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Characterization of the molecular distribution of drugs in glassy solid dispersions at the nano-meter scale, using differential scanning calorimetry and gravimetric water vapour sorption techniques

D.J. van Drooge *, W.L.J. Hinrichs, M.R. Visser, H.W. Frijlink

Groningen University Institute of Drug Exploration (GUIDE), Department of Pharmaceutical Technology and Biopharmacy, Groningen, The Netherlands

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Abstract

The molecular distribution in fully amorphous solid dispersions consisting of poly(vinylpyrrolidone) (PVP)–diazepam and inulin–diazepam was studied. One glass transition temperature (T_g), as determined by temperature modulated differential scanning calorimetry (TMDSC), was observed in PVP–diazepam solid dispersions prepared by fusion for all drug loads tested (10–80 wt.%). The T_g of these solid dispersions gradually changed with composition and decreased from 177 °C for pure PVP to 46 °C for diazepam. These observations indicate that diazepam was dispersed in PVP on a molecular level. However, in PVP–diazepam solid dispersions prepared by freeze drying, two T_g's were observed for drug loads above 35 wt.% indicating phase separation. One T_g indicated the presence of amorphous diazepam clusters, the other T_g was attributed to a PVP-rich phase in which diazepam was dispersed on a molecular level. With both the value of the latter T_g and the ΔC_p of the diazepam glass transition the concentrations of molecular dispersed diazepam could be calculated (27–35 wt.%). Both methods gave similar results. Water vapour sorption (DVS) experiments revealed that the PVP-matrix was hydrophobised by the incorporated diazepam. TMDSC and DVS results were used to estimate the size of diazepam clusters in freeze dried PVP–diazepam solid dispersions, which appeared to be in the nano-meter range. The inulin–diazepam solid dispersions prepared by spray freeze drying showed one T_g for drug loads up to 35 wt.% indicating homogeneous distribution on a molecular level. However, this T_g was independent of the drug load, which is unexpected because diazepam has a lower T_g than inulin (46 and 155 °C, respectively). For higher drug loads, a T_g of diazepam as well as a T_g of the inulin-rich phase was observed, indicating the formation of amorphous diazepam clusters. From the ΔC_p of the diazepam glass transition the amount of molecularly dispersed diazepam was calculated (12–27 wt.%). In contrast to the PVP–diazepam solid dispersions, DVS-experiments revealed that inulin was not hydrophobised by diazepam. Consequently, the size of diazepam clusters could not be estimated. It was concluded that TMDSC enables characterization and quantification of the molecular distribution in amorphous solid dispersions. When the hygroscopicity of the carrier is reduced by the drug, DVS in combination with TMDSC can be used to estimate the size of amorphous drug clusters.

Keywords: Mode of incorporation; Amorphous drug clusters; Solid solution; Solid suspension; Molecular incorporation; Carrier; Non-proportional water vapour sorption

1. Introduction

In spite of promising progress in biotechnology, most of the new drug substances that currently have to be formulated in dosage forms are small and hydrophobic molecules (Lipinski et al., 2001). Problems related to poor water solubility, slow dissolution and concomitantly low bioavailability (Löbenberg and Amidon, 2000) can be overcome by using solid dispersions (Chiou and Riegelman, 1971; Fawaz et al., 1996; Kai et al., 1996; Torrado et al., 1996; Kohri et al., 1999; Kushida et al., 2002; Gohel and Patel, 2003). Solid dispersions consist of a hydrophilic carrier in which a hydrophobic drug is incorporated. The carrier can be either crystalline or amorphous and the drug can be dispersed either molecularly, in amorphous particles or in crystalline particles (van Drooge et al., 2004a). In this study, we focus on fully amorphous solid dispersions. Previous studies

* Corresponding author at: Groningen University Institute of Drug Exploration (GUIDE), Department of Pharmaceutical Technology and Biopharmacy, Ant. Deusinglaan 1, 9713AV Groningen, The Netherlands. Tel.: +31 50 363 2397; fax: +31 50 363 2500.
E-mail address: D.J.van.Drooge@rug.nl (D.J. van Drooge).

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showed that fully amorphous solid dispersions can be used to increase the dissolution rate (Yoo et al., 2000) and that they are suitable for formulation of a tablet for gastro-intestinal delivery (van Drooge et al., 2004c), a sublingual tablet (van Drooge et al., 2005) or as a powder for inhalation (Simonelli et al., 1969).

Based on their molecular distribution, three different types of fully amorphous solid dispersions can be distinguished:

(1) Fully amorphous solid solutions.
(2) Fully amorphous solid suspensions.
(3) Combination of (1) and (2).

The first type, an amorphous solid solution, consists of an amorphous carrier in which the drug is molecularly distributed (Chiou and Riegelman, 1969, 1971). This type of solid dispersion is homogeneous on a molecular level. Therefore, only one phase is present and only one glass transition temperature (T_g) will be observed. The second type is a fully amorphous suspension. It consists of an amorphous carrier in which the drug is dispersed as amorphous clusters. This type of solid dispersion is not homogeneous on a molecular level and consists of two phases. Hence, glass transitions of both carrier and drug are observed. In literature both types of solid dispersions are referred to as glassy solid solutions and amorphous glassy suspensions, respectively (Chiou and Riegelman, 1971). However, this terminology is equivocal, because both types can become rubbery when exposed to temperatures above their T_g's. Therefore, in this study, the term glassy is omitted and the terms amorphous solid solution and amorphous suspension are used. The third type is a combination of both. Two phases are present: one consists of carrier in which a part of the drug is molecularly dispersed; the other consists of amorphous drug clusters. Which one of the three types is obtained depends on the miscibility of drug and carrier and on the preparation method (Breitenbach et al., 1999).

The detection of the molecular arrangement of the incorporated drug is a prerequisite for comprehension of stability and dissolution of solid dispersions. Many techniques have been developed to investigate the molecular arrangement in solid dispersions. The vast majority of the techniques focus on discrimination between amorphous and crystalline. Only a few attempts to discriminate between amorphous clusters and molecular distribution have been reported. Confocal Raman Spectroscopy was used to measure the homogeneity of drug distribution in a solid dispersion of ibuprofen and PVP (Six et al., 2004). In pixels of $2\,\mu m^3$, the drug content was quantified. It was stated that when the standard deviation in drug content was smaller than 10%, a homogeneous distribution was obtained. However due to the limited resolution, uncertainty remains about the presence of amorphous clusters in the nano-meter range up to now. The most powerful and straightforward technique to assess the degree of mixing of an incorporated drug is thermal analysis. In case of amorphous carriers, DSC has been used to prove the presence of amorphous clusters of drug molecules (Vasanthavada et al., 2004). Furthermore, DSC has been used to quantify the concentration of molecularly dispersed material (Leuner and Dressman, 2000). Generally, the T_g of a homogeneous solid dispersion is somewhere between the T_g of the carrier and the T_g of the drug (Gordon and Taylor, 1952). This is described by the Gordon–Taylor equation (Cilurzo et al., 2002; Kaushal et al., 2004; van Drooge et al., 2004c; Weuts et al., 2004). Using this equation, the measurement of the T_g of the homogeneous phase reveals its composition. A good fit of the Gordon–Taylor equation with experimental data, indicates ideal mixing and absence of specific interactions (van Drooge et al., 2004b,c).

In this study, temperature modulated differential scanning calorimetry (TMDSC) and water vapour sorption (DVS) were used to assess the mode of incorporation and to quantify the size of detected amorphous clusters by combining these two techniques. Two carriers were evaluated: poly(vinylpyrrolidone) (PVP) or the oligosaccharide inulin. In all solid dispersions diazepam was used as a lipophilic model drug. The effect of various preparation methods (fusion, freeze drying, spray freeze drying and amorphous physical mixtures) on the molecular structure is discussed.

2. Materials and methods

2.1. Materials

Tertiary butanol (TBA) was purchased from Sigma–Aldrich Chemie GmbH, Steinheim, Germany, inulin type TEX!803, having a number average degree of polymerization of 23 (inulinDP23), was a gift from Sensus, Roosendaal, The Netherlands. Polyvinylpyrrolidone K30 (PVP) and diazepam were provided by BUFA B.V. Uitgeest, The Netherlands. The water used was demineralised in all cases.

2.2. Preparation of solid dispersions by vial freeze drying

The preparation of the glassy solid dispersions was based on a procedure described before (van Drooge et al., 2004a). Shortly, diazepam was dissolved in tertiary butyl alcohol (TBA) at a fixed concentration of 25 mg/ml and the carrier, i.e. PVP or inulin, was dissolved in water. The solutions were mixed in a TBA/water ratio of 40/60 (v/v). Subsequently, the mixture was immersed in liquid nitrogen until it was fully frozen. Various concentrations of diazepam in the resulting solid dispersions (drug loads) were obtained by adjusting carrier concentrations, while maintaining diazepam concentrations constant. The frozen solutions were lyophilized using a Christ lyophilizer, type Alpha 2-4 (Salm and Kipp, Breukelen, The Netherlands) with a condenser temperature of $-53\,^{\circ}C$. Lyophilization was performed according to a two-step procedure. Firstly, the pressure was set at 0.220 mbar and the shelf temperature at $-35\,^{\circ}C$ for 1 day. Subsequently, the pressure was decreased to 0.05 mbar, while the shelf temperature was gradually raised to $20\,^{\circ}C$. 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.

2.3. Preparation of solid dispersions by spray freeze drying

Solutions for spray freeze drying, were prepared as described above. The solutions were sprayed with a 0.5 mm two-fluid noz-

zle of a benchtop Buchi spray dryer into liquid nitrogen. The liquid feed rate was 10.5 ml/min and the atomising airflow was set at 500 l_n/h, i.e. the equivalent of 500 l of air of 1 atm and 0 °C. The outlet of the nozzle was positioned about 10 cm above liquid nitrogen. Hot water (about 90 °C) was pumped through the jacket of the nozzle in order to avoid freezing of the solution inside the nozzle. The resulting suspension (frozen droplets of the solution in liquid nitrogen) was transferred to the lyophilizer. The lyophilization procedure described above was started as soon as all liquid nitrogen was evaporated.

2.4. Preparation of solid dispersions by fusion

Solid dispersions containing PVP and diazepam prepared by freeze drying were heated to 190 °C which is well above the T_g of PVP and the melting temperature of diazepam, in a standard aluminium sample pan using a differential scanning calorimeter (DSC2920, TA Instruments, Ghent, Belgium). The samples were annealed for 10 min to allow for fusion of PVP and diazepam. Subsequently the sample was cooled to 20 °C, and immediately used for further calorimetric analysis.

2.5. Preparation of physical mixtures

Physical mixtures with various amounts of crystalline diazepam were prepared by mixing amorphous carrier with the appropriate amount of diazepam, using a spatula and a mortar. The diazepam was untreated and used as supplied.

A physical mixture containing amorphous diazepam was prepared by heating a physical mixture of amorphous PVP or inulin and crystalline diazepam to 135 °C for about 10 min to melt the diazepam and then rapidly cooling to room temperature.

2.6. Temperature modulated differential scanning calorimetry (TMDSC)

A differential scanning calorimeter (DSC2920, TA Instruments, Ghent, Belgium) was used to measure glass transitions in the solid dispersions. A heating rate of 2 °C/min (modulation amplitude 0.318 °C, modulation period 60 s, resulting in heating-only conditions) was used. Indium was used for calibration. Pure nitrogen gas, i.e. without water vapour, was purged through the sample cell continuously. The samples, weighing 5–10 mg, were analysed in open aluminium pans. Residual moisture was removed from the samples by pre-heating them to a temperature of about 10–20 °C below the first glass transition for 30 min. Control experiments revealed that this procedure results in complete evaporation of all moisture and completely dry samples, since the T_g's thus obtained corresponded well with literature values. The dried samples were scanned from 10 to 180 °C. The inflection point in the step change visible in the reversing heat flow was taken as the T_g.

The difference in specific heat between the glassy and the liquid state (ΔC_p) was also measured using the differential scanning calorimeter. The ΔC_p was determined from the reversing heat flow using software from TA Instruments. Fully amorphous diazepam was used as a reference for the solid dispersions. It was prepared by melting crystalline diazepam at 135 °C and annealing for 10 min to assure complete melting followed by rapid cooling. The absence of a melting endotherm upon reheating confirmed that indeed all diazepam was in the amorphous state. In solid dispersions, the ΔC_p of the glass transition of amorphous diazepam clusters was related to the ΔC_p of pure amorphous diazepam after correction for the drug load. Measurements were performed 3–4 times.

2.7. Measurement of water vapour sorption

To investigate the hygroscopicity of the solid dispersions, the water uptake was measured using a gravimetric sorption analyser (DVS-1000 Water Sorption Instrument, Surface Measurement Systems Limited, London, UK). Samples, weighing 5–10 mg, were initially dried by exposing them to 25 °C and 0% relative humidity (RH). When the change of sample mass was less than 0.00050 wt.% per minute during a 10 min-period, equilibrium was assumed and the humidity was changed to 30% RH until again equilibrium was reached. The amount of water absorbed was expressed as the mass percentage of water relative to the dry sample mass.

3. Results and discussion

3.1. Physicochemical properties of diazepam, PVP and inulin

In the thermograms of crystalline diazepam, a melting endotherm at 132 ± 0.1 °C was observed. The heat of fusion, calculated by integration of the melting endotherm in the total heat flow, was 84.6 ± 1.0 J/g. Melting crystalline diazepam followed by rapid cooling yielded fully amorphous material as the thermogram showed a T_g at 46.2 ± 0.13 °C and no melting endotherm at 132 °C. The change in specific heat at the glass transition (ΔC_p) in amorphous diazepam was 0.739 ± 0.012 J/g/°C. The T_g's of PVP and inulin were 176.7 ± 2.6 and 154.5 ± 3.3 °C, respectively.

In attempts to prepare a physical mixture of PVP and amorphous diazepam, a physical mixture of crystalline diazepam and PVP was heated to 135 °C. In contrast to the fusion-method, where the components are heated to a temperature above the T_g of PVP, at 135 °C diazepam melts but PVP remains in the glassy state. After cooling such a mixture, no glass transitions of amorphous diazepam at 46 °C and amorphous PVP at 177 °C were detected. Instead, one T_g representing a homogeneous PVP-diazepam mixture was observed indicating that diazepam was molecularly incorporated in PVP. However, the same experiment with inulin instead of PVP resulted in the formation of two separate amorphous phases with T_g's at 46 and 155 °C. It was found that, in contrast to PVP, inulin in the glassy state does not, or only partly, dissolves in molten diazepam. Furthermore, it was found before that the aqueous solubility of diazepam was increased by the addition of PVP, whereas diazepam solubility remained unchanged in aqueous inulin solutions (Hancock and Zografi, 1994). Both observations illustrate

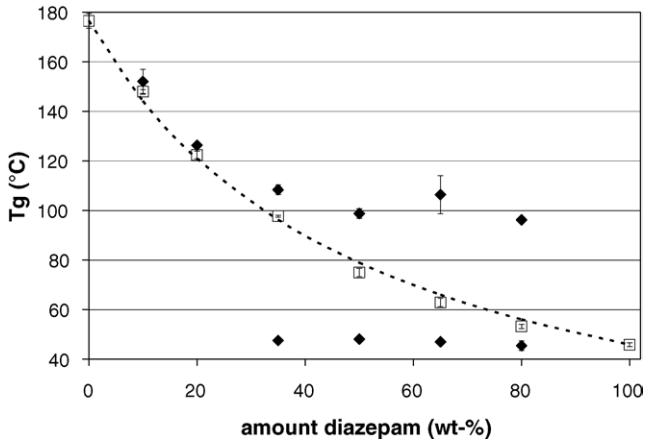

Fig. 1. Glass transitions in solid dispersions prepared by freeze drying using PVPK30 as carrier. Key: (□) solid dispersion prepared by fusion method; (♦) solid dispersion prepared by freeze drying. Dotted line: best fit with Gordon–Taylor equation, $K = 0.34 \pm 0.02$, $r^2 = 0.997$ ($n = 3$–4, error bars represent standard deviations).

that PVP and inulin interact with diazepam in a different manner.

3.2. Glass transitions in PVP solid dispersions

Solid dispersions of PVP–diazepam were prepared by either freeze-drying or by using the fusion method. The T_g's of the dry solid dispersions as measured with TMDSC are presented in Fig. 1. In solid dispersions prepared by the fusion method, only one T_g was observed for all compositions, indicating that homogeneous solid dispersions were obtained in which diazepam is molecularly distributed. The T_g gradually decreased with increasing drug loads, which is often referred to as plasticization of the carrier by a drug (Gordon and Taylor, 1952; Leuner and Dressman, 2000). The composition of solid dispersions can be expressed by the total drug load DL defined in Eq. (1):

$$\mathrm{DL} = \frac{m_\mathrm{D}}{m_\mathrm{D} + m_\mathrm{P}} \tag{1}$$

in which m_D is the mass of the drug diazepam and m_P is the mass of the polymer PVP. This gradual decrease in T_g is described by the well-known Gordon–Taylor equation (Six et al., 2003):

$$T_{g_\mathrm{MIX}} = \frac{T_{g_\mathrm{D}}\mathrm{DL} + T_{g_\mathrm{P}}K(1-\mathrm{DL})}{\mathrm{DL} + K(1-\mathrm{DL})} \tag{2}$$

in which T_{g_MIX} is the glass transition of the solid dispersion, T_{g_D} and T_{g_P} are the glass transition temperatures of pure diazepam and PVP, respectively, and DL is expressed as weight fraction. As shown in Fig. 1, the data could indeed be fitted quite well. In solid dispersions prepared by freeze drying, no melting endotherm of diazepam was observed in the TMDSC scans. This indicated that the solid dispersions were fully amorphous. Furthermore, for drug loads up to and including 20 wt.%, only one T_g was observed. This T_g corresponded with the T_g of the solid dispersions prepared by fusion indicating that these materials were amorphous solid solutions (Fig. 1). However, in solid dispersions prepared by freeze drying with higher drug loads, two T_g's were observed. One T_g was found at 46 °C. Since this T_g corresponds to the T_g of pure diazepam, it was concluded that clusters of amorphous diazepam were formed. The other T_g, being lower than that of pure PVP (176 °C), was attributed to a phase in which the remaining diazepam was molecularly dispersed in PVP. Apparently, these solid dispersions are a combination of an amorphous solid suspension and an amorphous solid solution. Because the T_g of this phase was roughly constant (100–108 °C), it was concluded that the amount of molecularly dispersed diazepam was more or less constant and independent of the total drug load and thus the amount of diazepam present in clusters increases with the drug load. This indicates that during the freeze drying process, a limited amount of diazepam can be molecularly incorporated in PVP. A similar threshold has been reported before for Eudragit/itraconazole solid dispersions (van Drooge et al., 2004b). The amount of molecularly dispersed diazepam in freeze dried solid dispersions ($\mathrm{DL_M}$) as defined in Eq. (3) could be calculated:

$$\mathrm{DL_M} = \frac{m_\mathrm{M}}{m_\mathrm{M} + m_\mathrm{P}} \tag{3}$$

in which m_M is the mass of molecularly dispersed diazepam and m_P is the mass of the carrier.

For this type of solid dispersions, $\mathrm{DL_M}$ can be calculated in two different ways. Firstly, because the relation is known between the T_g of the amorphous solid solution and its composition (Fig. 1), the T_g at around 100 °C can be used to calculate the composition of the PVP-phase in which a part of the diazepam is molecularly dispersed. In the second technique to calculate $\mathrm{DL_M}$, the amount of amorphous diazepam present in the other phase is quantified. The ΔC_p of the amorphous diazepam clusters that result in a T_g at around 46 °C is used to calculate the mass of amorphous diazepam clusters. The observed value is related to the ΔC_p of pure amorphous diazepam (0.739 J/g/°C). When for example in a solid dispersion with a drug load of 50 wt.% a ΔC_p of 0.1 J/g/°C is found, then $0.1/(0.739 \times 0.5) = 27$ wt.% of the total sample consists of diazepam present in clusters. In this example, 23 wt.% of the sample consists of randomly distributed diazepam. The results of both methods of calculation are depicted in Table 1. It can be seen that in three out of four measurements nearly the same molecular drug loads are found with two different methods (see Table 1). The corresponding values indicate reliability of the two methods. It can be concluded that, for solid dispersions in which the carrier is plasticized, the amount of the drug present in clusters can be quantified by either using the T_g of the PVP-phase in which a part of the drug is molecularly dispersed or by using the ΔC_p of the amorphous drug clusters.

From these results, the weight fraction of molecularly dispersed diazepam compared to the total mass of diazepam was calculated. The weight fraction molecularly dispersed diazepam (W_M) is given by Eq. (4):

$$W_\mathrm{M} = \frac{m_\mathrm{M}}{m_\mathrm{M} + m_\mathrm{C}} \tag{4}$$

in which m_M is the mass of molecularly dispersed diazepam and m_C is the mass of clustered diazepam. The results are depicted

Table 1
Amount of molecularly incorporated diazepam in solid dispersions with PVP and inulin (FD, freeze dried; SFD, spray freeze dried) calculated using the T_g of the molecular dispersion phase and using the ΔC_p of the amorphous diazepam phase ($n = 3–4 \pm$ S.D.)

DL (wt.%)	PVP (FD)				Inulin (FD) [DL_M, ΔC_p-method (wt.%)]	Inulin (SFD) [DL_M, ΔC_p-method (wt.%)]
	T_g (°C)	ΔC_p (mJ/g/°C)	DL_M, T_g-method (wt.%)	DL_M, ΔC_p-method (wt.%)		
10			10	10	10	10
20			20	20	12 ± 1.0	20
35	108.4 ± 2.0	58 ± 16	27 ± 1.2	29 ± 1.7	19 ± 6.8	35
50	98.8 ± 1.9	222 ± 35	33 ± 1.3	28 ± 4.9	24 ± 3.2	42 ± 4.7
65	106.4 ± 7.7	294 ± 21	28 ± 4.9	42 ± 2.7	27 ± 4.9	56 ± 3.3
80	96.2 ± 0.8	509 ± 4	35 ± 0.6	36 ± 1.2	25 ± 16	53 ± 18

in Fig. 2. As can be seen from this figure all diazepam is homogeneously dispersed up to drug loads of at least 20 wt.%. When drug loads were further increased, a part of diazepam is present as amorphous clusters, thus lowering W_M.

3.3. Glass transitions in inulin solid dispersions

To investigate the mode of incorporation of diazepam in inulin glass dispersions, four different samples were analysed with TMDSC: a physical mixture of crystalline diazepam and inulin; a physical mixture of amorphous diazepam and inulin; a solid dispersion prepared by freeze drying; a solid dispersion prepared by spray freeze drying. All four samples consisted of 10 wt.% diazepam and 90 wt.% inulin. Nevertheless, large differences were observed in the thermograms of the reversing heat flow (Fig. 3). The first trace, representing a physical mixture of crystalline diazepam and amorphous inulin, clearly showed the melting endotherm of diazepam at 132 °C and the glass transition of inulin at 155 °C. The absence of the melting endotherm of diazepam in trace 2 indicated that in this physical mixture diazepam was completely amorphous. Two glass transitions were observed: one at 46 °C for the diazepam phase and the other at 155 °C for the inulin phase. This measurement implies that, even at the relatively low drug load of 10 wt.%, TMDSC is sensitive enough to detect an amorphous diazepam phase. Trace 3 shows that a solid dispersion prepared by freeze drying did not contain any crystalline diazepam: it is fully amorphous as was concluded in previous work (Tong and Zografi, 1999). However, the absence of a T_g of diazepam is remarkable. This indicates that at a drug load of 10 wt.%, no clusters of amorphous diazepam are present. Therefore, the sample should consist of one phase consisting of diazepam molecularly dispersed in the inulin matrix and thus should be an amorphous solid solution. However, the glass transition temperature of this phase was 158 °C, which is higher than what can be expected based on the composition. An increase in T_g has been observed previously, but only as a result of ionic interactions (Weuts et al., 2004). Deviations from gradual T_g changes as a function of the composition can be attributed to the presence of specific hetero-molecular interactions (Chan et al., 1999). The current observations lead to the hypothesis that the large difference in polarity between diazepam and inulin causes a decrease in mobility of both molecules, whereas for diazepam and PVP, being similar in polarity the mobility is much greater. Trace 4 shows that inulin glass dispersions prepared by spray freeze drying also yield only one T_g at around 155 °C. At a drug load of 10 wt.%, no differ-

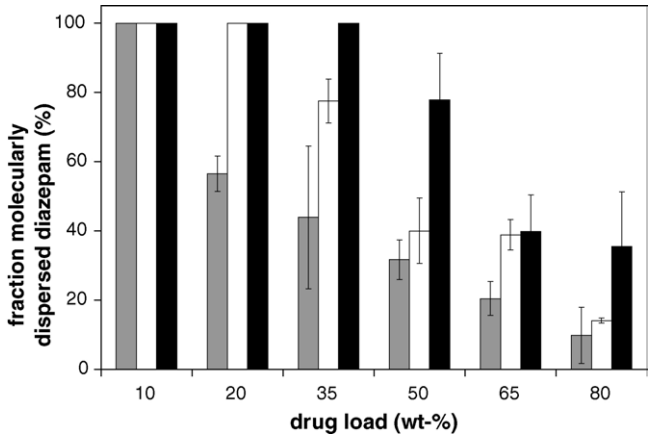

Fig. 2. Amount of molecularly dispersed diazepam. Key: shaded columns, inulin solid dispersions (freeze drying); white columns, PVP solid dispersions (freeze drying); black columns, inulin solid dispersions (spray freeze drying) ($n = 4$, error bars represent standard deviations).

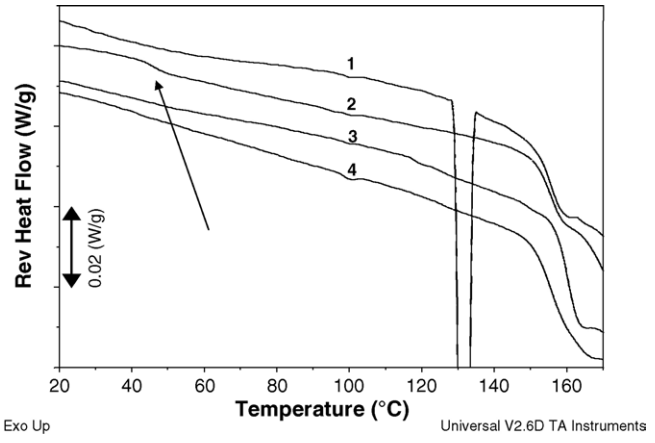

Fig. 3. Thermograms 10 wt.% diazepam and 90 wt.% inulin: (1) physical mixture crystalline diazepam and amorphous inulin; (2) physical mixture amorphous diazepam and amorphous inulin; (3) solid dispersion prepared by freeze drying; (4) solid dispersion prepared by spray freeze drying.

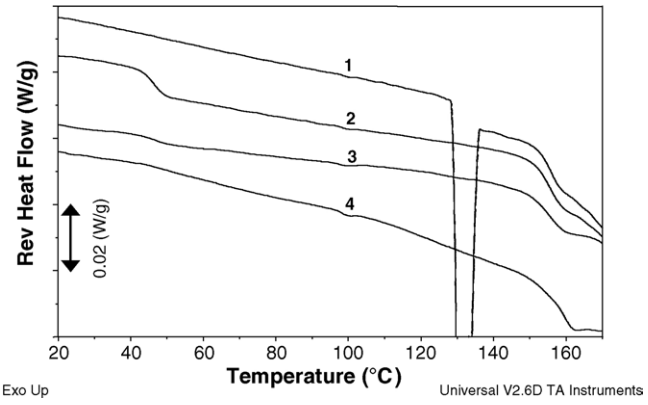

Fig. 4. Thermograms 35 wt.% diazepam and 65 wt.% inulin: (1) physical mixture crystalline diazepam and amorphous inulin; (2) physical mixture amorphous diazepam and amorphous inulin; (3) solid dispersion prepared by freeze drying; (4) solid dispersion prepared by spray freeze drying.

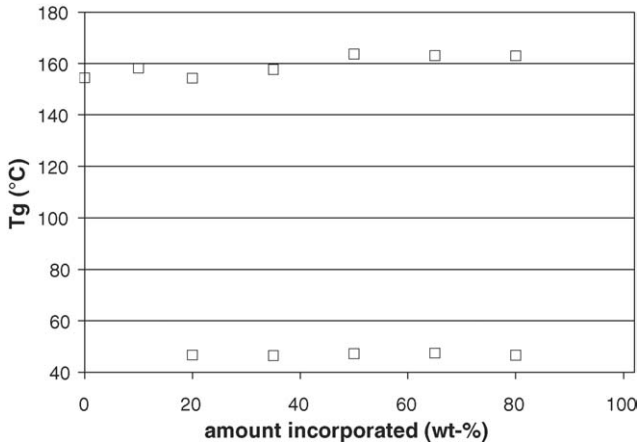

Fig. 5. Glass transitions in solid dispersions containing inulin and diazepam prepared by freeze drying (n = 2–4).

ences could be observed between freeze drying and spray freeze drying.

When the drug load was increased to 35 wt.% (Fig. 4), the surface area of the melting endotherm in the physical mixture containing crystalline diazepam increased proportionally (trace 1). In the amorphous physical mixture (trace 2) the difference in specific heat before and after the glass transition of diazepam (ΔC_p) also increased proportionally. At this drug load also the freeze dried sample showed a T_g of diazepam around 46 °C (trace 3). This indicates that at a drug load of 35 wt.% amorphous diazepam clusters are present in freeze dried inulin glass dispersions. Furthermore, it was observed that the ΔC_p in the freeze dried solid dispersions was less than 35% of the ΔC_p of pure amorphous diazepam. This implicates that diazepam is partly molecularly dispersed and partly present as amorphous clusters. However, in inulin glass dispersions prepared by spray freeze drying (trace 4), no glass transition could be discerned in the thermogram. This implicates that no phase separation occurred during spray freeze drying and that all diazepam is molecularly dispersed in the inulin carrier. Apparently, during spray freeze drying phase separation is inhibited, which results in molecular dispersion of the lipophilic drug in the carrier.

3.4. Effect of freezing rate

TMDSC-experiments were repeated for inulin solid dispersions with other drug loads. No melting endotherm of diazepam was observed, indicating that all solid dispersions were amorphous. For the freeze dried samples, the number and the value of the T_g's are depicted in Fig. 5. It can be seen that in the thermograms of diazepam containing solid dispersions with a drug load of 10 wt.%, only one T_g was observed at around 158 °C. In solid dispersions with a drug load of 20 wt.% or more, also the T_g of diazepam at 46 °C was observed while the second T_g remained constant at around the same value with a slight increase for the higher drug loads. The spray freeze dried samples showed the same behaviour, except that a T_g of diazepam was observed at a drug load of 50 wt.% or more. The amount of molecularly incorporated diazepam in both freeze dried and spray freeze dried samples was calculated from the ΔC_p values and the results are given in Table 1.

To investigate the effect of the preparation method, the inulin solid dispersions prepared by freeze drying were compared with inulin solid dispersions prepared by spray freeze drying. The results are given in Table 1 and Fig. 2. As discussed before, at a drug load of 10 wt.% no clusters are present in both freeze dried and spray freeze dried inulin solid dispersions. This was concluded because no T_g of diazepam was observed. Therefore, the fraction molecular diazepam (W_M) is 100 wt.%. However, when drug loads were increased to 20 wt.% in freeze dried inulin solid dispersions, the fraction molecularly dispersed diazepam sharply dropped and decreased with increasing drug loads. This was concluded from the ΔC_p of the amorphous diazepam present in clusters. When solid dispersions were prepared by spray freeze drying, up to and including 35 wt.% drug load, no T_g of diazepam was observed, indicating that all diazepam is molecularly dispersed. When the drug load was further increased to 50 wt.%, a T_g of diazepam could be seen. Therefore, it was concluded that phase separation occurred only at drug loads of 50 wt.% or higher when spray freeze drying was used. Apparently, during spray freeze drying phase separation is inhibited compared to freeze drying, but also in case of spray freeze drying, the amount of molecularly dispersed diazepam is decreasing for increasing drug loads (Fig. 2). The decrease of W_M can be explained as follows. Higher drug loads were obtained by lowering the inulin concentration in the solution. Therefore, a larger amount of solvent has to crystallize. Furthermore, crystallization is exothermic. Both aspects decelerate the formation of the maximally freeze concentrated fraction in which carrier and drug are vitrified. Therefore, in the concentrated meta-stable and yet unfrozen solution, phase separation between diazepam and inulin will be more pronounced resulting in a lower fraction molecularly dispersed diazepam. During spray freeze drying the cooling rate is much higher, due to direct contact between the solution and liquid nitrogen and the large surface area of the small droplets of solution. From this perspective, the higher fraction of molecularly dispersed diazepam in spray freeze dried material can be explained: because the solute molecules are

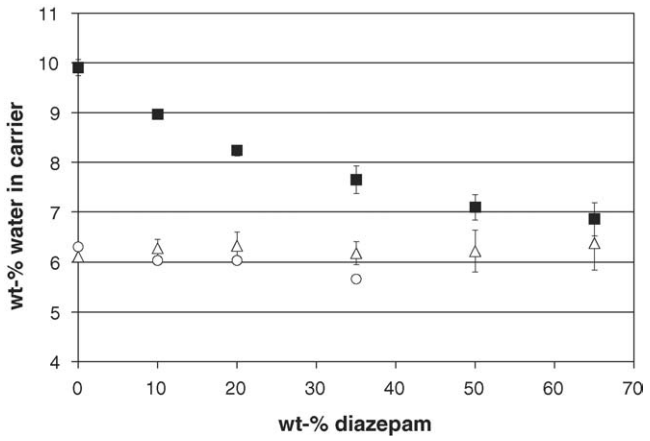

Fig. 6. Water vapour sorption in carriers of solid dispersions. Key: (■) solid dispersion with PVP as carrier (freeze dried), $n = 3–4 \pm$ S.D.; (△) solid dispersion with inulin as carrier (freeze dried), $n = 3–4 \pm$ S.D.; (◯) solid dispersion with inulin as carrier (spray freeze dried), $n = 1–2$.

faster vitrified, shorter time is available for phase separation. Consequently, a higher fraction diazepam is molecularly dispersed.

When comparing inulin and PVP solid dispersions prepared by freeze drying, represented by the shaded and the white columns respectively (Fig. 2), it is observed that more diazepam is homogeneously dispersed in PVP compared to inulin. The phase separation between PVP and diazepam is less pronounced, which can be ascribed to the smaller difference in polarity between PVP and diazepam.

3.5. Water vapour sorption in PVP and inulin solid dispersions

The hygroscopicity of the solid dispersions was determined by measuring the amount of water vapour sorption gravimetrically at 25 °C/30% RH. This humidity was chosen, because all samples tested do not absorb too much water and remain in the glassy state. Because pure amorphous diazepam is hydrophobic, the amount of absorbed water in solid dispersions decreased at increasing drug loads. To compare samples with different drug loads, the amount of water absorbed in the solid dispersions will be corrected for the drug load. Furthermore, a correction was made for the relatively small amount of water (0.3%) absorbed in pure amorphous diazepam. The weight fraction water absorbed in the carrier was calculated using the following equation:

$$W_{\mathrm{w}}\,(\text{in carrier}) = \frac{W_{\mathrm{w}}\,(\text{in solid dispersion}) - \mathrm{DL}\,W_{\mathrm{w}}\,(\text{in pure amorphous diazepam})}{1 - \mathrm{DL}} \quad (5)$$

in which W_{w} is the weight fraction of water. Eq. (5) considers the carrier as a separate matrix with certain hygroscopicity. When for example the calculated hygroscopicity is constant, this implies that the water uptake is not affected by incorporated diazepam. A similar approach has been used before (Crowley and Zografi, 2002). The results are depicted in Fig. 6. It can be seen that PVP absorbs more water than inulin for all compositions tested, showing its higher hygroscopicity. However, water vapour sorption in PVP was not constant but decreased upon increasing drug load, whereas the hygroscopicity of inulin remained unchanged. This indicates that water vapour sorption in PVP is less than what is expected based on the composition of the solid dispersion. This means that the water uptake is not proportional to the drug load. Apparently, PVP becomes less hygroscopic as more diazepam is incorporated. The reduced water uptake can be considered as hydrophobisation of PVP and is due to additional physical-chemical effects. Hydrophobisation and the resulting non-proportional water vapour sorption in PVP solid dispersions containing hydrophobic drugs has been reported previously by Crowley and Zografi (Imamura et al., 2002; Lopez-Diez and Bone, 2004). Deviations from proportional water vapour sorption can be related to interactions between carrier and incorporated molecule. Apparently, diazepam reduces the water uptake of the PVP matrix resulting in faster than linear decrease in hygroscopicity of PVP in solid dispersions with increasing drug loads.

On the other hand, inulin solid dispersions show a proportional water vapour sorption decrease. This can be concluded from the constant hygroscopicity of the inulin carrier as can be seen in Fig. 6. Apparently, diazepam does not affect the hygroscopicity of inulin. The most obvious explanation would be a complete phase separation between inulin and amorphous diazepam. However, it was shown with TMDSC-experiments that this is not the case. This implies that even though there is substantial amount of diazepam molecularly dispersed, no effect on water uptake was observed. Moreover, in spite of the larger fraction of molecularly dispersed diazepam in spray freeze dried inulin glass dispersions (see Fig. 5), water vapour sorption was similar to that of the freeze dried samples. Therefore, it can be concluded that a different mode of incorporation of diazepam does not affect water uptake in inulin. This is another indication that diazepam does not affect the hygroscopicity of inulin. Thus in agreement with TMDSC measurements, water vapour sorption measurements indicate that diazepam can alter hygroscopicity of PVP whereas with inulin changes are not observed.

3.6. Estimation of the size of amorphous diazepam clusters in PVP solid dispersions

Currently, no appropriate techniques are available to measure directly the size of amorphous drug clusters in solid dispersions like the ones presented in this study. Therefore, we suggest a method to calculate the mean cluster size based on the results of TMDSC and DVS. The calculations require geometrical assumptions as explained as follows.

From water vapour sorption experiments, it was concluded that water uptake of PVP was reduced due to the presence of incorporated diazepam. It is assumed that PVP that is in contact to any diazepam molecule is ‘hydrophobised’. This means that no water molecules will be present immediately next to hydrophobic diazepam molecules, but gradually the amount of water molecules will increase with increasing distance from the

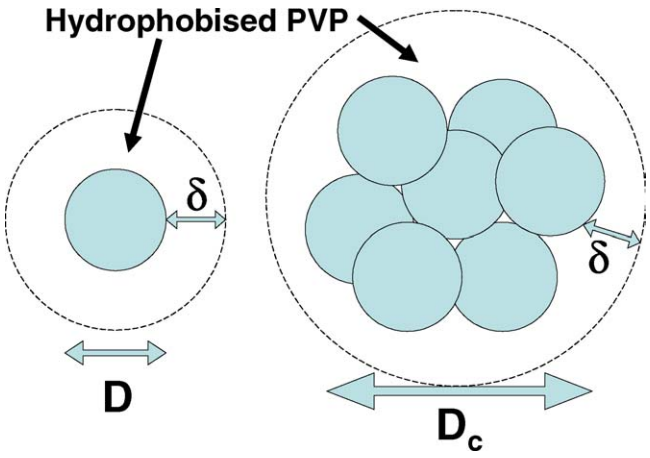

Fig. 7. Schematic representation of lipophilic drug molecules hydrophobising the carrier. Left: a separate molecule as in homogeneous solid dispersions in which drug is molecularly dispersed. Right: a cluster of molecules as in solid dispersions containing amorphous drug clusters.

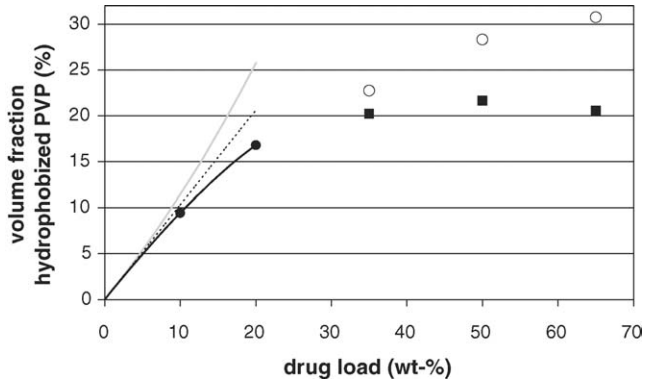

Fig. 8. Volume fraction hydrophobised PVP as a function of drug load for $\delta = 5.48 \times 10^{-11}$ m. Key: gray line, molecular solid dispersion with $p = 0$; dashed line, molecular solid dispersion with $p = 1$; black line, best fit for homogeneous freeze dried solid dispersions with PVP up to 20 wt.% ($p = 1.737$); (●) volume fraction hydrophobised PVP in homogeneous solid dispersion; (■) volume fraction PVP hydrophobised only by molecularly dispersed diazepam calculated using the TMDSC data in Table 1; (◯) total volume fraction hydrophobised PVP.

diazepam molecules. To model the hydrophobisation, we define a layer around diazepam in which no water is present while beyond that layer the hygroscopicity is the same as pure PVP. It can be argued that the extent hydrophobisation depends on the mode of incorporation of diazepam. Molecularly dispersed diazepam, for example, reduces water uptake of the carrier more than clustered molecules, because clusters have a relatively smaller contact area with the carrier. The modelled hydrophobisation of PVP by separate diazepam molecules and diazepam clusters is visualised in Fig. 7. By dividing the total volume of all layers by the total volume of PVP, the volume fraction hydrophobised PVP $\varphi_{\mathrm{H.PVP.TOT}}$ is obtained.

The thickness of the hydrophobised layer and the size of the clusters can be found when the volume fraction hydrophobised PVP ($\varphi_{\mathrm{H.PVP.TOT}}$) is measured. The volume fraction of hydrophobised PVP can be derived from water vapour sorption measurements according to the following equation:

$$\varphi_{\mathrm{H.PVP.TOT}} = 1 - \frac{W_{\mathrm{w}}\,(\text{in carrier})}{W_{\mathrm{w}}\,(\text{in pure PVP})} \tag{6}$$

Firstly, the freeze dried homogeneous solid dispersions of PVP and diazepam are considered, i.e. up to a drug load of 20 wt.%. In these solid dispersions, PVP is only hydrophobised by molecularly dispersed diazepam molecules ($\varphi_{\mathrm{H.PVP.M}}$). Since no clusters are present: $\varphi_{\mathrm{H.PVP.M}} = \varphi_{\mathrm{H.PVP.TOT}}$. The volume fraction PVP hydrophobised by molecularly dispersed diazepam ($\varphi_{\mathrm{H.PVP.M}}$) is given by:

$$\varphi_{\mathrm{H.PVP.M}} = \frac{V_{\mathrm{layers}}}{V_{\mathrm{PVP}}} = \frac{\pi}{6}\left((D+2\delta)^3 - D^3\right)\frac{\rho_{\mathrm{PVP}} N_{\mathrm{Avo}}}{M_{\mathrm{W.Dia}}}\frac{\mathrm{DL_M}(1-\mathrm{DL_M}p)}{1-\mathrm{DL_M}} \tag{7}$$

in which D is the diameter of the diazepam molecule (1.5 nm assuming spherical molecule shape), δ is the thickness of the hydrophobised layer as indicated in Fig. 7, N_{Avo} is Avogadro's number, ρ_{PVP} is the density of PVP (1170 kg/m^3), $M_{\mathrm{W.Dia}}$ is the molar mass of diazepam (0.2847 kg/mole), $\mathrm{DL_M}$ is the molecular drug load, which is in this case equal to the total drug load (DL) since a glassy solid solution is considered. The factor p is introduced to incorporate the likelihood of a neighbouring diazepam molecule. Statistically, diazepam molecules can become neighbours, even in solid solutions. This will result in overlapping hydrophobised layers and thus depletion of hydrophobised PVP volume for which is corrected in Eq. (7). It is assumed that the probability of a drug–drug contact increases proportionally with the drug load. To calculate the chance of a neighbouring drug molecule the drug load was multiplied by a proportionality factor p. The probability of a drug–drug contact will then be equal to $\mathrm{DL_M}p$. When $p = 1$, all molecules are randomly distributed and the chance of a neighbouring drug molecule is exactly proportional to the drug load. When $p < 1$, a drug molecule prefers a carrier environment, and when $p > 1$ it prefers drug–drug contacts. When $p = 0$ all diazepam molecules are always completely surrounded by carrier molecules. The implications on water vapour sorption are depicted in Fig. 8. The thickness of the hydrophobised layer δ (Fig. 7) and the value of p could now be calculated by fitting Eq. (7) using water vapour sorption measurements up to a drug load of 20 wt.% because in this region no clusters are present. It can be seen that p should be larger than 1, since the volume fraction hydrophobised PVP is lower than the theoretical line for $p = 1$. According to this model, diazepam molecules have a preference to neighbour other diazepam molecules because fitting yielded a value of 1.737 for p. The thickness of the hydrophobised layer δ as defined above was found to be 5.48×10^{-11} m, which is 3.65% of the molecule diameter. This implies that the volume of the layer is about 24% of the volume of a diazepam molecule.

In the solid dispersions with drug loads of 35 wt.% and higher, PVP is hydrophobised by both molecular dispersed diazepam and by clusters of diazepam. Using the molecular drug loads ($\mathrm{DL_M}$) calculated by from the T_{g} values of the PVP phase (Table 1), the fraction PVP hydrophobised by molecularly dis-

Table 2
Calculated mean cluster diameters and mean number of molecules per cluster of diazepam in freeze dried solid dispersions with PVP

DL (wt.%)	DL_M (wt.%)	$\varphi_{H.PVP.TOT}$	$\varphi_{H.PVP.M}$	$\varphi_{H.clusters}$	Cluster diameter, D_C (nm)[a]	Cluster size (number of molecules)
10	10	0.095	0.095	–	–	–
20	20	0.168	0.168	–	–	–
35	27	0.228	0.204	0.025	9.6	265
50	33	0.283	0.217	0.067	11.0	393
65	28	0.308	0.206	0.102	20.8	2674

[a] Estimated R.S.D. in $D_C \leq 10\%$.

persed diazepam could be calculated for solid dispersions containing 35 wt.% or more diazepam (Fig. 8). The remaining part of the hydrophobised PVP is due to amorphous diazepam clusters ($\varphi_{H.clusters}$):

$$\varphi_{H.clusters} = \varphi_{H.PVP.TOT} - \varphi_{H.PVP.M} = \frac{\pi}{6}\left(\left(1+\frac{2\delta}{D_C}\right)^3 - 1\right)\frac{\rho_{PVP} D^3 N_{Avo}}{M_{W.Dia}}\frac{m_C}{m_P}$$

$$= \frac{\pi}{6}\left(\left(1+\frac{2\delta}{D_C}\right)^3 - 1\right)\frac{\rho_{PVP} D^3 N_{Avo}}{M_{W.Dia}}\frac{DL - DL_M}{(DL-1)(DL_M-1)} \quad (8)$$

in which D_C is the diameter of a cluster. At this point D_C is the only unknown parameter and can be calculated. In Eq. (8) depletion of hydrophobised volume by clusters–molecule contact as well as cluster–cluster contact was neglected. Furthermore, it is assumed that the clusters are mono disperse. Therefore, the calculated cluster diameter represents the volume average diameter. The results, given in Table 2, suggest that clusters of 10–20 nm are present and that the diameter of the clusters increases with increasing drug load.

Since inulin is not hydrophobised by diazepam, the size of the amorphous diazepam clusters in solid dispersions with inulin could not be calculated according to this method.

4. Conclusion

In this study, it was shown that TMDSC could be used to distinguish between homogeneous solid dispersions and solid dispersions containing amorphous drug clusters. Furthermore, the influence of the carrier-type could be characterized. Water vapour sorption experiments yielded additional information about the effect of a hydrophobic drug on the water uptake of the carrier. When the drug reduced the hygroscopicity of the carrier, the size of amorphous drug clusters could be estimated.

During the development of these methods, the following differences between the carriers PVP and inulin were observed:

- PVP and diazepam mixed spontaneously during heating, whereas inulin and diazepam did not.
- The T_g of PVP was reduced significantly by molecularly incorporated diazepam, whereas a slight increase was observed in inulin solid solutions.
- In PVP solid dispersions more diazepam was molecularly incorporated compared to inulin solid dispersions.
- The hygroscopicity of the PVP was reduced by diazepam, whereas the water uptake in inulin was exactly proportional to the drug load.

Furthermore, the effect of freezing rate during preparation of inulin glass solid dispersions could be measured. It was shown that inulin–diazepam solid dispersions prepared by spray freeze drying contained less diazepam present in clusters and consequently more molecularly dispersed diazepam than freeze dried solid dispersions.

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