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Moisture absorption and desorption of different rubber lyophilisation closures

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Abstract

Rubber closures form a critical barrier in the protection of freeze-dried products against the uptake of moisture. In this study, the moisture absorption of different rubber lyophilisation closures at different temperatures and relative humidities (RH) was evaluated, using a Karl Fischer titration–oven combination. Also, the moisture absorption during steam sterilisation and the moisture desorption during subsequent drying of the stoppers was investigated. Five chlorobutyl and three bromobutyl rubber stoppers were used in this study. The moisture level from the stoppers stored during 85 days at 95% RH–40°C was in the range 0.85–1.49% for the bromobutyl stoppers and in the range 1.71–1.99% for the chlorobutyl stoppers, depending on the stopper formulation. The same trend in moisture absorption was seen during steam sterilisation, where the moisture uptake of the chlorobutyl rubber closures was higher (0.82–0.9%) compared with the bromobutyl closures (0.41–0.57%). Moisture desorption after steam sterilisation, during drying at 100°C, depended on the treatment of the stopper, e.g., siliconation. Finally the moisture absorption of a freeze-dried formulation was evaluated after venting the lyophilisation chamber with air, dry nitrogen, dry helium or closing the vials under vacuum with two different rubber closures. There was no moisture desorption in the rubber closures during the lyophilisation process. Moisture uptake of the freeze-dried cakes depended on the venting procedure of the lyophilisation chamber after freeze-drying. © 1997 Elsevier Science B.V.

Keywords: Rubber stoppers; Moisture absorption; Lyophilisation

1. Introduction

Because of the limited stability in aqueous solution, many pharmaceutical protein and polypeptide formulations are freeze-dried to achieve long term stability (Manning et al., 1989; Pikal, 1991).

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Table 1
Lyophilisation stoppers used in the study

Stopper	Supplier	Type	Polymer	Treatment
W4018	Pharma Gummi	1092	Bromobutyl	Grade A emulsion siliconised
FM357/1	Helvoet Pharma	V9032		SN1 emulsion siliconised
FM257/2		V9032		SNO emulsion siliconised
FM257/2 Omniflex		V9032		Omniflex coated (Fluor-polymer)
W1888	Pharma Gummi	1097	Chlorobutyl	Grade 1/2A emulsion siliconised
PH701/50		1319		Grade A emulsion siliconised
FM140/1	Helvoet Pharma	V9032		SAF1 oil siliconised

Such lyophilised products are presented in a vial and are reconstituted prior to use. The dry products obtained after lyophilisation are highly hygroscopic and must be protected against the uptake of moisture. Rubber closures form a critical barrier in the protection of freeze-dried products against moisture or oxygen uptake. However, moisture absorbed by the rubber closures during autoclave sterilisation, can be transfered to the freeze-dried product, resulting in stability problems (Pikal and Shah, 1991). The capacity of different rubber lyophilisation closures to absorb or desorb moisture is affected by several factors such as rubber formulation, coating and sterilisation procedure, etc.

In this study, the moisture absorption of different rubber lyophilisation closures at different temperatures and relative humidities (RH) was evaluated. Also, the moisture absorption during steam sterilisation and the moisture desorption during subsequent drying of the stoppers were investigated. Finally, the moisture absorption of a freeze-dried formulation was evaluated after venting the lyophilisation chamber with air, dry nitrogen, dry helium or closing the vials under vacuum with different rubber closures.

2. Materials and methods.

2.1. Rubber stoppers

The study was performed using 20 mm diameter lyophilisation closures. Seven different rubber stoppers were evaluated: bromobutyl stoppers W4018 1092 Grade A (Pharma Gummi France, Fourqueux, France), FM357 V9032 SN1, FM257 V9032 SNO and FM257 V9032 Omniflex (Helvoet Pharma, Alken, Belgium); chlorobutyl stoppers PH701/50 1319 Grade A and W1888 1097 Grade 1/2A (Pharma Gummi) and FM140 V9032 SAF1 (Helvoet Pharma). An overview of the stoppers is listed in Table 1. Before each experiment the stoppers were dried at 100°C in a hot-air oven for 24 h.

2.2. Moisture analysis

The moisture content of the stoppers was determined by Karl Fischer titration using a Mettler DL34 in combination with a DO337 drying oven (Mettler Toledo, Lot, Belgium). The Karl Fischer instrument was calibrated using a water standard and disodium tartrate (Riedel-de-Haen, Seelze, Germany). The rubber closures were divided into eight equal-sized pieces and inserted in the drying oven at a temperature of 250°C. The water was vapourized in the oven and transfered into the titration vessel using a dry nitrogen flow rate of 300 ml/min, a conditioning time of 20 min and was subsequently titrated. Hydranal® Composite 2 (Riedel-de-Haen, Seelze, Germany) was used as the Karl Fischer reagent and dry methanol as the solvent. The moisture content of the lyophilised cakes was tested using Karl Fischer titration with direct addition of the powder inside the titration vessel, using a stirring time of 4 min. All results are presented as the mean $\pm$ S.D. ($n = 3$).

2.3. Moisture absorption experiment

The seven different lyophilisation stoppers were stored in dessicators at 30°C–75% RH and 40°C–95% RH and analysed for moisture absorption as described previously over a period of 195 days. The RH inside the dessicators was installed using saturated salt solutions (NaCl: 75% RH and KNO_3: 95% RH) and continously monitored by a humidity sensor (Testostor® 171, Testo, Lenzkirch, Germany).

2.4. Steam sterilisation experiment

The rubber closures were dried at 100°C for 24 h, before steam sterilisation. The rubber closures were then packed in a SPS Pealpack® sterilisation bag (SPS Laboratoires, Coulommiers, France), each containing 200 stoppers. The bags were sterilised in an autoclave (Wesa IPP144-40) for 30 min at 121°C and 1 atm overpressure using saturated steam. After sterilisation, the stoppers were put in stainless steel boxes and dried in a hot air oven at 100°C.

The moisture content of the different stoppers was analysed before sterilisation, immediately after sterilisation and at different time intervals (2, 4, 8 and 24 h) during the drying process. The moisture content was determined by Karl Fisher titration and by a gravimetric method. The weight gain during sterilisation and the weight loss during drying was determined on 20 stoppers of each type.

The results were statistically evaluated using ANOVA and Tukey's Multiple comparison test ($P = 0.001$).

2.5. Lyophilisation experiments

Two millilitres of a 10% w/v solution of maltodextrin DE 22 (Eridania-Beghin Say-Cerestar, Vilvoorde, Belgium), was placed into 8 ml Type I glass vials (Gaasch Packaging, Mollem, Belgium). Bromobutyl 20 mm stoppers FM257/2 SN0 and chlorobutyl 20 mm stoppers FM140/1 SAF1 (Helvoet Pharma) were dried at 100°C for 24 h and partially inserted into the vials. Next the solutions were lyophilised in a Amsco-Finn Aqua GT4 freeze-dryer. The samples were frozen on the lyophiliser shelves to −40°C in 25 min and kept at this temperature for 1 h. Primary drying was performed by keeping the vials for 8 h at a pressure of 0.5 mb, a shelf temperature of −10°C and a condenser temperature of −60°C. Secondary drying was carried out by increasing the shelf temperature to 25°C and reducing the pressure to 0.1 mb. The secondary drying time was 6 h. The lyophilisation process was carried out in quadruplicate using four different venting procedures: venting the drying chamber with air, dry nitrogen ($H_2O < 2$ ppm) or dry helium ($H_2O < 2$ ppm) (L'Air Liquide Belge NV, Gent, Belgium) and sealing the vials or sealing the vials under vacuum by automatic stoppering in the freeze-dryer. The freeze-dried samples were stored at 95% RH–40°C for a period of 300 days. The moisture content of the closures was determined before freeze-drying and the moisture content of the closures and the lyophilised powders was determined immediately after lyophilisation and at different times during the storage period.

3. Results and discussion

Common elastomers used in the pharmaceutical packaging industry include butyl/halobutyl rubber, natural rubber, neoprene a.o. (Avis et al., 1986). Moisture vapour transmission (MVT) is an important consideration when a closure is selected for hygroscopic materials, such as lyophilised powders (PDA). Butyl/halobutyl elastomers provide excellent MVT protection in comparison with natural rubber, making it a good choice for vial closures to package lyophilised drugs (Swarbrick and Boylan, 1992). The moisture absorption of the investigated halobutyl rubber lyophilisation closures at 95% RH–40°C is shown in Fig. 1. The moisture levels of the rubber closures stored at 95% RH–40°C increased rapidly during the first 20 days and then slowly reached a plateau. After 85 days of storage under these conditions (95% RH–40°C) saturation was achieved. The moisture saturation level was a function of the composition of the rubber stoppers. For the chlorobutyl stoppers FM140, W1888 and PH701/50, a saturation

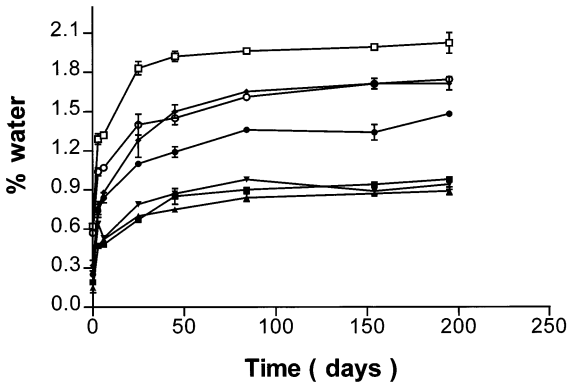


Fig. 1. Water content of the rubber stoppers (% w/w) in function of time. The stoppers were stored at 95% relative humidity and 40°C. FM 357/1 SN1 (■); FM 257/2 SN0 (▲); FM 257/2 omniflex (▼); FM 140/1 SAF1 (◆); W4018 (●); W1888 (□); PH701/50 (o).

moisture level of $1.71 \pm 0.05\%$, $1.50 \pm 0.02\%$ and $1.99 \pm 0.01\%$, respectively was reached. It was impossible to differentiate between the moisture sorption behaviour of the FM257 SNO emulsion siliconised, the FM257 omniflex coated and the FM 357 SN1 emulsion siliconised stoppers. The W4018, which was a bromobutyl stopper with silicate as a filler, reached an intermediate saturation moisture level of $1.34 \pm 0.06\%$. To obtain a better idea of the influence of the stopper formulation on the rate of moisture uptake which is in turn is influenced by the diffusion coefficient of vapour molecules inside the stopper, the results from Fig. 1 were plotted on a semi logarithmic scale. The transformation of the data did not show a clear linear relation between the water uptake and ln(time). However, clear differences were observed between the slopes of the (linear) trend lines as plotted in Fig. 2. Table 2 shows the slopes being in the range 0.22–0.29 for the chlorobutyl stoppers and 0.13–0.19 for the bromobutyl stoppers. This indicated that not only the maximal water absorption but also the rate of water absorption was higher for the chlorobutyl stoppers compared with the bromobutyl stoppers.

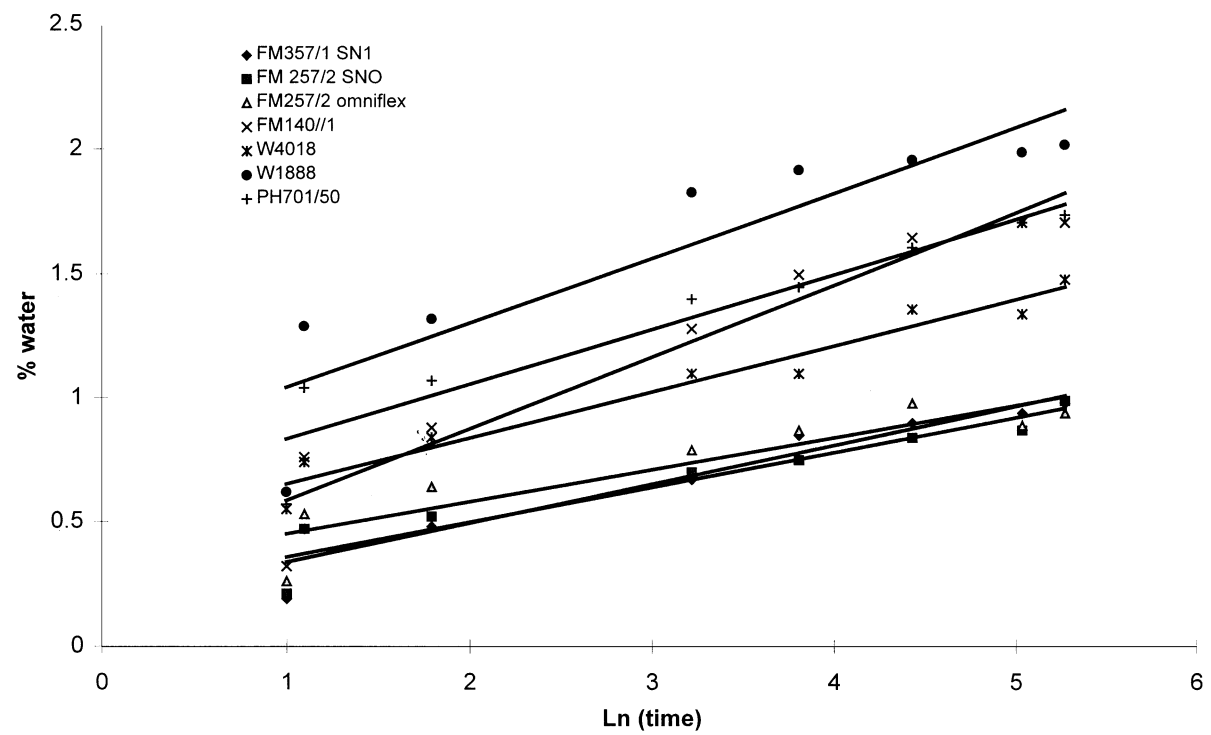


Fig. 2. Water content of the rubber stoppers (% w/v) vs. Ln (time). The stoppers were stored at 95% relative humidity and 40°C.

Table 2
Slope of the linearised curve fitted exponential equation of the moisture absorption of the different rubber stoppers at 95% RH–40°C

Stopper	Slope
W4018	0.187
FM357/1	0.157
FM257/2	0.142
FM257/2 Omniflex	0.131
W1888	0.261
PH701/50	0.222
FM140/1	0.291

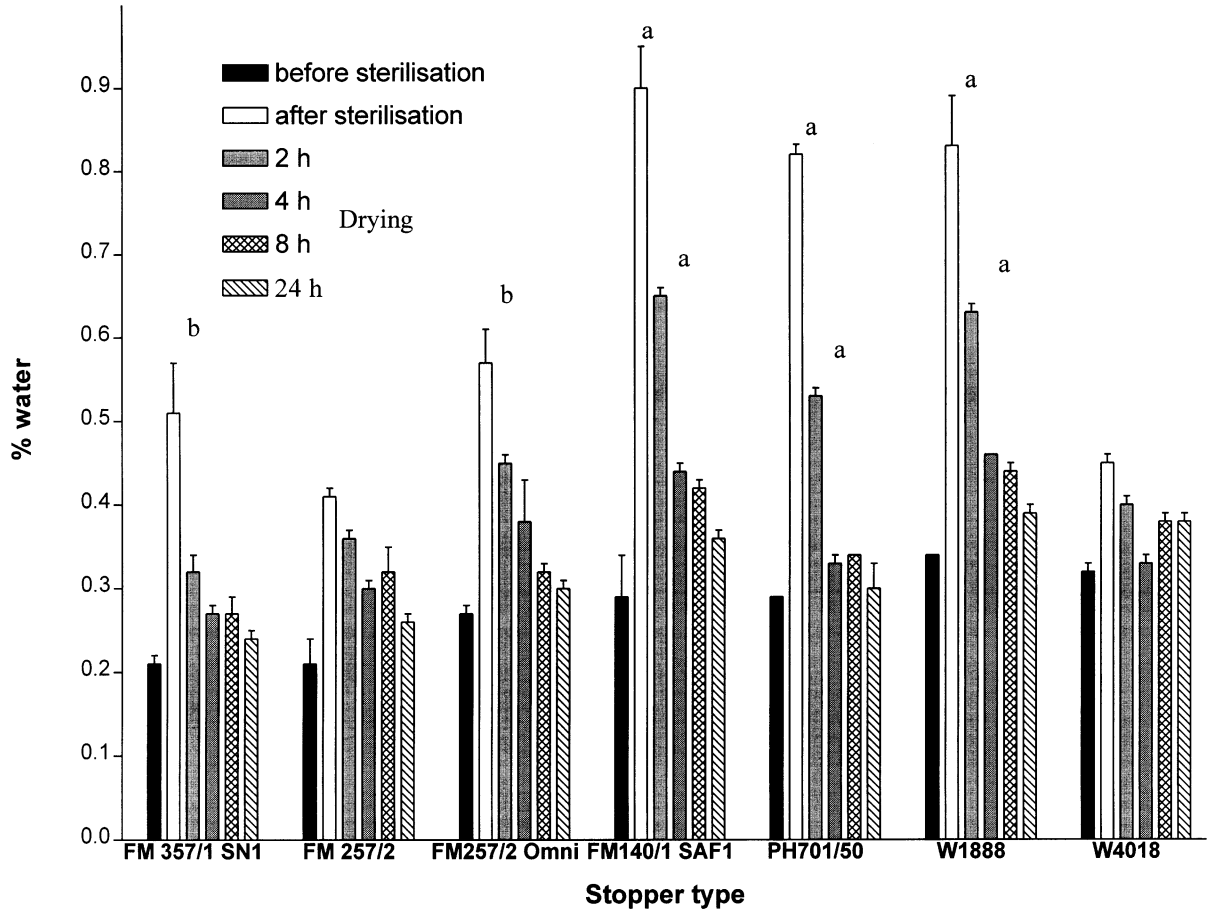
When the stoppers were stored at 75% RH–30°C it was impossible to differentiate between the moisture absorption profile of the rubber closures (data not shown). It was impossible to curve-fit the data to a first order logarithmic equation with an acceptable correlation coefficient. Vromans and Van Laarhoven (1992) reported that new types of rubbers can be evaluated with respect to their barrier properties under stress conditions such as 40°C–95% RH. It was reported that the uptake capacity of large amounts of water was not an indication for higher water permeability of the stoppers: rubber closures with a low permeability were able to take up significant amounts of water (Vromans and Van Laarhoven, 1992).

The moisture content of the different rubber stoppers before and after steam sterilisation and at different times during drying at 100°C are shown in Fig. 3. Compared with the moisture absorption study, the same trend in water uptake was seen after steam sterilisation of the stoppers in a Pealpack® sterilisation bag. The moisture content of the chlorobutyl rubber closures, measured by Karl Fischer titration, was $0.90 \pm 0.05\%$, $0.82 \pm 0.01\%$ and $0.83 \pm 0.06\%$ for the FM140, PH701/50 and W1888 stoppers, respectively. The moisture content of the bromobutyl stoppers after steam sterilisation was in the range 0.41–0.57%. The moisture decrease of the stoppers, during air drying at 100°C, depended on the stopper formulation. For the FM357/1 SN1 and the 1319 A stoppers, there was a rapid decrease in moisture content of 0.20% and 0.28%, respectively, during the first 2 h of the drying process being equivalent to 61 and 49%, respectively, of the moisture taken up during steam sterilisation. For the other rubber closures, the moisture content decreased more gradually during the total drying period of 24 h at 100°C.

During the lyophilisation experiments, no moisture loss of the rubbers stoppers, which were partially inserted into the vials, was observed. This is not unexpected, since the water inside the rubber stoppers is present in a vapour state and there is no direct contact between the rubber stopper and the lyophiliser shelf, the water inside the rubber stopper is not frozen and no sublimation occurs in the rubber stopper during drying. One would rather expect to have water vapour diffusion through the rubber material comparable with what happens to the freeze-dried cake during the secondary drying stage of the lyophilisation process. At low temperature and low pressure however, no water diffusion occurs in the rubber material, since the porosity of the rubber is very low compared with the freeze-dried cake.

The water absorption of the rubber stoppers and the freeze-dried cakes of a 10% w/v maltodextrin DE22 formulation, during a storage period of 300 days at 40°C–95% RH is shown in Fig. 4a,b,c. For the vials vented with dry nitrogen, surprisingly the drying of the freeze-dried cakes continued inside the vials: the moisture content of the cake decreased during 150 days, then the cakes began to take up moisture (Fig. 4a). Moreover, during storage an increase in moisture content of the stoppers was observed. However, the uptake of water occurs more slowly in comparison with the moisture absorption as described in Fig. 1. This could be expected as, in the 'storage after lyophilisation experiments' (Fig. 4), the stoppers were inserted into the vials. Consequently, only the upper part of the surface of the stoppers was in direct contact with the 95% RH while the whole surface of the stoppers was surrounded by the 95% RH air in the experiments described in Fig. 1.

To understand moisture absorption/desorption during storage after lyophilisation, Fig. 5 shows schematically the physicochemical phenomena which might influence the water transport in the cake, the head space and the stoppers of the vials.



a significantly higher than FM257/2 omniflex (p=0.001)
b significantly higher than FM257/2 and W4018 (p=0.001)

Fig. 3. Water content of the rubber stoppers (% w/w) during steam sterilisation and drying at 100°C.

At the beginning of the storage, few water molecules are present in the stopper. However, as described above (Fig. 1), water molecules from the 95% RH environment (E) begin to penetrate the stopper (S). As explained earlier, this water flux (F_{ES}) occurs relatively slowly partially due to the limited surface (S_{exp}) exposed to the humid environment. Other parameters which might influence F_{ES} are, the composition of the stopper, which influences the diffusion coefficient (D), and the difference in partial pressure of the vapour molecules across the stopper ($\Delta P/l$).

$$F_{ES} \approx D\, S_{exp}\, (\Delta P/1) \quad (1)$$

$$\Delta P = P_E - P_H \quad (2)$$

A second type of water transport occurs from water molecules which evaporate (EV_{CH}) from the cake (C) into the head space (H) of the vials. They move in the head space of the vial (H) and tend to diffuse into the stopper. This flux is indicated in Fig. 5 by F_{HS}. The diffusion coefficient of water vapour into air is 0.24 cm^2/s (Handbook of chemistry and physics, 1971) which also states that, the

diffusion of water into the gases studied and vacuum occurs very fast and cannot be a rate limiting step in the vapour flux from the cake into the stopper. However, considering the slow moisture absorption, as observed in Fig. 1, it is assumed that F_{HS} is the rate limiting step in the overall process. Fig. 4a,b show that freeze-dried cakes loose water during the first weeks of storage. As described in Fig. 5, this is attributed to EV_{CH} and F_{HS}. It seems that the rate of water loss by the freeze-dried cakes, as calculated from the slope of the linear decrease of the water content of the cakes, is independent on the kind of gas in the head space. The daily decrease of the procentual water content of the cakes equals 0.0043% and 0.0045% for the nitrogen and helium vented vials, respectively. Moreover, Fig. 4a,b shows that, independently of the gas used, freeze-dried cakes start to take up water when the moisture level is around $0.73 \pm 0.3\%$ (in the case of the FM140 chlorobutyl stoppers) and $0.51 \pm 0.2\%$ (in the case of the 257 bromobutyl stoppers; data not shown). The same rate of water loss by the freeze-dried cakes, on one hand, and the same water content of the stoppers at the moment the cakes begin to absorb water, on the other hand, indicate that part I in Fig. 4a,b is independent on the gas used. Part I of the curves is governed by the interplay

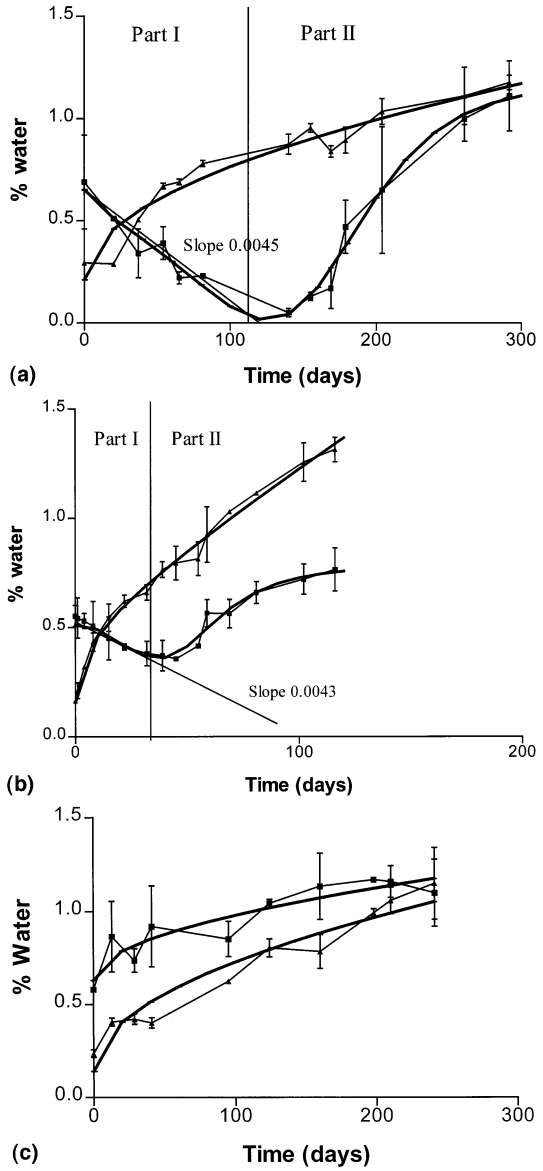


Fig. 4. Water content (% w/w) of the rubber stoppers FM140/1 SAF1 (▲) and freeze-dried cakes (■) during a storage period of 300 days at 95% RH–40°C, using different venting procedures: dry nitrogen venting (4a); dry helium venting (4b); vacuum sealing (4c).

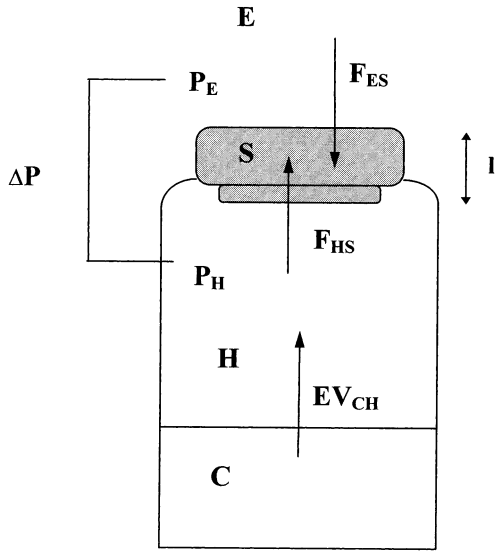


Fig. 5. Schematical overview of the physicochemical phenomena influencing water transport in the cake, the head space and the stopper of the vials. Environment (E), headspace (H), stopper (S), cake (C).

between F_{ES} and F_{HS}: as long as the water content in the stoppers is lower than a critical water content ($\%_{crit}$) being 0.73% in the case of chlorobutyl stoppers, the lyophilised cakes loose water due to F_{HS}. However, as the water content in the stoppers increase above $\%_{crit}$, water molecules from F_{ES} arrive in the head space and become available for absorption by the lyophilised cake.

Fig. 4a,b clearly illustrate that the water uptake by the FM140 chorobutyl stoppers occured faster when helium was used than when nitrogen was used as headspace gas. The reason is unclear. However, it means that $\%_{crit}$ is obtained for the helium vented vials quicker and that lyophilised cakes stored under helium begin to adsorb water much faster than when stored under nitrogen. This is clearly observed from Fig. 4a,b.

The observation of moisture loss by the lyophilised cake was not confirmed when the vials were vented with air or closed under a vacuum. A moisture increase both for the rubber stoppers and the freeze-dried cakes was seen (Fig. 4c). When the vials were vented with air it can be expected that EV_{CH} does not occur as the equilibrium partial pressure of water vapour in the headspace of the vial is already present. Consequently, the lyophilised cakes do not loose water but absorb moisture which is present in the air in the headspace. When the vials are closed under vacuum, all the water in the cakes might evaporate instantaneously. This might explain the absence of the gradual decrease of water content in the cakes stored under vacuum.

Although the Karl Fischer titration was used in this study to evaluate the moisture content of the rubber stoppers, a gravimetric method can also be used. There was a good correlation between the gravimetric method (weight increase) and the Karl Fischer titration for the determination of the moisture absorption during steam sterilisation of the stoppers. For example for the FM140 closure, using the Karl Fischer analysis, the moisture content before sterilisation was $0.29 \pm 0.05\%$ and after sterilisation $0.90 \pm 0.05\%$. This is equivalent with an increase in moisture content of 0.61%. The average weight increase of the stoppers was 0.266 g, equivalent with a weight gain of 0.58%, which correlated good with the increase in water content of 0.61%. Using a gravimetric method to evaluate the weight loss of rubber stoppers, it is not possible to differentiate between water and other volatile compounds, whereas the Karl Fischer titration is specific for water. Using the gravimetric analysis, an average weight loss of 0.301% was seen after 2 h drying time, whereas the decrease in moisture content, measured with KF titration was only 0.25%. The same observations were made by DeGrazio et al. (1992), when the correlation between Karl Fischer titration and gravimetric analysis was evaluated.

It can be concluded that not only the choice of rubber stopper formulation is an important parameter in moisture control of freeze-dried formulations, but the processing of the stoppers (e.g. sterilisation and drying) and the aeration procedure of the vials before sealing are also critical parameters.

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