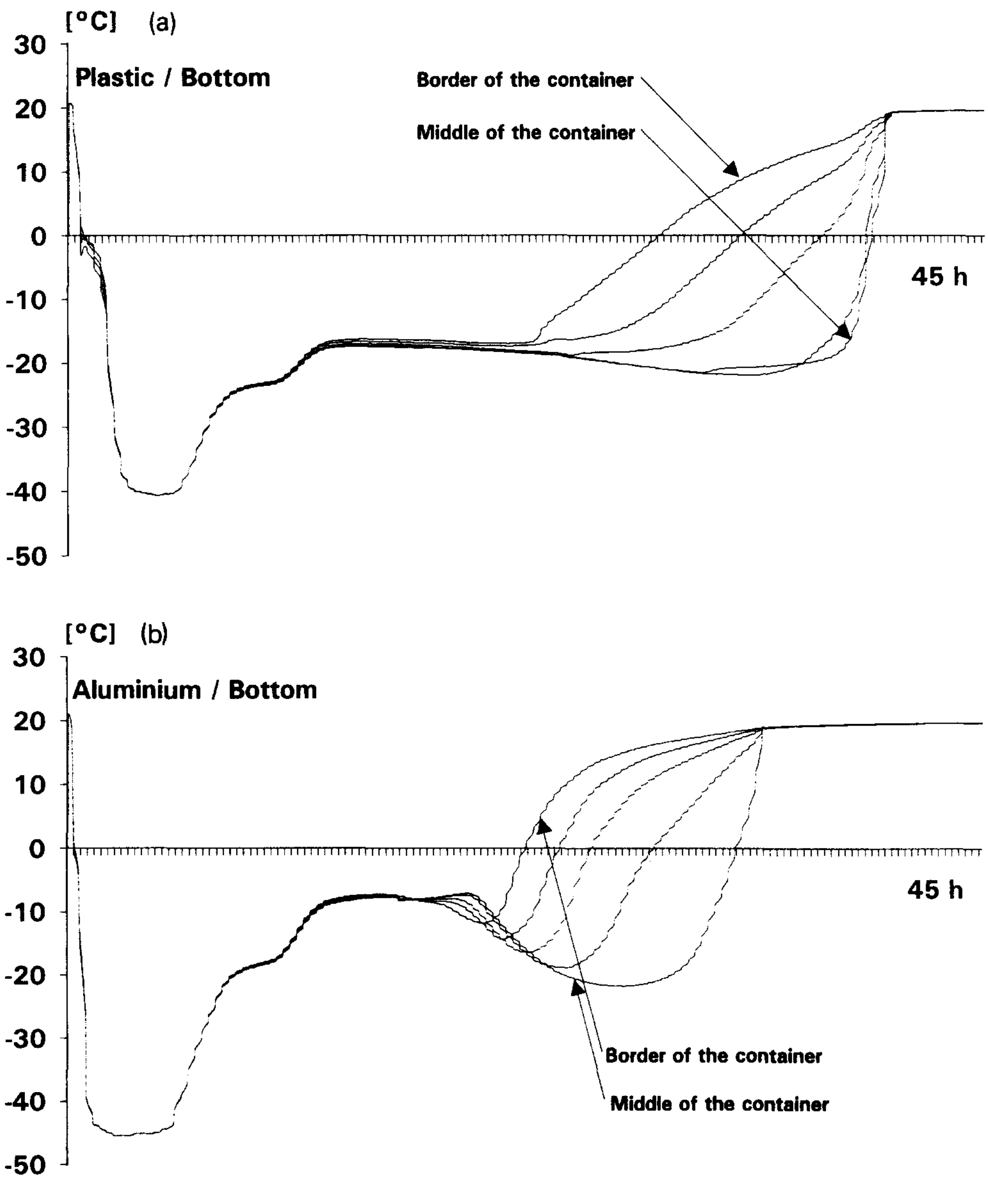


Fig. 2. Temperature profile of 30 ml manntiol solution during lyophilization in the bottom region of a plastic and aluminium container, respectively.

could be explained by the curvature of the sublimation front and the thereby enlarged boundary between ice and dried product. The energy transfer being kept constant leads to an increase in the energy required for sublimation so that energy is drawn from the ice core, resulting in a decrease in temperature in the ice core.

Apart from glass containers as used in the pharmaceutical industry, plastic and aluminium containers were tested. Fig. 2 shows that the material, i.e., its thermal conductivity, has a considerable influence on the drying process.

While plastic, as expected, slows down the process of drying, the process is much more accelerated in an aluminium container compared with a glass container. Due to its high thermal conductivity, aluminium can feed more sublimation energy to the frozen product than, e.g., glass, thus increasing the rate of drying.

Generally, in all graphics (Fig. 1 and 2) two

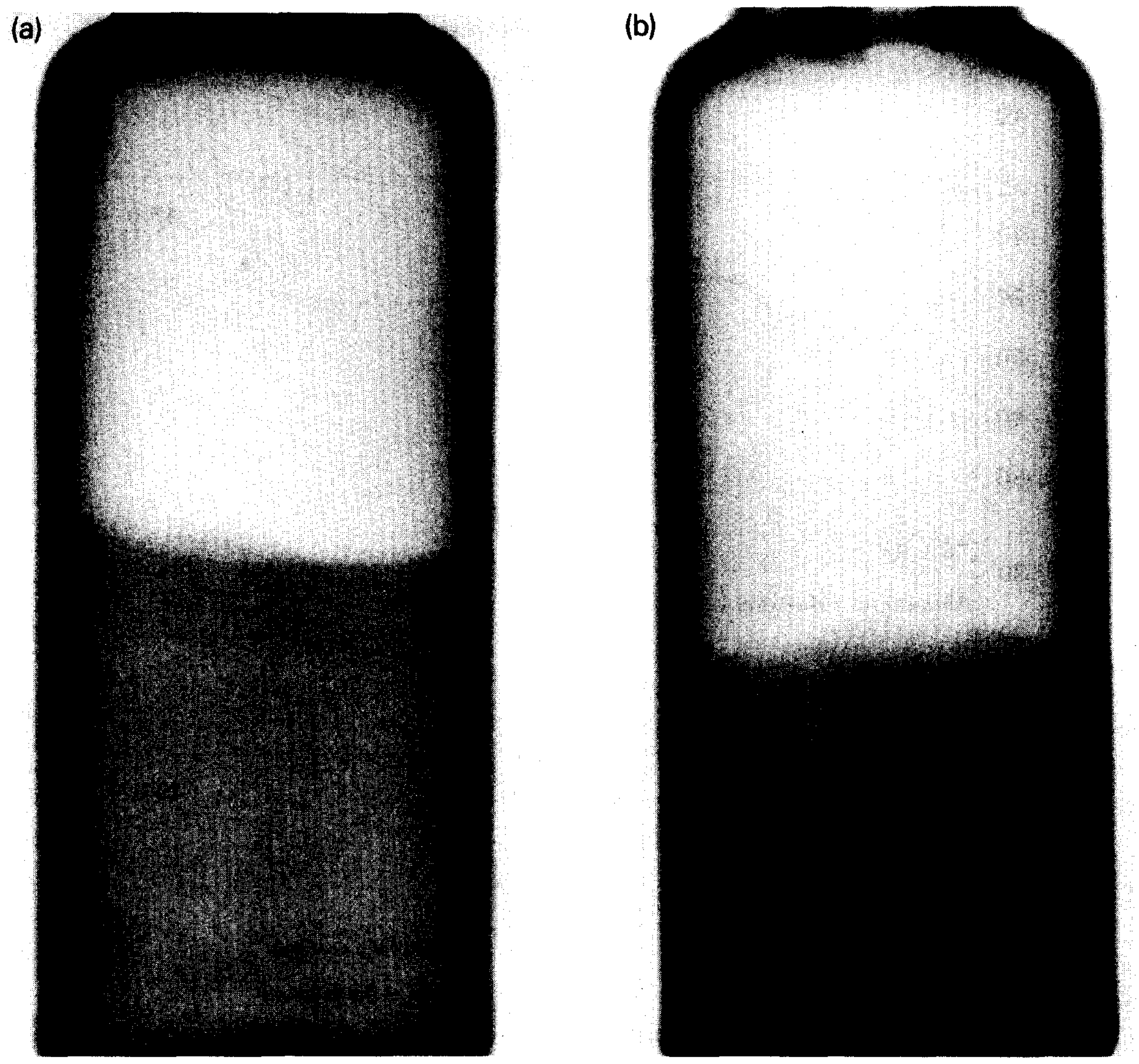


Fig. 3. X-ray representation of the primary drying phase. Pictures were taken at the beginning of primary drying after 4, 6 and 8.5 h.

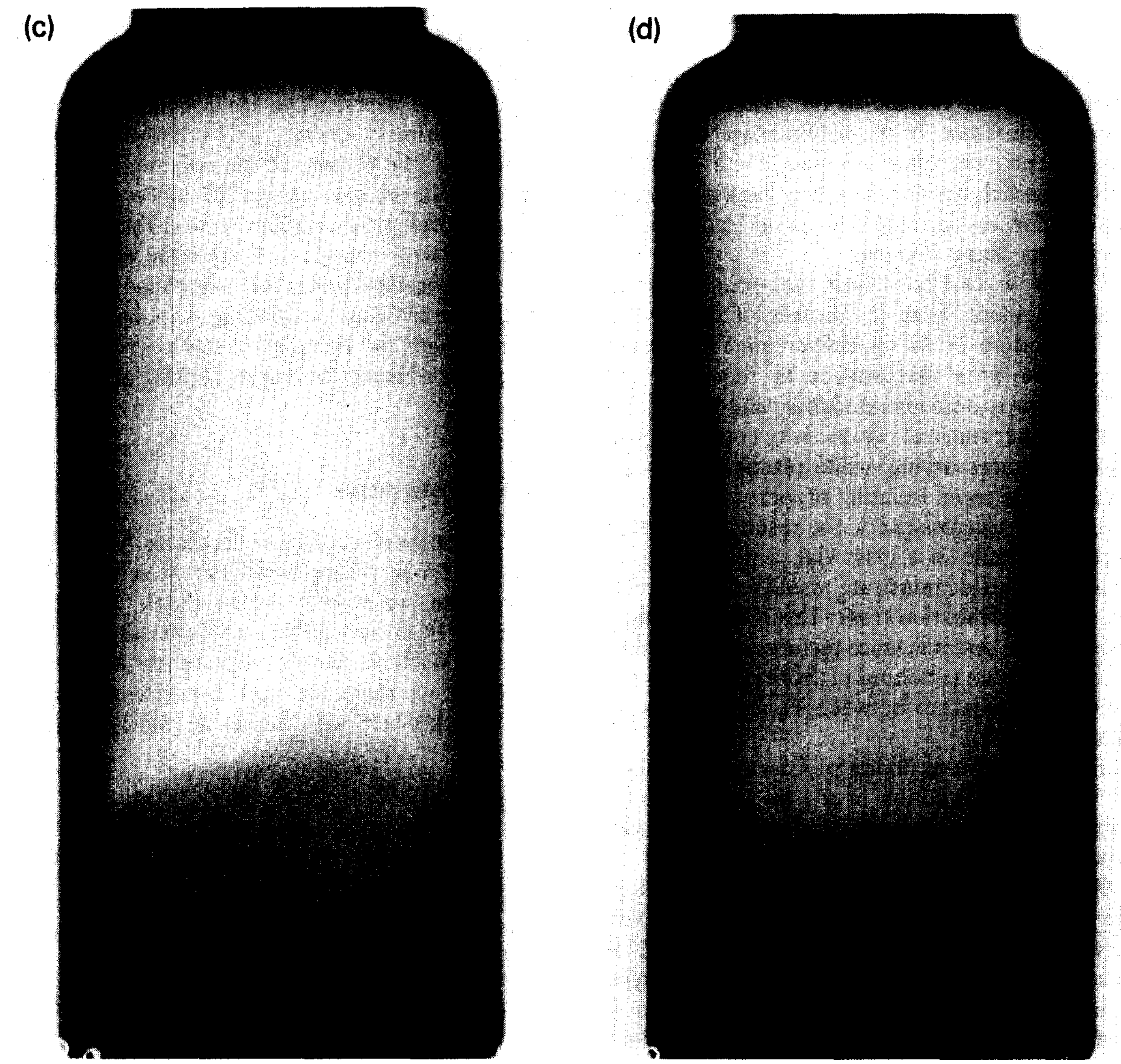


Fig. 3 (continued).

level phases at the beginning of primary drying can be recognized. The first one can be explained by the constant phase at 0°C in the drying program. While the product in the glass vial at this point has a temperature of approx. −20°C, the product in the isolating plastic container, as expected, has a lower temperature (approx. −25°C), and that in the highly conductive aluminium container a slightly higher temperature (approx. −18°C). The second level represents the phase during drying where supply and consumption of energy are almost equal. During this phase, the material of the container also influences the temperature of the product: while temperatures of −10 to −13°C are established in the vial, they are again lower for the plastic (approx. −18°C) and higher for the aluminium (approx. −8 °C).

Particularly astonishing in Fig. 2 is also the second distinct decrease in temperature in the ice core inside the aluminium container. The greater heat supply from the sides and the thus enhanced drying from the outside to the inside result in a stronger curvature of the sublimation front. As a consequence, the sublimation surface is considerably extended, leading to a very high demand of sublimation energy, which is gained from the ice by a temperature decrease.

Finally, it can be stated that primary drying proceeds mainly from the surface of the product to the bottom of the container, and to a lesser extent also from the outside to the centre. In addition, it is demonstrated that aluminium, due to the higher thermal conductivity compared with glass, enhances drying, while plastic retards this process, due to its isolating property.

In order to cross-check the results of the temperature profile in a glass vial, a procedure was established to demonstrate visually the proceeding of the sublimation front. The X-ray technique proved to be optimal, since pictures can be quickly obtained without melting of frozen products and a significant contrast between ice and dried products is visible.

Fig. 3 shows the drying process of 2.5 ml of a 2.5% mannitol solution in a 4 ml vial. The photographs were taken every 2.0 h during the drying process.

As suggested from the temperature profiles, at the beginning a plane boundary surface exists between ice and dried product. After a short time a convex curvature appears, caused by drying from the sides. This curvature is enhanced during drying and remains until the end of the sublimation phase.

The X-ray photographs thus confirm the results of the temperature measurements for the vial and hence the statements concerning the sublimation process.

## 4. Conclusion

The tests described above do not confirm the previous assumption that a frozen product dries symmetrically from all sides during primary drying, resulting in an isolated ice core. On the contrary, it is demonstrated that drying of a frozen mannitol solution proceeds mainly from the surface of the product to the bottom of the container and, to a lesser extent, also from the outside to the centre. The ice remaining until the end of primary drying, i.e., sublimating last, is located at the bottom of the container. The container material also has an influence on the process of drying. Aluminium proved to be the best material with respect to its thermal conductivity. For practical purposes this advantage is counterbalanced by glass, exhibiting a poorer thermal conductivity, but being more stable without corrosion and offering the visual control of the product.

## Acknowledgements

The authors would like to thank ASTA Medica AG for providing facilities and substances; in particular, we would like to thank the departments PEGB and BTW and especially Dr Rawert, Dr Wichert, Mr Kerber and Mr Bollweg. In addition, we thank Dr med. dent. Bätcher, Bielefeld, for his help with the X-ray photographs.

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