Drug Development and Industrial Pharmacy, 25(9), 1005–1013 (1999)

RESEARCH PAPER

Stability of Freeze-Dried Tablets at Different Relative Humidities

Sam Corveleyn and Jean Paul Remon*

Laboratory of Pharmaceutical Technology, University of Gent, Harelbekestraat 72, 9000 Gent, Belgium

ABSTRACT

The purpose of the study was to evaluate the stability of two different freeze-dried tablet formulations at different relative humidities (RHs). The tablets contained 25 mg hydrochlorothiazide (HCT) as a model drug and were prepared by freeze-drying a suspension and an oil-in-water (o/w) emulsion. Formulation A was a rapidly disintegrating tablet and consisted of 80 mg of maltodextrine DE38; 8 mg of polyethyleneglycol (PEG 6000), 8 mg of xanthan gum, and 25 mg of HCT. Formulation B was a lyophilized dry emulsion tablet that consisted of 160 mg of Miglyol® 812, 80 mg of maltodextrin DE38, 16 mg of methylcellulose (Methocel®) A15LV, and 25 mg of HCT. Tablets were packaged in different packing materials: polyvinylchloride (PVC)/aluminum blister packs, PVC-polyvinylidenechloride (PVDC)/aluminum blister packs, closed containers with a dessicant tablet, and open containers. The tablets were stored at three relative humidities (45%, 60%, and 85% RH) and were characterized on mechanical strength, residual moisture, porosity, content uniformity, and scanning electron microscopy (SEM) during a period of 6 months. After 1 month at 60% and 85% RH, a strong increase in moisture content (from 2.7% to 6.8%) was seen for the tablets packed in the open and closed containers and for the PVC/aluminum blistered tablets. This increase was higher for formulation A compared to formulation B since B contained 160 mg of triglycerides and was more hydrophobic. This increase in water content was correlated with a decrease in mechanical strength. The tablets also showed a change in microstructure and porosity. At a moisture content of 7.2%, formulation A showed a structural ''collapse'' since water acts as a plasticizer for the amorphous glass, lowering the glass transition

* To whom correspondence should be addressed. Telephone: ++32 9 264 80 56. Fax: ++32 9 222 85 36. E-mail: jeanpaul.remon@rug.ac.be

temperature T_g*. This phenomenon even occurred in PVC/aluminum blister packs at 85% RH. The structural collapse was associated with a complete loss of microstructure as detected by porosimetric analysis and SEM. For the PVC-PVDC/aluminum blistered tablets, the increase in moisture content and decrease in mechanical strength at 85% RH occurred much slower, and the water uptake and strength loss were less intensive. No significant breakdown of HCT could be observed in both formulations with all of the packing materials. Packaging of freeze-dried tablets with PVC/aluminum blister packs, PVC/PVDC/aluminum blister packs, or closed containers did not offer protection against moisture uptake, mechanical strength loss, and structural collapse.*

INTRODUCTION

In previous work, the formulation and production of both rapidly disintegrating freeze-dried tablets (1,2) and lyophilized dry emulsion tablets (3) was described. These freeze-dried tablets were evaluated in vivo in a relative bioavailability study (3). It is known that freeze-dried tablets are very hygroscopic and should be protected from air and humidity (4).

Most of the freeze-dried pharmaceutical formulations are packed in vials closed with a rubber stopper. Freeze-dried tablets should be packed in another accurate barrier that protects the product from moisture absorption. The amount of water that is absorbed by a formulation is not only a function of the identity and polarity of the individual components, but also the relative humidity (RH) and temperature of the environment (5). Packing of freeze-dried tablets in blister packs seems a good choice; however, closed containers with a dessicant tablet would also be a possible choice.

In the use of blister packing, the choice of the blister material (polyvinylchloride [PVC], polyvinylidenechloride [PVDC], polypropylene, aluminum, etc.) is very important to minimize moisture permeation through the blisters (6). The glass transition temperature T_g of amorphous freeze-dried materials will decrease when the formulation absorbs water. This decrease in T_g can result in a glass-rubber transition (7,8). Water acts as a plasticizer, reducing the T_g of the formulation, thereby changing the texture characteristics of the freeze-dried tablets. This could result in a change in porosity and hardness of the freeze-dried tablets. In this study, the influence of different packaging materials on the characteristics of freeze-dried tablets stored at different relative humidities was examined. Five different parameters were evaluated: porosity, moisture content, hardness, content uniformity, and morphology of the fracture plane of the tablets.

MATERIALS AND METHODS

Materials

The spray-dried maltodextrin (C✯PUR01934, Eridania-Beghin Say-Cerestar, Vilvoorde, Belgium) was obtained by enzymatic hydrolysis of cornstarch and had a dextrose equivalent (DE) of 38. Xanthan gum was obtained from Ludeco (Brussels, Belgium). Polyethyleneglycol (PEG 6000) was obtained from Union Carbide (Danbury, CT). Solutions were made in distilled water. Hydrochlorothiazide (HCT) (batch no. 5327 B, Ludeco, Brussels, Belgium) was chosen as a model drug. HCT is a diuretic that is practically insoluble in water (25°C) and has a solubility of 250 mg/L in 0.1 N HCl (25°C). Methylcellulose (Methocel® A15LV, 2% aqueous solution viscosity 15 mPa · s at 20°C) was provided by Colorcon (Kent, UK). A medium-chain triglyceride Miglyol 812 (Federa, Belgium) was used as the oil phase in the emulsion tablets. Karl-Fischer reagents Hydranal® Composite 5 (Riedel-de-Haën, Seelze, Germany) and dried methanol (Riedel-de-Haën) were used for moisture content determination.

Methods

Formulations

Two lyophilized tablet formulations were evaluated in this stability study. Formulation A was a freeze-dried, rapidly disintegrating tablet that consisted of 80 mg of maltodextrin DE38, 8 mg of PEG 6000, 8 mg of xanthan gum, and 25 mg of HCT. Formulation B was a lyophilized dry emulsion tablet that consisted of 160 mg of Miglyol 812, 80 mg of maltodextrin DE38, 16 mg of Methocel A15LV, and 25 mg of HCT.

Preparation of the Tablets

For the manufacturing of formulation A, 12.5 g of HCT was suspended in 400 ml of a solution containing

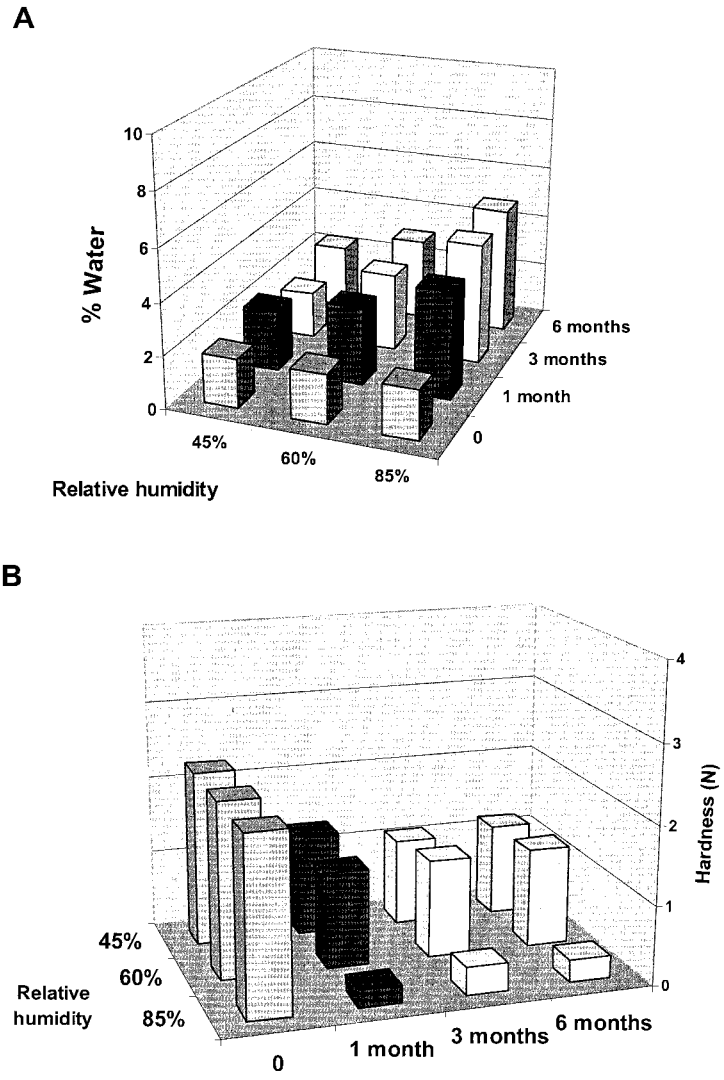

Figure 1. (A) Moisture content and (B) tablet hardness as a function of time of formulation A packed in PVC/aluminum blister packs and stored at three different relative humidities.

maltodextrin in a concentration of 20% (w/v), PEG 6000 in a 1% (w/v) concentration, and xanthan gum in a 1% (w/v) concentration. For the preparation of the emulsion formulation B, an aqueous solution was prepared containing 20% (w/v) maltodextrin; this solution was emulsified with Miglyol 812 using 2% (w/w) Methocel A15LV, and 12.5 g of HCT was mixed with the emulsion. The ratio water phase/oil phase was 80/20 (w/w). The emulsion was prepared using a Silverson mixer (Silverson Machines, Waterside, UK) according to a standardized production protocol described by Kiekens et al. (9).

The freeze-dried tablets were prepared as follows: 0.8 g of the emulsion or the suspension was used to fill PVC blisters with a diameter of 15 mm and a depth of 6 mm. The blisters were placed on the shelves of the freeze-dryer (Amsco-Finn Aqua GT4, Amsco, Brussels, Belgium). The samples were frozen to $-45°C$ at a rate of $0.5°C\ min^{-1}$ and kept at this temperature for 1.5 hr. Primary drying was performed by keeping the blisters for

8 hr at a pressure of 1 mbar, a shelf temperature of −10°C, and a condensor temperature of −60°C. Secondary drying was carried out by reducing the pressure to 0.1 mbar and increasing the shelf temperature to 25°C. Secondary drying time was 6 hr. Lyophilization was terminated by venting the drying chamber with air.

Packaging and Storage

Three different packaging materials were evaluated in this stability study. Unpacked tablets were tested as a control. Both tablet formulations were packed in PVC/aluminum blister packs and in PVC-PVDC/aluminum blister packs using an EAS unit dose blistermachine (Klöckner Pentapack, Hammont-Achel, Belgium). The PVC film had a thickness of 120–400 μm (Pentapharm PH 170/01, Kale Pentaplast, Germany). PVC/PVDC is a PVDC-coated PVC film having lower water vapor permeability. The aluminum film thickness was 20 μm (Patz 38/Alu H20, Patz, Laipersbach, Austria). The other packaging material was a closed container (50 ml volume) with a dessicant tablet; it was filled with 50 tablets of the respective formulations. The tablets were stored at three relative humidities (45%, 60%, and 85%) at room temperature (22°C). The relative humidities were installed using saturated salt solutions. The relative humidity was

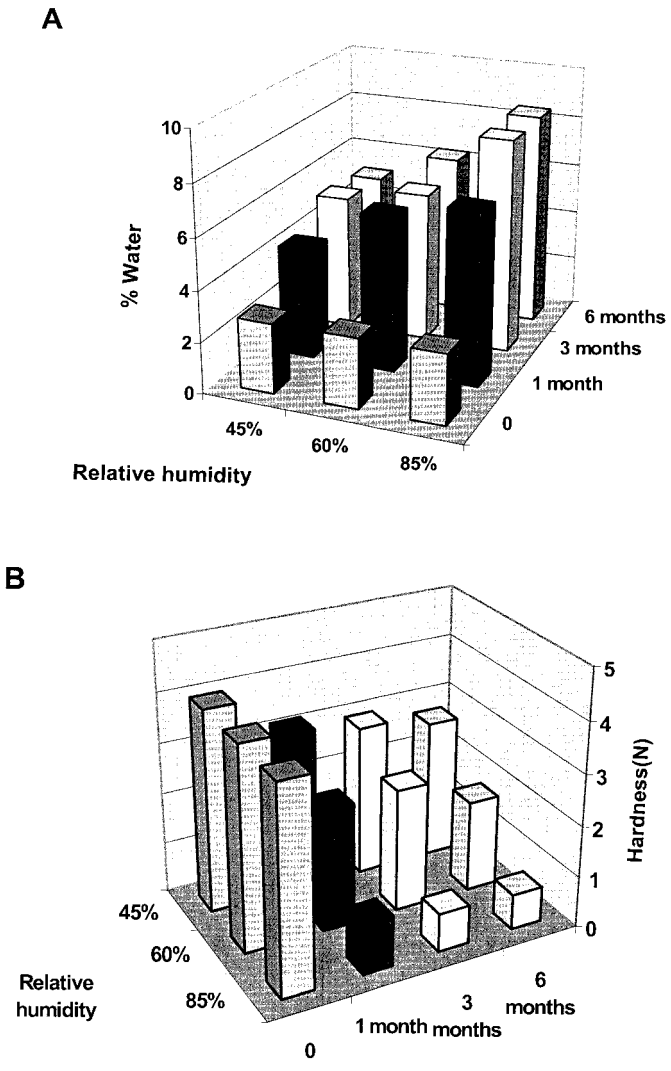

Figure 2. (A) Moisture content and (B) tablet hardness as a function of time of formulation B packed in PVC/aluminum blister packs and stored at three different relative humidities as a function of time.

monitored using a Testostor® relative humidity sensor (Tsto, Lenzkirch, Germany).

Content Uniformity Analysis

Five tablets were each weighed to obtain the mean tablet weight. An accurately weighed portion of the homogenized powder corresponding to 25 mg HCT and 25 mg of hydroflumethiazide (HFMT) (Sigma Chemical Co., St. Louis, MO) as the internal standard (IS) were treated with methanol of high-performance liquid chromatography (HPLC) grade (Merck, Darmstadt, Germany) for the formulation A tablets or with diluted sodium hydroxide (NaOH; 0.2 M) (Vel, Leuven, Belgium) for the formulation B tablets. This was next put in the ultrasonic generator for 1 hr and then diluted to 50 ml with the same solvent. Next, 10 ml of the solution was filtered through a 0.2-μm membrane filter (Schleicher and Schuell FP030/3). The first 5 ml of the filtrate was discarded, and 2.5 ml of the filtrate was diluted with mobile phase to 50.0 ml, providing a theoretical concentration of 25 μg/ml HCT and 25 μg/ml IS. Of this solution, 100 μl was injected in the HPLC system. The HCT concentrations were determined using a validated HPLC procedure described by Vervaet and Remon (10). An RP-C18 column (250 × 4 mm, 5 μm) (Lichrospher 100, Merck) equipped with a precolumn (RP-C18, 4 × 4 mm, 5 μm) was used. Both were kept at a constant temperature of 40°C. The mobile phase was 0.2 M phosphate buffer (pH 7.0; buffer/tetrahydrofuran/acetonitrile 85/10/5 v/v/v). The ultraviolet (UV) detector was set at a wavelength of 273 nm. The HFMT (Sigma) was used as the IS.

Scanning Electron Microscopy

Scanning electron micrographs (SEMs) of the fracture plane of the lyophilized tablets were taken using an XL3 scanning electron microscope (Philips, Eindhoven, The Netherlands).

Porosimetric Analysis

The pore size and porosity of the tablets were determined using mercury porosimetry (AutoPore III 9420 System, Micromeretics Instrument Corp., Norcross, GA). Results are presented as means ± SD ($n = 3$).

Tablet Strength Testing

Because of the plastic deformation, it was impossible to test the hardness of the tablets on a conventional hardness tester; the strength of the tablets was determined with a texture analyzer (TA-XT2 analyser, Stable Micro Systems, Godalming, UK). The tablet was placed on a support and deformed in a defined, controlled manner by a cone penetration probe (diameter 1.5 mm, height 5 mm)

Table 1

Statistical Evaluation of the Correlation Between the Mean Hardness and Mean Moisture Content of the Different Tablet Formulations

	45% RV, Formulation		60% RV, Formulation		85% RV, Formulation	
Packaging	A	B	A	B	A	B
PVC/aluminum blister						
r	−0.979	−0.446	−0.994	−0.985	−0.941	−0.988
P	0.021[a]	0.554	0.006[a]	0.015[a]	0.059	0.012[a]
PVC-PVDC/aluminum blister						
r	−0.968	−0.777	−0.978	−0.925	−0.930	−0.924
P	0.032[a]	0.223	0.022[a]	0.075	0.070	0.076
Closed recipient						
r	−0.858	−0.862	−0.852	−0.968	−0.998	−0.960
P	0.142	0.138	0.148	0.032[a]	0.002[a]	0.040[a]
Open recipient						
r	−1.000	−0.698	−0.927	−0.929	−0.992	−0.976
P	0.000[a]	0.302	0.073	0.071	0.008[a]	0.024[a]

The values are the Pearson correlation coefficient *r* and the significance *P*. A negative *r* value shows a negative correlation.

[a]Significant correlation ($p < .05$).

over a constant distance of 1 mm using a speed of 0.1 mm/sec. A force *N*-versus-distance (mm) diagram was recorded. The maximal force *N* after 1 mm of penetration was determined. The results are presented as mean values ± SD ($n = 6$).

Moisture Analysis

The tablets were analyzed for their residual moisture content after lyophilization using Karl Fischer titration (Mettler DL35, Mettler Toledo, Lot, Belgium). The instrument was calibrated using disodium tartrate and water as a standard (Riedel-de-Haen, Seelze, Germany). Each tablet was pulverized, inserted in the titration vessel, and analyzed after a stirring time of 3 min. Results are presented as mean values ± SD ($n = 6$).

Statistical Evaluation

Changes in water content and tablet hardness as function of time were evaluated statistically by a one-way analysis of variance (ANOVA) and Dunnets posttest ($p < .05$). A correlation between water content and tablet hardness was determined by a Pearson correlation coefficient ($p < .05$) (SPSS-Base 7.0 for Windows, Sybex, San Francisco, CA).

RESULTS AND DISCUSSION

The results of the moisture content determination and the tablet hardness of formulation A and formulation B packed in PVC/aluminum blister packs are shown in Fig. 1 and Fig. 2, respectively. There was a significant increase ($p < .05$) in moisture content of formulation A packed in PVC/aluminum blister packs and stored in closed containers (data not shown) during the first month of the storage period. This increase in moisture content depended on the relative humidity. The moisture content of formulation A was 2.75% ± 0.14% at time T_0 and increased significantly to 4.34% ± 0.37%, 6.09% ± 0.59%, and 6.75% ± 0.84% after 1 month of storage at 45%, 60%, and 85% RH, respectively. The moisture content of formulation B increased, although not intensively, since formulation B is a dried emulsion tablet containing the triglyceride Miglyol 812, which results in a more hydrophobic and less hygroscopic formulation compared to formulation A. The moisture content of formulation B was 1.88% ± 0.16% at time T_0 and increased to 1.75% ± 0.23%, 2.95% ± 0.25%, and 4.08% ± 0.64% after 1 month of storage at 45%, 60%, and 85% RH, respectively. The PVC-PVDC/aluminum blister packs offered better protection against moisture uptake compared to the other packaging. After 1 month of storage in PVC-PVDC/aluminum blister packs, the moisture content of formulation A was 4.13% ± 0.21%, 4.36% ± 0.25%, and 5.26% ± 0.24% at 45%, 60%, and 85% RH, respectively.

It is clear from Figs. 1 and 2 that the hardness of the tablets decreases as a function of time and relative humidity. For the rapidly disintegrating tablets (formulation A) packed in PVC/aluminum blister packs, a significant reduction in tablet hardness was seen from 2.26 ± 0.12 N at time T_0 to 0.35 ± 0.09 N during a storage period of 3 months at 85% RH. This decrease in hardness was smaller and less intensive for the tablets packed in PVC-

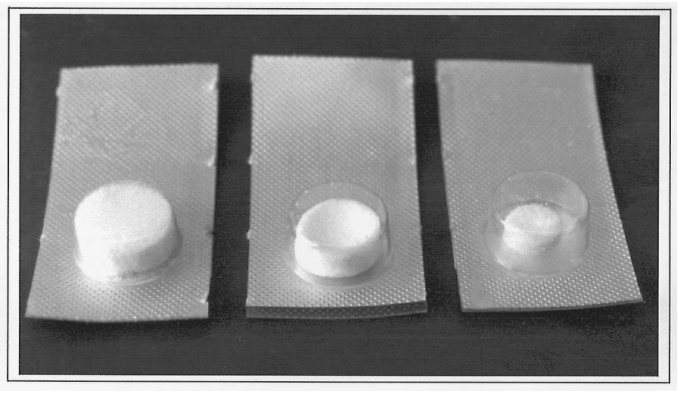

Figure 3. Formulation A packed in PVC/aluminum blister packs at time T_0 and after storage for 1 month and 3 months at 85% relative humidity.

PVDC/aluminum: For formulation A, a significant reduction in tablet hardness was seen from 2.26 ± 0.12 N at time T_0 to 1.24 ± 0.24 N during a storage period of 3 months at 85% RH.

The increase in water content of the freeze-dried tablets is correlated with a decrease in tablet hardness. This correlation was evaluated using the Pearson correlation coefficient (Table 1). There is a clear negative correlation between both parameters. The water uptake by the freeze-dried tablets is also associated with a loss in microstructure and tablet porosity. At a water content of 7.2% after 1 month of storage at 85% RH, a structural collapse was seen for formulation A. The absorbed water acts as a plasticizer, reducing the glass transition temperature T_g of the amorphous material (maltodextrin and xanthan gum) in the freeze-dried tablet. This results in a glass-rubber transition when the T_g of the formulation decreases under the storage temperature. This was reported by Levine and Slade (11) in a study of the influence of moisture on the glass transition temperature of amorphous products. The glass transition, or the transition from a glassy state to a rubbery state, occurs when the water content of an amorphous system increases at constant temperature or when the temperature of the system increases at constant water content (12).

To avoid the structural collapse of the freeze-dried tablets, the moisture content of the amorphous polymers in the formulation should be kept below T_g. Hatley (13) recently reported that the T_g of a dried (<0.01% water) anhydric trehalose glass (116°C) decreased by 20° when the moisture content increased by 0.75%. When the T_g of an amorphous glass decreases under the storage temperature, the amorphous matrix will lose its rigidity and will change into a more deformable rubbery state, resulting in physical collapse, crystallization of individual compounds, or breakdown of the active component (14). It is clear that packaging of freeze-dried tablets with PVC/aluminum blister packs, PVC-PVDC/aluminum blister packs, or closed containers did not offer protection against moisture uptake and mechanical strength loss. Packing of freeze-dried tablets into polypropylene blisters or aluminium-aluminium blisters could offer a solution to this problem. However, problems of thermic instability with polypropylene blisters are known (6).

The structural collapse of the freeze-dried tablets into the PVC/aluminum blister packs is shown in Fig. 3. From the SEM pictures of the fracture plane of the tablets of

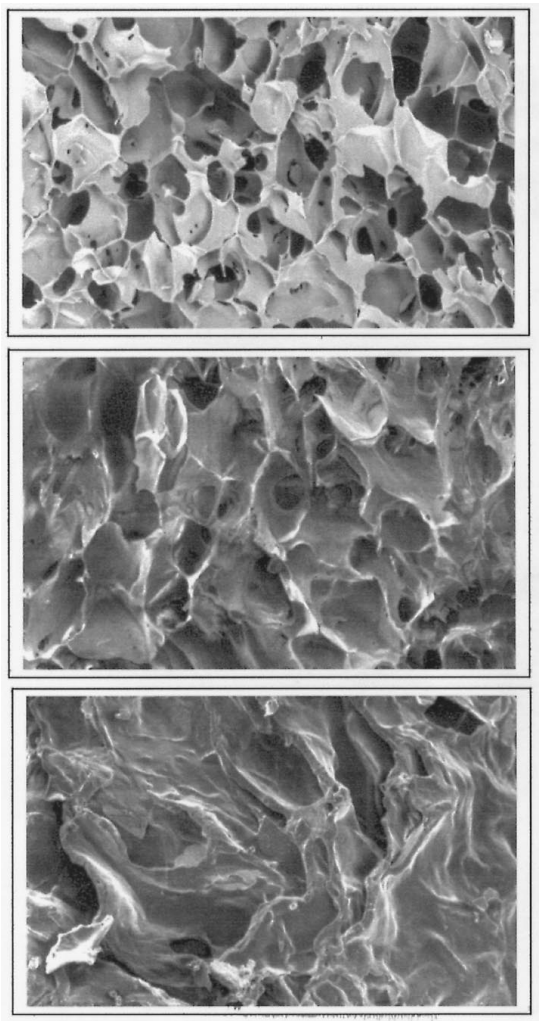

Figure 4. SEM picture of the fracture plane of rapidly disintegrating freeze-dried tablets at time T_0 and after storage for 1 month and 3 months at 85% RH.

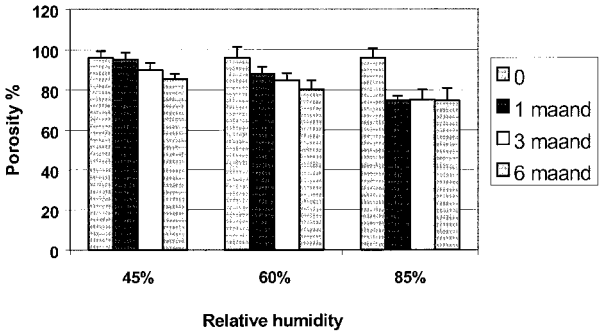

Figure 5. Porosity of the lyophilized emulsion tablets (formulation B) packed in PVC/aluminum blister packs stored at three different relative humidities as a function of time.

Table 2

Content Uniformity Analysis (mg HCT) (Mean ± SD; n = *3) of Formulation A and Formulation B at* T_0 *and* $T_{6\ months}$

	Formulation A		Formulation B	
Packaging	T_0	$T_{6\ months}$	T_0	$T_{6\ months}$
45% RH				
PVC/aluminum blister	25.1 ± 0.1	25.1 ± 0.1	25.0 ± 0.3	25.0 ± 0.1
PVC-PVDC/aluminum blister	25.3 ± 0.2	24.5 ± 0.2	24.8 ± 0.4	24.7 ± 0.2
Closed recipient	25.1 ± 0.1	25.0 ± 0.2	24.7 ± 0.3	24.8 ± 0.4
Open recipient	25.1 ± 0.1	25.0 ± 0.1	24.8 ± 0.4	25.0 ± 0.2
60% RH				
PVC/aluminum blister	25.1 ± 0.1	25.0 ± 0.1	24.8 ± 0.5	24.8 ± 0.3
PVC-PVDC/aluminum blister	25.3 ± 0.2	24.8 ± 0.2	24.9 ± 0.1	24.7 ± 0.4
Closed recipient	25.1 ± 0.1	25.1 ± 0.2	24.6 ± 0.2	25.0 ± 0.2
Open recipient	25.1 ± 0.1	25.1 ± 0.3	24.8 ± 0.4	25.2 ± 0.3
85% RH				
PVC/aluminum blister	25.1 ± 0.1	24.5 ± 0.4	[a]	[a]
PVC-PVDC/aluminum blister	25.3 ± 0.2	[a]	24.9 ± 0.3	24.5 ± 0.2
Closed recipient	25.1 ± 0.1	[a]	[a]	[a]
Open recipient	25.1 ± 0.1	[a]	[a]	[a]

[a]Tablets showed structural collapse and were not analyzed.

formulation A (Fig. 4), it is clear that the microstructure of the maltodextrin–xanthan gum network is completely lost after 3 months of storage at 85% RH because of structural collapse and swelling of the xanthan gum polymer due to an increased water content.

The porosity of formulation B packed in PVC/aluminum blister packs as a function of time and storage conditions is shown in Fig. 5. The porosity of the tablets decreases as a function of time. A decrease in porosity of the rapidly disintegrating tablets was measured from 97.5% to 78.9% after 6 months of storage at 45% RH in PVC/aluminum blister packs. For formulation B, a decrease in porosity was measured from 96.02% to 86.9% after 6 months of storage at 45% RH in PVC/aluminum blister packs. None of the emulsion tablets showed structural collapse independent of the storage conditions. The results of the content uniformity analysis at time T_0 and time $T_{6\ months}$ are shown in Table 2. No significant breakdown of the HCT was detected.

The packaging of rapidly disintegrating tablets in PVC/aluminum blister packs, PVC-PVDC/aluminum blister packs, or closed containers does not offer adequate protection against moisture uptake, loss of tablet hardness and microstructure, and decrease in porosity. The use of other polymeric blister films, such as polypropylene or aluminum-aluminum blisters, could be interesting for packaging of freeze-dried oral dosage forms.

REFERENCES

1. S. Corveleyn and J. P. Remon, Formulation and production of rapidly disintegrating tablets by lyophilisation using hydrochlorothiazide as a model drug, Int. J. Pharm., 152, 215–225 (1997).
2. S. Corveleyn and J. P. Remon, Formulation of a lyophilized dry emulsion tablet for the delivery of poorly soluble drugs, Int. J. Pharm., 166, 65–74 (1997).
3. S. Corveleyn and J. P. Remon, Bioavailability of hydrochlorothiazide: conventional versus freeze-dried tablets, Int. J. Pharm., 173, 149–155 (1998).
4. B. Vennat, D. Gross, A. Pourrat, and P. Legret, Mise au point de lyophilisats oreaux de procyanidines, J. Pharm. Belg., 48, 430–436 (1993).
5. G. Zografi and B. C. Hancock, Water-solid interactions in pharmaceutical systems, In *Topics in Pharmaceutical Sciences 1993* (D. J. A. Crommelin, K. K. Midha, and T. Nagai, Eds.), Medpharm Scientific, Stuttgart, 1994, pp. 405–419.
6. B. Guise, The material benefits of blister packaging, Manuf. Chem., 18–21 (April 1995).
7. F. Franks, Freeze-drying: from empiricism to predictability, Cryo-Letters, 11, 93–110 (1990).
8. F. Franks, R. H. M. Hatley, and S. F. Mathias, Materials science and the production of shelf stable biologicals, Pharm. Technol. Int., 3, 24–34 (1991).
9. F. Kiekens, A. Vermeire, N. Samyn, J. Demeester, and J. P. Remon, Optimisation of electrical conductance mea-

surements for the quantification and prediction of phase separation in o/w emulsions, containing hydroxypropylmethylcellulose as emulsifying agents, Int. J. Pharm., 146, 239–245 (1997).
10. C. Vervaet and J. P. Remon, Bioavailability of hydrochlorothiazide from pellets containing PEG, made by extrusion spheronisation, Pharm. Res., 14(11), 1644–1646 (1997).
11. H. Levine and L. Slade, in *Water Science Reviews*, Vol. 3 (F. Franks, Ed.), Cambridge University Press, Cambridge, UK, 1987, pp. 79–185.
12. C. A. Oksanen and G. Zografi, The relationship between the glass transition temperature and water vapor absorption by poly(vinyl-pyrrolidone), Pharm. Res., 7, 654–657 (1990).
13. R. M. Hatley, Glass fragility and the stability of pharmaceutical preparations—excipient selection, Pharm. Dev. Technol., 2(3), 257–264 (1997).
14. M. P. te-Booy, R. A. de-Ruiter, and A. L. de-Meere, Evaluation of the physical stability of freeze-dried sucrose-containing formulations by differential scanning calorimetry, Pharm. Res., 9(1), 109–114 (1992).