Incorporation of Lipophilic Drugs in Sugar Glasses by Lyophilization using a Mixture of Water and Tertiary Butyl Alcohol as Solvent

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ABSTRACT: In this study, a new and robust method was evaluated to prepare physically stable solid dispersions. Trehalose, sucrose, and two inulins having different chain lengths were used as carrier. Diazepam, nifedipine, Δ^9-tetrahydrocannabinol, and cyclosporine A were used as model drugs. The sugar was dissolved in water and the drug in tertiary butyl alcohol (TBA). The two solutions were mixed in a 4/6 TBA/water volume ratio and subsequently freeze dried. Diazepam could be incorporated at drug loads up to 63% w/w. DSC measurements showed that, except in some sucrose dispersions, 97–100% of the diazepam was amorphous. In sucrose dispersions with high drug loads, about 10% of the diazepam had crystallised. After 60 days of exposure at 20°C and 45% relative humidity (RH), diazepam remained fully amorphous in inulin dispersions, whereas in trehalose and sucrose crystallization of diazepam occurred. The excellent physical stability of inulin containing solid dispersions can be attributed to the high glass transition temperature (T_g) of inulin. For the other drugs similar results were obtained. The residual amount of the low toxic TBA was only 0.1–0.5% w/w after freeze drying and exposure to 45% RH and 20°C. Therefore, residual TBA will not cause any toxicity problems. This study provides a versatile technique, to produce solid dispersions. Inulin glasses are preferred because they provide an excellent physical stability of the incorporated amorphous lipophilic drugs. © 2004 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 93:713–725, 2004

Keywords: amorphous; solid dispersion; crystallization; physical stability; freeze drying; residual TBA; inulin; solubility; cyclosporine A; Δ^9-tetrahydrocannabinol (THC)

INTRODUCTION

Dissolution of drugs is a prerequisite for absorption from the gastrointestinal tract. However, lipophilic drugs often dissolve slowly, poorly, and irregularly in the aqueous environment of the gastrointestinal tract. Especially for class II drugs, this results in poor and variable bioavailability after oral administration.[1] Many dissolution improving technologies have been described[2–4] but only a few reached the market. Problems related to large-scale production and physical stability are the cause of this limited success. Therefore, the development and production of a stable formulation for fast dissolution is still one of the major challenges in pharmaceutical technology.

In the literature, three different strategies can be distinguished to produce a formulation for rapid dissolution of lipophilic drugs. At first micronization of the drug particles to enlarge the specific surface area is used to accelerate dissolution rate.[5] Second, administration of lipophilic drugs in liquid form, for example, oil-filled capsules, surfactant

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solutions, emulsions, or self-(micro)emulsifying systems, is gaining increasing attention.[2,3] The third strategy to accelerate dissolution is the use of solid dispersions. They consist of a water-soluble carrier containing very small areas of lipophilic drug preferably in the amorphous state.[6] Solid solutions refer to a system in which drug molecules are mono-molecularly dispersed throughout the carrier. Although the exact dissolution mechanism of solid dispersions is not yet fully understood, the accelerated dissolution is generally attributed to the large surface area and increased wetting of the lipophilic drug.[7] Furthermore, for fast dissolution the drug should be amorphous.[8]

Two techniques have been described to produce solid dispersions. In fusion techniques both carrier and drug are melted and subsequently cooled to solidify the system. However, drug and carrier may be incompatible, for example, two liquid phases may appear during melting.[9,10] Also, when a homogeneous melt is obtained, the melt bears the risk of phase separation during cooling, which may result in crystallization. Surfactants like Gelucire® can be applied to overcome these problems.[8,11] Furthermore, fusion techniques are only suitable for heat stable materials. Allen and coworkers[12] needed temperatures of at least 169°C to melt their sugar carriers. The second technique to produce solid dispersions is the solvent technique. It requires dissolution of both drug and carrier in one solution followed by drying, for example, vacuum drying, spray drying, or freeze drying. Several strategies have been used. Low drug concentrations are used to dissolve both carrier and drug in water,[13,14] but this requires evaporation of tremendous amounts of solvent, making the process uneconomic and impractical. Solubilizers like cyclodextrin or surfactants like Tween80® increase the aqueous solubility of the drug substantially. However, the amount of solubilizers or surfactants in the final product are often eminent. This results in solid dispersions that mainly consist of solubilisers or surfactants, which therefore largely determine the physical properties of the carrier. Moreover, they are not always tolerated well in the body, or may even be toxic. Chloroform[15] or dichloromethane[8] are used to dissolve both drug and poly(vinyl-pyrrolidone) (PVP) as carrier simultaneously. However, with these solvents, freeze drying is impractical because their melting points are very low, and vacuum or spray drying solutions with these solvents should be avoided because of the risk for phase separation of drug and carrier during drying. Furthermore, due to their toxicity, residual solvents of this kind present after drying are unacceptable. Supercritical CO_2 has been used by some scientists to dissolve both the drug and carrier.[16] However, because most pharmaceutical compounds dissolve poorly in CO_2, it is mostly used as an antisolvent. Conventional but toxic solvents like acetone, dichloromethane, and methanol are frequently used to dissolve the drug and carrier.[17] Subsequently, these solutions, brought into contact with supercritical CO_2, become supersaturated, and the solutes recrystallize.[16,18–20] The last strategy for the dissolution of both the drug and carrier is the use of solvent mixtures. Water and ethanol,[21] or methylene chloride and ethanol[22] are examples encountered in literature. However, dissolution of the drug and carrier in these mixtures is not always possible. Moreover, during drying crystallization of the lipophilic drug is commonly encountered.[23]

In this study water will be used to dissolve the carrier, whereas a cosolvent will be used to dissolve the drug. Subsequently, both solutions will be mixed, and if a clear mixture is obtained, the solution will be dried. To minimize the risk of crystallization during evaporation of solvents, freeze drying is chosen as drying method. Therefore, an organic cosolvent is required that is miscible with water, has a relatively high melting point, and a high vapor pressure to assure that sublimation proceeds at an acceptable rate. Table 1 enlists some physical properties of solvents that could be appropriate to produce solid dispersions. DMSO is also included in this table because it has a relatively high melting point, which makes it suitable for freeze drying. However, the vapor pressure of DMSO is extremely low, and a slow drying process can be expected. Indeed, in a pilot experiment it has been shown that after 3 days of freeze drying, only a very small amount of DMSO was removed from a frozen solution. 2-Methyl-2-propanol, also called tertiary butyl alcohol (TBA), meets both melting temperature and vapor pressure requirements relevant for freeze drying. Furthermore, the toxicity of TBA is low. According to the ICH Guidelines, solvents are divided into three different categories: class 1, class 2, and class 3 solvents, with class 1 indicating extremely high toxicity, and class 3 indicating a very low toxicity. Although not listed in the ICH Guidelines for Residual Solvents, TBA is likely to fall in the category of a class 3 solvent based on its similarity of LD_{50} toxicity data for other class 3 solvents.[24] Therefore, TBA will be used in this study.

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Table 1. Atmospheric Melting and Boiling Points and Vapor Pressures of Relevant Solvents

Solvent	Melting Point (°C)	Boiling Point (°C)	Vapor Pressure at 25°C (kPa)
Water	0	100	3.16
Methanol	−93.9	65	16.9
Ethanol	−117	78.5	5.79
1-Propanol	−85.8	97.4	2.27
2-Propanol	−127	82.4	5.85
2-Methyl-2-propanol (TBA)	25	82	5.49
Dimethylsulphoxide	19	189	0.08

Four sugars will be used. Sucrose and trehalose were selected because they are frequently used as an excipient to stabilize proteins during drying and storage. Recently, also the fructose oligomer inulin was found to stabilize proteins.[25,26] Two different inulins having a number average degree of polymerisation (DP) of 11 and 23 were tested in this study. To evaluate the versatility of the production method four lipophilic drugs are included: nifedipine,[27] Δ^9-tetra-hydrocannabinol,[28] and cyclosporine A,[29] because they all have a low oral bioavailability. Physical stability is determined with diazepam because it easily crystallizes.

MATERIALS AND METHODS

Materials

The following materials were used as supplied: TBA, trehalose, sucrose, and cyclosporine A from Sigma-Aldrich Chemie GmbH, Steinheim, Germany. Diazepam from BUFA B.V. Uitgeest, The Netherlands, and nifedipine from Bayer AG, Werk Leverkusen, Germany. Two types of inulin, TEX!803 and HD001111, having a number average degree of polymerization of 23 (inulin DP23) and 11 (inulin DP11), respectively, were a gift of Sensus, Roosendaal, The Netherlands. Purified Δ^9-tetra-hydrocannabinol (THC) was a gift of Unimed Pharmaceuticals Inc., Marietta, United States. The water used was demineralised in all cases.

Methods

Determination of TBA/Water Ratio for Optimal Physical Stability of Sugar/Drug Solutions

In preliminary experiments it was found that the sugars and lipophilic drugs could not be dissolved in appropriate concentrations in mixtures of water and TBA. However, when a solution of sugar in water was mixed with a solution of a lipophilic drug in TBA, the resulting mixtures remained clear for some time before they became cloudy. Apparently, the mixtures are not thermodynamically stable, but it takes some time before phase separation starts.

To investigate the physical stability of solutions of the sugars and the lipophilic drugs in mixtures of water and TBA, light-scattering experiments were performed. The sugar of interest was dissolved in water by heating for 15 min to about 80°C. Subsequently, the solution was cooled in a water bath to 37°C for 10 min and then TBA was added. The final sugar concentrations were in all cases 90 mg/mL while the water/TBA ratio was varied. The drug of interest was dissolved in TBA and then the solution was mixed with water. The final drug concentrations were 10 mg/mL (for THC 4 mg/mL) while the water/TBA ratio was varied; 190 μL of the solutions was transferred in a 96-wells plate. The absorption was measured in time at an arbitrarily chosen wavelength of 655 nm in a plate reader (Benchmark Platereader, Bio-Rad, Hercules, CA). At least four wells were measured simultaneously. The average absorption of these measurements was plotted against time. The intercept with the time axis of the tangent through the inflection point of the graph was defined as clouding time. A typical example of clouding in an inulin solution is given in Figure 1. Clouding times were determined at least in duplicate. Clouding in trehalose and sucrose solutions could not be obtained in this way. Solutions of trehalose and sucrose either remained clear for a few days at low TBA contents or became cloudy immediately at high TBA contents; 90 mg/mL solutions were prepared and the threshold TBA content was determined by increasing the TBA content until clouding appeared.

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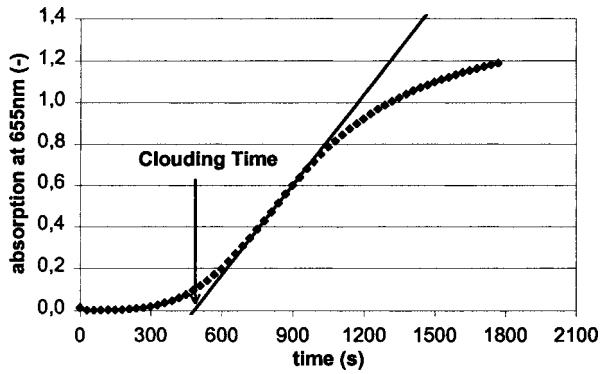

Figure 1. Clouding in a solution of inulin DP23 in 40% v/v TBA.

Production of Solid Dispersions by Freeze Drying

To prepare a solid dispersion with 10% w/w drug, 1.20 mL of a 150 mg/mL sugar in water solution was added to 0.80 mL of a 25-mg/mL drug solution in TBA in a 20 mL vial. This resulted in a concentration of 90 mg/mL of sugar and 10 mg/mL of drug in a solvent that consisted of 40% v/v TBA. For the THC containing products solutions of 160 mg/mL of sugar in water were mixed with 10 mg/mL THC solutions in TBA. Immediately after mixing, the vial was immersed in liquid nitrogen for a few minutes. To prepare solid dispersions with higher drug loads the same procedure was followed except that the sugar concentration was decreased. Placebo material was produced in the same way except that in the TBA no drug was dissolved. A typical lyophilization cycle involved the freezing of the solution by immersing it in liquid nitrogen followed by placing it in a Christ model Alpha 2-4 lyophilizer (Salm and Kipp, Breukelen, The Netherlands) with a condenser temperature of −53°C. Lyophilization was performed according to a two-step procedure. First, the pressure was set at 0.220 mbar and the shelf temperature at −35°C for 1 day. Subsequently, the pressure was decreased to 0.05 mbar, while the shelf temperature was gradually raised to 20°C. These conditions were maintained for another day. After removing the samples from the freeze drier, they were placed in a vacuum desiccator over silica gel at room temperature for at least 1 day.

Differential Scanning Calorimetry

Ccrystallinity of solid dispersions: to determine the degree of crystallinity of the lipophilic drugs diazepam and nifedipine in the solid dispersions, a differential scanning calorimeter (DSC2920, TA Instruments, Gent, Belgium) was used. A linear heating rate of 10°C/min as well as modulated DSC (MDSC 2°C/min, modulation amplitude 0.318°C, modulation period 60 s, heat-only conditions) were used. Control experiments showed that both methods gave the same results. The heat of fusion of crystallized drug in a solid dispersion was calculated from the peak area of the melting endotherm. The heat of fusion of pure crystalline drug was determined in a separate experiment. The ratio of these fusion energies was used to calculate the crystallinity of drug in the solid dispersions.

Glass transitions of pure components: to determine the glass transition temperature (T_g) of sugars and lipophilic drugs modulated DSC (MDSC was used at 2°C/min at a modulation amplitude of 0.318°C every 60 s, heat-only conditions). Glass transition temperatures of pure drugs were measured by melting and quench cooling the drug in the sample pan, prior to the DSC measurement. Glass transition temperatures were taken as the inflection point of the transition. The glass transition temperature of the maximally freeze concentrated fraction (T'_g) was measured in 96 mg/mL solutions at 2°C/min without modulation. Samples were analyzed in open aluminium pans.

Glass transitions of humidified sugars: humidified sugars were obtained by exposing them to different Relative Humidities (RHs) in desiccators with different saturated salt solutions at 20°C. After "equilibration" (see section Physical properties of model drugs and sugars) the T_gs of these samples were analyzed using closed aluminium sample pans.

Dynamic Vapor Sorption

Water sorption isotherms of freeze dried inulin, trehalose, and sucrose were measured at ambient pressures and 25°C using a gravimetric sorption analyser (DVS-1000 Water Sorption Instrument, Surface Measurement Systems Limited, London, UK). The uptake of water by the different sugars was measured from 0 to 80% RH with steps of 10% RH. The initial sample weight was about 10 mg. It was assumed that equilibrium was reached when the change of weight was less than 0.9 μg during a 10-min period.

Determination of Residual TBA

The amount of residual TBA in freeze dried samples was determined by means of gas chro-

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matography (GC). Samples were weighed in 10-mL GC vials (glass vials with crimpcap with silcon/teflon septum 20 mm, Chromacol Herts, United States). After dispersion in 2 mL demineralised water, the vial was centrifuged for 5 min at 1600 rpm and 60°C to increase the sensitivity. Headspace gas chromatography quantificated the concentration of TBA in 200 μL of the vapor. The injection temperature was 250°C. A DB wax column, 0.53 mm and 30 m with a film thickness of 1 μm was used at 50°C. A flame ionisation detector (Carlo Erba Instruments GC8000 series) operating at 275°C yielded quantitative data. Control experiments showed that the peak areas were not affected by presence of sugar nor drug. Calibration of pure water/TBA mixtures was used for all experiments.

Testing Robustness of Production Process

The effect of freezing rate on crystallinity of drug in the freeze dried product, was investigated with solid dispersions containing 10 and 20%w/w diazepam incorporated in inulin DP23. Solutions were frozen in refrigerators at −18 and −37°C, instead of immersing them in liquid nitrogen. Second, the effect of shelf temperature during the first step of lyophilization was investigated. Instead of −35°C it was set at −5°C. Furthermore, the effect of clouding was investigated. Solutions were left for 1.5 h at room temperature to induce partial phase separation. After that, the clouded solutions were frozen in liquid nitrogen and lyophilized (initial shelf temperature −5°C). The crystallinity of diazepam in the products was determined by means of DSC.

Determination of Physical Stability

To determine the physical stability of solid dispersions, 10, 20, 40, or 62.5% w/w of diazepam, was incorporated in sucrose, trehalose, inulin DP11, and inulin DP23, respectively. Also, pure diazepam was prepared by freeze drying. The samples were placed in a climate chamber of 20°C and 45% RH. After 60 days the crystallinity of diazepam in the samples was determined by means of DSC.

RESULTS AND DISCUSSION

Physical Properties of Model Drugs and Sugars

As can be seen from Table 2, the drugs used in this study exhibit low aqueous solubilities. Because their octanol/water partition coefficients are very high, oral bioavailability is most likely dissolution rate limited.[1] Furthermore, it can be seen from Table 2 that the T_g of three of the four drugs used in this study is above room temperature. However, it was noted that the tendency of amorphous diazepam to crystallize at room temperature was largest. In control experiments diazepam was found to be completely amorphous after freeze drying, but had crystallized completely after only a few hours at ambient conditions. Apparently, the molecular mobility at 25°C below the T_g is high enough to allow for crystallization. This was confirmed in literature.[30] The tendency of THC and CSA to crystallize was much less. No melting of crystalline THC or CSA could be observed under any circumstance, which was reported before for THC.[31] For CSA, three very small endotherms were found at 108, 124, and 146°C in the total heat flow with enthalpies of 1.45, 0.90, and 1.23 J/g, respectively. The origin of these small endothermic events is unknown. However, a glass transition was clearly visible at 125.6°C in the reversing heat flow. Apparently, CSA is supplied in the amorphous state.

Table 2. Physical Properties of Drugs Used in This Study ($n = 2$–4, ±SD)

Compound	Solubility in Water at 37° (mg/L)	log P (Water/Octanol)	Glass Transition Temperature (°C)	Melting Temperature (°C)
Diazepam	65.2[a]	2.82[a]	46.6 ± 0.6	131.8 ± 0.4
Nifedipine	9.3[d]	3.14[b]	45.3 ± 1.6	173.8 ± 0.3
Cyclosporine A	7.3[e]	3.00[a]	125.6 ± 0.5	Not detected
THC	2.8[f]	3.78[c]	9.3 ± 0.97	Not detected

[a]Ref. 45.
[b]Pharmaceutical Codex.
[c]http://www.marinol.com.
[d]Ref. 46.
[e]Ref. 47.
[f]Ref. 48.

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Table 3. Thermal Properties of Sugars ($n = 2–4$, SD, <1.5°C in All Cases)

Sugar	T_g Dry (°C)	T'_g 40% v/v TBA (°C)	T'_g 100% Water (°C)
Inulin DP23	155.0	−24.6	−19.4
Inulin DP11	130.7	−28.3	−22.3
Trehalose	121.6	−36.0	−31.8
Sucrose	76.1	−38.9	−34.7

The T_gs of the four sugars used in this study as carrier are given in Table 3. To prevent collapse and crystallization during primary drying, samples should be below their T'_g.[32,33] The T'_g of all four sugars is lowered by the presence of TBA. Apparently, TBA is present in the freeze concentrated fraction. The effect of TBA/water ratios on T'_g was also investigated. The ratios ranged from 50% v/v to 5% v/v TBA. It turned out that the value of the T'_g was the same for all water/TBA ratios tested (data not shown). This indicates that a constant amount of TBA is present in the freeze concentrated fraction. Similar effects have been found before.[34]

As expected, the T_g for the dry sugars was the highest for inulin DP23 followed by inulin DP11.[25] The T_g values found for trehalose and sucrose agreed with the literature.[35]

Furthermore, T_gs of humidified sugars were determined. The decrease of the T_g due to plasticizing by absorbed water is depicted in Figure 2. At 20°C, the two inulins are in the glassy state upon exposure to ambient or high humidities, whereas trehalose is in the rubbery state at about 42% RH and higher and sucrose at 35% RH and higher. To determine the T_g of humidified amorphous trehalose or sucrose at high humidities, only a short period of exposure was allowed, because otherwise those sugars had crystallized. Melting peaks of crystalline trehalose and sucrose was observed by DSC for trehalose after prolonged exposure to 45% RH and higher and for sucrose at 33% RH and higher (data not shown).

Because sorption isotherms of crystalline sugars are different from isotherms of amorphous sugars, crystallization can also be measured gravimetrically (see Fig. 3). At low RH, the weight gain of amorphous samples was more or less the same for all four sugars indicating that all glasses under investigation have a similar hygroscopicity. However, during vapor sorption experiments a bend was seen in the absorption isotherms of trehalose and sucrose. Sucrose expelled most of its absorbed water, during crystallization. For trehalose, crystallising as a dihydrate, the measured weight gain of about 11.5% closely corresponds to a trehalose/water mol ratio of 1/2. Similar effects are found in literature.[30,35,36] But both inulins show no such behavior, although the T_g must have been passed at some point. This indicates that inulin does not crystallize easily.

Stability of Solutions Containing Sugar and Lipophilic Drug

To produce fully amorphous solid dispersions, only clear and homogeneous solutions in which no phase separation has occurred, can be used for

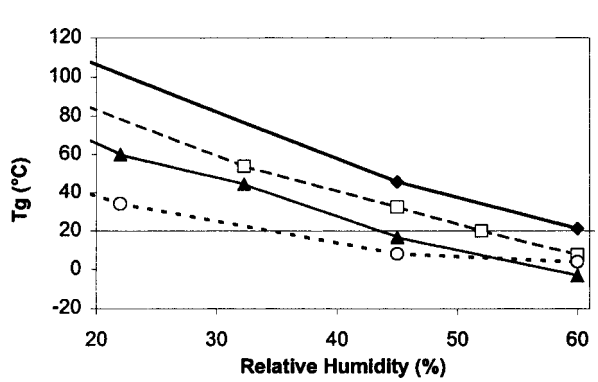

Figure 2. T_gs of the sugars used after conditioning at different RHs (key: ◆: inulin DP23; □: inulin DP11; ▲: trelalose; ○ sucrose).

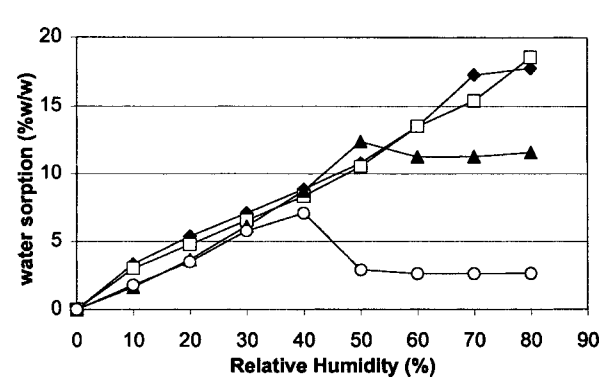

Figure 3. Water vapor sorption isotherms of lyosphilized sugars at 24°C (key: ◆: inulin DP23; □: inulin DP11; ▲: trehalose; ○: sucrose).

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lyophilization. To find the optimal TBA/water ratio, the physical stability of solutions of drugs and sugars in water/TBA mixtures was determined. Trehalose and sucrose solutions remained clear up to 60% v/v TBA and 72% v/v, respectively. Clouding times of solutions containing inulins or lipophilic drugs are depicted in Figure 4. Both inulins, being polar in nature, were more stable in the solutions with higher water contents. Inulin DP23 solutions appeared to be less stable than inulin DP11 solutions, which can be explained by the difference in chain length. As was expected, the lipophilic drugs showed phase separation more quickly as the water content of the solution was increased. The difference in stability in water/TBA mixtures between the four drugs could be related to their aqueous solubility. Higher aqueous solubility corresponded with a higher physical stability in water/TBA solutions (see also Table 2). As can be seen from Figure 4, THC solutions are stable only at high TBA contents, even though the concentration was lowered to 0.4% w/v.

It can be concluded that the most critical combination is inulin DP23 and THC. To produce a solution in which both inulin DP23 and THC remain dissolved for a significant period of time, 40% v/v TBA is needed. This TBA/water ratio was maintained throughout this study for all other drug–sugar combinations.

Solid Dispersions of Diazepam, Nifedipine, THC, and CSA with Inulin DP23

Solid dispersions with all four drugs were prepared. Inulin DP23 was used as a carrier. Drug loads were 10% w/w for diazepam, nifedipine, and CSA. THC was incorporated at a 4% w/w content. By lowering the inulin concentration in the solution that was freeze dried, the THC content could be increased to 8% w/w. The absence of melting peaks in DSC thermograms for diazepam (see Fig. 5) and nifedipine, indicates the absence of crystalline drug. Therefore, it can be concluded that these drugs were incorporated in inulin to yield solid dispersions that were fully amorphous. No glass transitions of pure amorphous drugs could be observed for diazepam, nifedipine, CSA, or THC. This may implicate mono-molecular inclusion of the drugs and formation of a so-called amorphous solid solution or glass solution.[4,37] But if drug and carrier would have been molecularly mixed, the T_g of the mixture would be somewhere between that of the drug and carrier,[4] as predicted by the Gordon-Taylor equation.[38] However, in this study no significant decrease of the T_g of inulin DP23 was observed in any of the samples, which may implicate the formation of a solid dispersion. The reasons for these conflicting results remain unclear, and based on these results no definite statement can be made whether these drugs were distributed in the carrier monomolecularly or as small amorphous particles.

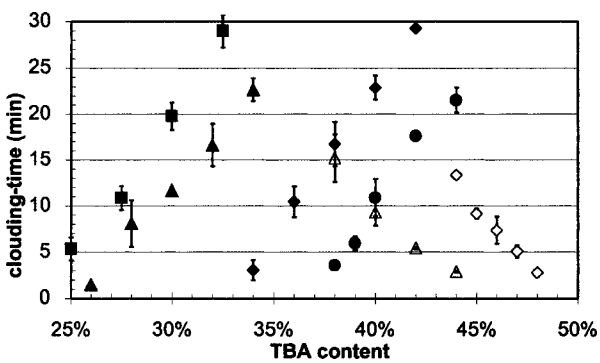

Figure 4. Clouding times of inulins and lipophilic rugs in different water/TBA solutions. (key: ■: diazepam 1.0% w/v; ▲: nifedipine 1.0% w/v; ◆: CSA 1.0% w/v; ●: THC 0.4% w/v; △: inulin DP23 9.0% w/v; ◇: inulin DP11 9.0% w/v; $n = 8–12$; error bars indicate standard deviation).

Solid Dispersions with Other Sugars

Nifedipine was incorporated in all four sugars at a drug load of 10% w/w. After freeze drying, the amount of amorphous nifedipine was determined by means of DSC. At least 98% of the incorporated nifedipine was present in the amorphous state. Solid dispersions with higher nifedipine contents could also be prepared. But in these products, significant crystallization of nifedipine was

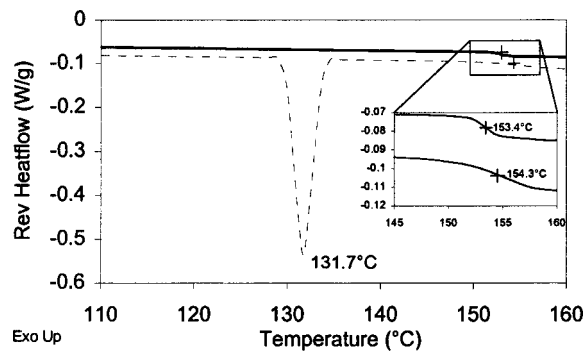

Figure 5. DSC thermogram of inulin DP 23 and 20% w/w diazepam. Solid dispersion (thick, solid line), physical mixture (thin, dashed line).

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observed during the DSC scan, and no reliable statements could be made about the amount of crystalline nifedipine before scanning (data not shown). However, diazepam in the solid dispersions does not crystallize during DSC measurements at higher drug loads. This is probably because diazepam has a much lower melting point (131.8°C instead of 173.8°C for nifedipine). Therefore, after passage of the T_g of the carrier, nifedipine has more time to crystallize during a DSC measurement. Another advantage of diazepam is that in pure amorphous form (thus not incorporated), it crystallizes rapidly under ambient conditions, as mentioned before. For those reasons, diazepam was chosen as a model drug for further experiments.

Diazepam was incorporated in all four sugars with drug loads of 10, 20, 40, and 63% w/w. The amount of amorphous diazepam is depicted in Figure 6. All solid dispersions containing 10% w/w or 20% w/w diazepam were fully amorphous. At higher drug loads (40% w/w and 63% w/w) at least 97% of the diazepam could be incorporated amorphously in the inulin and trehalose glasses, while in sucrose solid dispersions about 89% of the diazepam was amorphous.

Minimization of TBA Content after Freeze Drying

It is known that during secondary drying not all TBA will evaporate from the freeze concentrated fraction.[24] The residual amount of TBA after freeze drying was investigated for inulin DP23, inulin DP11, trehalose and sucrose, with and without incorporated drugs. The residual amounts of TBA are given in Table 4. It can be seen that the TBA content was slightly higher when a drug is incorporated in the matrix. Apparently, more TBA is retained when the sugar contains lipophilic drugs. The TBA contents in the sugars without drug were similar to literature values, and the difference in TBA retention between small sugars and poly-saccharides was seen before.[24,39,40] Although TBA is not very toxic: a maximal daily dose of 50 mg is allowed,[24,41] several attempts were made to minimise the amount of TBA present after freeze drying.

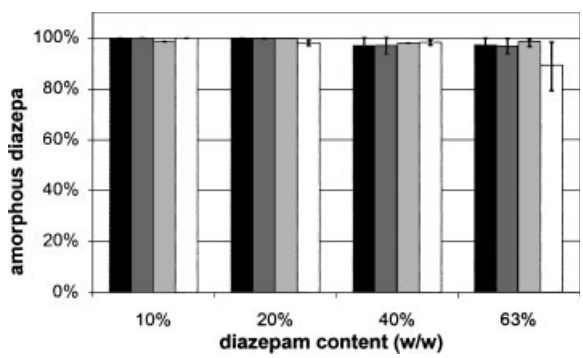

Figure 6. Fraction amorphous diazepam in solid dispersions after freeze drying (key: dark columns: inulin DP23; medium-dark columns: inulin DP11; shaded columns: trehalose; open columns: sucrose; average values; $n = 2–5$; bars indicate lowest and highest values).

Several factors that possibly affect TBA retention were considered. However, changing the TBA/water ratio, freezing rate, sample size, freeze drying time did not result in lower amounts of residual TBA (data not shown). Only in a very extreme lyophilization process, in which the pressure was 0.42 mbar instead of 0.22 mbar and the shelf temperature was −5°C during the first lyophilization step and 40°C during the second step, the residual TBA content was reduced to 0.6% w/w for inulins without collapse. Trehalose and sucrose did collapse and crystallize, resulting in almost complete expulsion of TBA.

It was also evaluated whether a postlyophilization treatment could be successful. The influence of moisture on the TBA content was investigated. As can be seen in Table 4, it turned out that solid dispersions containing 10% w/w diazepam lost almost all of the TBA after 7 days exposure to 20°C/45% RH. Similar behavior was observed when no drugs were incorporated in the sugars. Assuming a TBA content of 0.5% w/w a patient may take up to 10,000 mg of solid dispersion, because the maximally allowed dose for class 3 solvents is 50 mg/day. Obviously, toxicity is not an issue.

To investigate this remarkable loss of TBA in more detail, inulin DP23, inulin DP11, trehalose, and sucrose were exposed to 11, 33, and 45% RH all at 20°C. The TBA contents after 7 days exposure are depicted in Figure 7. Figure 7 shows that a certain amount of absorbed water can facilitate TBA removal. Two different mechanisms have been described in literature, which may explain the release of TBA after exposure of the dry samples to humidified air. In some studies, concentration-dependent diffusivities of cosolvents are used to explain their retention after freeze drying. During drying the free volume of the amorphous sugar decreases, resulting in a decrease of diffusivity of water but a more pronounced decrease of larger organic molecules like TBA.[24,42] In other studies, the behavior of organic volatiles in amorphous sugars is ascribed to

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Table 4. TBA Content in Solid Dispersions ($n = 2–4 \pm SD$)

	TBA Content (% w/w) after Freeze Drying			
Carrier	Inulin DP23	Inulin DP11	Trehalose	Sucrose
Incorporated drug:				
Diazepam 10% w/w	3.14 ± 0.18	2.73 ± 0.43	1.34 ± 0.05	1.64 ± 0.04
Nifedipine 10% w/w	3.37 ± 0.10	2.23 ± 0.15	1.23 ± 0.09	1.78 ± 0.23
CSA 10% w/w	3.18 ± 0.03	2.13 ± 0.06	1.14 ± 0.00	1.73 ± 0.07
THC 4% w/w	3.08 ± 0.34	1.88 ± 0.04	1.42 ± 0.14	1.46 ± 0.02
No drug	2.43 ± 0.17	1.56 ± 0.03	0.92 ± 0.06	1.49 ± 0.19
	TBA Content (% w/w) after 7 days at 45% RH/20°C			
Diazepam 10% w/w	0.39 ± 0.06	0.06 ± 0.01	0.08 ± 0.00	0.23 ± 0.08
No drug	0.26 ± 0.00	0.06 ± 0.00	0.24 ± 0.01	0.45 ± 0.03

intermolecular hydrogen bonding between sugar molecules during lyophilization.[39,43] This can give rise to the formation of so-called microregions within the strongly hydrogen-bonded sugar carrier in which organic cosolvent molecules are entrapped. Even after grinding of freeze dried material, organic volatiles remain entrapped in microregions.[44]

In this study, the release of TBA from inulin DP23 at 45% RH and the high retention of TBA at lower humidities, shows that diffusivity of TBA is not gradually increased by increasing amounts of water. Apparently, there is a threshold water content, above which TBA is released. Such a threshold has also been observed for maltose.[40] Concentration-dependent diffusivities can not explain the release of TBA, because then a gradual decrease in TBA content should have been observed upon increasing the water content in the samples. Because a threshold is observed, the behavior of TBA can be best described in terms of microregions. Due to water vapor sorption sugar–water hydrogen bonds are starting to replace sugar–sugar hydrogen bonds. The microregions in which TBA was entrapped are opened and TBA is released. It can be proposed that a certain minimum amount of water has to be absorbed to open these microregions. For both inulins it is clear that this occurs when the inulin glasses are exposed to an RH between 33–45%, which corresponds to a water uptake of 7.4–9.6% w/w, respectively (Fig. 3).

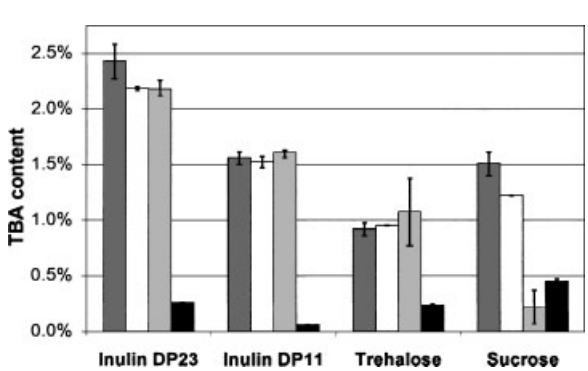

Figure 7. Content of TBA from freeze dried sugars before and after 7 days storage (key: medium-dark columns: before storage; open columns: 20°C/11% RH, shaded columns: 20°C/33% RH, dark columns: 20°C/45% RH; average values, $n = 2–3$; bars indicate lowest and highest values).

It is found that most TBA is released from sucrose already upon exposure to 11–33% RH. Because equilibrium moisture contents of all four sugars are the same (Fig. 3), it may be concluded that the threshold water content to open the microregions is lower for sucrose than for inulins. However, in contrast to the inulins that remain in the glassy state upon exposure to RHs much higher than 45%, sucrose already crystallises upon exposure to 33% RH. This implies that microregions are not opened but simply disappear by which TBA evaporation is facilitated. Because trehalose crystallizes at 45% RH, it may be assumed that TBA release is here also induced by the crystallization of the sugar.

It can be concluded that the release of TBA from trehalose and sucrose occurred together with crystallization, whereas TBA could be removed from both inulins while maintaining the glassy state. Clearly, for inulins the threshold water content for TBA removal did not coincide with the undesired glass-to-rubber transition.

Robustness of Freeze Drying Process

The robustness of the freeze drying process, was tested with the inulin DP23 and diazepam. Table 5 presents an overview of the experiments. Control

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Table 5. Effect of Different Freeze Drying Conditions on Crystallinity of Diazepam

Experiment Number	Solution before Freezing	Freezing Temprature (°C)	Freezing Time (min)	Shelf Temprature (°C)	Diazepam Content (% w/w)	Fraction Amorphous Diazepam (% w/w)
C 1	Clear	−196	<1	−35	10	100
C 2	Clear	−196	<1	−35	20	100
1	Clear	−196	<1	−5	10	100
2	Clear	−196	<1	−5	20	99.7
3	Clouded	−196	<1	−5	10	100
4	Clouded	−196	<1	−5	20	99.9
5	Clear	−37	15	−35	10	96.7
6	Clear	−37	15	−35	20	96.9
7	Clear	−18	45	−35	10	99.6
8	Clear	−18	45	−35	20	97.9

experiments C1 and C2 are given first. In experiments 1 and 2 the shelf temperature during the first 24 h was −5°C. Although this is well above the T'_g of inulin DP23 (−24.6°C) no collapse and hardly any crystallization of diazepam was observed. Most likely, the enthalpy of sublimation was such that the sample temperature remained below the T'_g despite of the high shelf temperature. In experiments 3 and 4 solutions were left for 1.5 h at room temperature to induce partial phase separation in the water/TBA solutions. The cloudy solutions were frozen at −196°C. Also in these samples no or hardly any crystalline diazepam was detected. Apparently, the clouding in the solutions was mainly caused by precipitation of small amounts of inulin. In experiments 4 to 8 the freezing rate was decreased. As can be seen from Table 5, this results in crystallization of a very small fraction of the diazepam. In all cases the fraction of crystalline diazepam was very low, and the freeze drying process can be considered as very robust.

Physical Stability of Solid Dispersions Containing Diazepam

As mentioned before diazepam is a suitable model drug for a physical stability study, because amorphous diazepam, obtained after freeze drying diazepam from a 40% v/v TBA solution, crystallized within 1 day of storage at 20°C and 45% RH. Solid dispersions containing 10, 20, 40, or 63% w/w diazepam were subjected to a physical stability study. All four sugars were tested for physical stability of incorporated diazepam by storing them at 20°C and 45% RH. The fraction of amorphous diazepam still present after 60 days is depicted in Figure 8. Physical stability of diazepam in inulin glass dispersions proved to be substantially higher than in the disaccharide glass dispersions. Already at low drug contents, the diazepam incorporated in solid dispersions with trehalose or sucrose was significantly crystallized, whereas the diazepam in the two inulin glasses stayed almost completely amorphous. It is generally known that seed crystals, can strongly affect the rate of crystallization of amorphous material. However, in our case, seed crystals do not play a role, because there is no difference in amount of seed crystals at, for example, 40% drug load immediately after lyophilization, whereas a large difference in crystallinity of diazepam in the different sugars is observed after 60 days at 45% RH/20°C. The difference in physical stability can be ascribed to difference in the glass transition temperature of the sugars. Due to the plasticizing effect of water (see Fig. 2), the T_g of sucrose

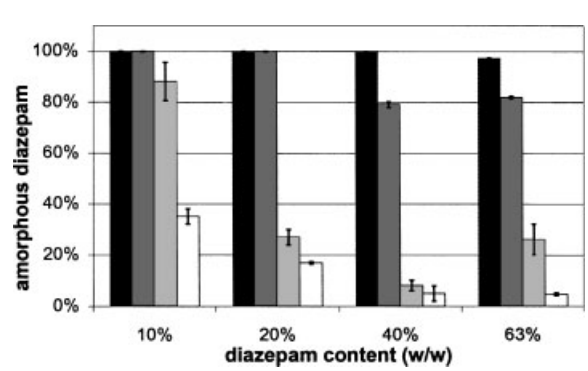

Figure 8. Fraction of amorphous diazepam after storage for 60 days at 20°C/45% RH (key: dark columns: inulin DP23; medium-dark columns: inulin DP11; shaded columns: trehalose; open columns: sucrose; average values, $n = 2–3$; bars indicate lowest and highest values).

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and trehalose at 45% RH dropped below 20°C, whereas both inulins are still in the glassy state. The rapid crystallization of the rubbery sucrose and trehalose was discussed in the section "physical properties of the model drugs and sugars." Both passage of the T_g and crystallization of the sugar will facilitate diazepam crystallization. In glassy inulin at 20°C DP11 the molecular mobility is low, but at high drug loads a part of the diazepam crystallizes. This is probably caused by the fact that inulin DP11 is only 12°C below its T_g. It has been argued that close to the T_g molecular mobility is already increased to a certain extent.[30] In the glassy inulin DP23, molecular mobility is even lower, thereby preventing crystallization completely up to 40% drug load and almost completely at 63% drug load. Clearly, the glassy state is crucial for optimal physical stability of incorporated lipophilic drugs.

CONCLUSIONS

In this study it is found that freeze drying using a mixture of water and TBA as solvents is a versatile technique for the production of solid dispersions of lipophilic drugs in sugar glasses. Solutions of sugars and lipophilic drugs will eventually become cloudy due to phase separation, but with an optimal TBA/water ratio of 40% v/v, solutions show no phase separation for at least 10 min. Solid dispersions obtained after freeze drying contain lipophilic drugs that are fully or almost fully amorphous. High physical stability is guaranteed when the carriers are in the glassy state. Therefore, carriers with high T_gs like inulin DP11 or inulin DP23 are preferred over trehalose and sucrose. Four different lipophilic drugs could be incorporated amorphously in the sugar carriers, which proofs the versatility of this technique. The amount of residual TBA can be strongly reduced by exposing the solid dispersions to air of 45% RH. The amount of TBA is such that toxicity is not an issue. With trehalose and sucrose dispersions, TBA removal is accompanied by crystallization of both sugars, while the inulin dispersions, being in the glassy state, remained fully amorphous.

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