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Research paper

Influence of particle size and preparation methods on the physical and chemical stability of amorphous simvastatin

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ABSTRACT

This study investigated the factors influencing the stability of amorphous simvastatin. Quench-cooled amorphous simvastatin in two particle size ranges, 150–180 μm (QC-big) and ⩽10 μm (QC-small), and cryo-milled amorphous simvastatin (CM) were prepared, and their physical and chemical stability were investigated. Physical stability (crystallization) of amorphous simvastatin stored at two conditions was monitored by X-ray powder diffractometry (XRPD) and diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS). Assessment of enthalpy relaxation of amorphous forms was conducted using DSC in order to link the physical and chemical stability with molecular mobility. Chemical stability was studied with high-performance liquid chromatography (HPLC). Results obtained from the current study revealed that the solubility of amorphous forms prepared by both methods was enhanced compared to the crystalline form. The rank of solubility was found to be QC-big = QC-small > CM > crystalline. For the physical stability, the highest crystallization rate was observed for CM, and the slowest rate was detected for QC-big, with an intermediate rate occurring for QC-small. QC exhibited lower molecular mobility and higher chemical degradation than CM. Therefore, the current study demonstrated that QC and CM have obvious differences in both physical and chemical properties. It was concluded that care should be taken when choosing preparation methods for making amorphous materials. Furthermore, particle size, a factor that has often been overlooked when dealing with amorphous materials, was shown to have an influence on physical stability of amorphous simvastatin.

1. Introduction

Amorphous substances lack three-dimensional long range order, which exists in crystalline materials. The amorphous state is a high-energy state that exhibits enhanced solubility and dissolution rate, and thus increased bioavailability [1]. However, the physical and chemical stability of amorphous solids are lower than those of the corresponding crystalline form, and thermodynamically, the amorphous form tends to transform to the more stable crystalline form during processing, storage or even administration.

Quench-cooling of the melt and mechanical activation are two methods commonly used to convert crystalline solids to the amorphous state. It has been reported that several drugs, for instance ursodeoxycholic acid, indomethacin, glibenclamide and dipyridamole, can be converted to the amorphous form using these two methods [2–5]. Depending on the preparation methods, the amorphous forms can show differences in properties such as thermal behavior [2], water vapour sorption [5], surface energy [5], and physical stability [4].

The effect of particle size on the solubility of crystalline solids is well documented [6–8]. It has been found that the solubility of crystalline drugs can be enhanced by reducing the particle size to submicron levels, but the solubility enhancement has been found insignificant if the particle size is not reduced below 10 μm [9]. It was reported that decreasing the size of aspirin particles from 10 to 0.02 μm and to 2 μm diameter led to a 58% and 0.4% solubility enhancement, respectively [6]. For polymorphic crystalline forms, particle size influences not only the solubility but also the physical stability, and plays an important role influencing the kinetics of solid-state transformations [10]. As the crystal size decreases, the surface to volume ratio increases which leads to faster transformation [10]. For amorphous substances, it has been reported that reducing particle size of amorphous indomethacin increases the crystallization rate [11].

Amorphous solids exhibit higher molecular mobility than the corresponding crystalline forms. Consequently, amorphous systems tend to lose excess enthalpy with time, and this enthalpy relaxation process is strongly temperature dependent. The lost

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enthalpy will be recovered upon heating showing an endothermic peak at the glass transition temperature (T_g) on a differential scanning calorimetry (DSC) thermograph. By conducting an enthalpy relaxation study, it is possible to compare the molecular mobility of amorphous systems. This technique has been used for single component amorphous materials [12,13] and binary mixtures [14,15]. Molecular mobility is one of the factors affecting the crystallization of the amorphous state and it is also important for chemical degradation reactions. Molecular mobility of the amorphous state in relation to chemical reactivity has been documented, in which increasing molecular mobility led to enhanced chemical degradation of amorphous forms [16,17].

It has been found that amorphous forms of simvastatin have differences in the thermodynamic parameters depending on the preparation method [2]. The authors reported a lower recrystallization temperature and recrystallization enthalpy for cryo-milled than for quench-cooled simvastatin. They suggested that the cryo-milled form was less disordered compared to the quench-cooled form. In this study, amorphous simvastatin prepared by two methods was further studied focusing on the influence of particle size and preparation method on the solubility as well as on the physical and chemical stability during storage.

2. Materials and methods

2.1. Materials

2.1.1. Preparation of amorphous simvastatin

The molecular structure of simvastatin is shown in Fig. 1. Crystalline simvastatin (Biocon Laboratories, Bangalore, India) with particle size $\leqslant$10 μm was used as the raw material. Quench-cooled amorphous simvastatin (QC) was prepared by melting crystalline simvastatin at 150 °C in an oven and thereafter solidifying it by pouring on liquid nitrogen. The obtained QC was ground gently using a mortar and pestle, and then sieved (Test sieves, Endecotts Ltd., London, England) to obtain a size range of 150–180 μm (QC-big). QC in a size range of $\leqslant$10 μm (QC-small) was achieved by grinding the yielded QC in an oscillatory ball mill (Mixer Mill MM301, Retsch GmbH & Co., Haan, Germany) at 30 Hz for 5 min. The particle size of the samples after milling was checked using light microscopy (Model 218502, Nikon, Tokyo, Japan). X-ray powder diffraction (XRPD) was used to confirm that the solid state of QC was unchanged after ball milling. Cryo-milled amorphous simvastatin (CM) was prepared by ball milling crystals under cryogenic conditions. Crystalline simvastatin (0.8 g) was placed in each milling chamber with 2×12 mm and 6×4 mm stainless steel balls and milled at 30 Hz for 90 min. The milling chambers were immersed in liquid nitrogen for 2 min prior to milling and at 15 min intervals during milling to ensure a low milling temperature. After preparation, all samples were stored at −20 °C over silica gel until further use.

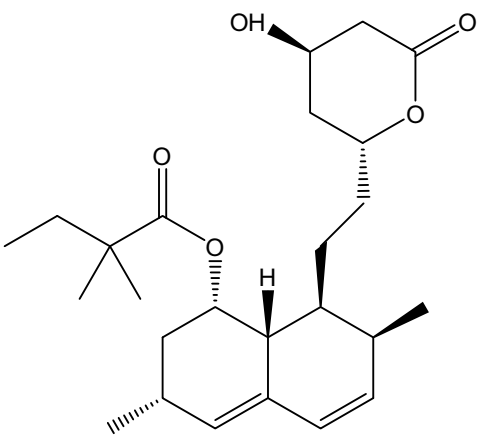

Fig. 1. Molecular structure of simvastatin.

2.2. Methods

2.2.1. X-ray powder diffraction (XRPD)

XRPD was performed using a PANalytical X'Pert PRO MPD system (PANalytical B.V., Almelo, The Netherlands) with Cu Kα radiation (λ = 1.542 Å) and a divergence slit of 1°. The X-ray generator was set to an acceleration voltage of 30 kV and a filament emission of 40 mA. Samples were scanned between 5° and 35° (2θ) using a step size of 0.008° and a count time of 2 s. Data were collected using X'Pert Data Collector and viewed using X'Pert Data Viewer (PANalytical B.V., Almelo, The Netherlands).

2.2.2. Infrared spectroscopy

Diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS) was performed using an FT-IR spectrometer (FTS 175 C, BioRad Laboratories, Massachusetts, IL, USA) with a diffuse reflectance accessory attached (Pike Technology Easidiff, Madison, WI, USA). A background scan was performed prior to analysis, and a reference sample of anhydrous KBr (200 mg) was ground gently for 2 min using an agate mortar and pestle. Samples with a drug content of 5% (w/w) were ground gently with KBr for 2 min using geometric dilution. The spectra were an average of 32 scans at a resolution of 4 cm^{-1} over the range of 500–4000 cm^{-1}.

2.2.3. Polarized light microscopy (PLM)

A phase contrast light microscope (Model 218502, Nikon, Tokyo, Japan) equipped with polarizer and analyzer (Optiphot, Nikon, Tokyo, Japan) was used to detect birefringence.

2.2.4. Differential scanning calorimetry (DSC)

A differential scanning calorimeter (TA-DSC Q100, TA Instruments, New Castle, DE, USA) with a refrigerated cooling system was used. The instrument was calibrated for temperature and enthalpy using indium. Samples (3–5 mg) were packed in non-hermetic aluminium pans and analyzed under dry nitrogen purge. The glass transition temperature was determined by heating samples from −10 to 170 °C at a heating rate of 10 °C/min. Data analysis was carried out using TA Universal Analysis 2000 Software.

The same DSC instrument was employed for the enthalpy relaxation study. Each sample (3–5 mg) was packed in a nonhermetic aluminium pan and heated to 10 °C above the T_g at a heating rate of 10 K/min followed by a 5 min isotherm. The sample was then cooled to 100 °C below T_g at a rate of 20 K/min to form a glass with a standardized thermal history. The glass was then heated to 0 °C and aged isothermally for 0–16 h. After aging for a predetermined time period, the sample was cooled at a rate of 20 K/min to 100 °C below T_g, then heated up to 170 °C with a heating rate of 10 K/min. The endothermic enthalpy recovery peak associated with enthalpy relaxation was analyzed. The area of the endothermic peak (ΔH) was determined by constructing a tangent to the heat curve in the region above T_g. By using the Kohlrausch–Williams–Watts equation [18], the mean relaxation time constant (ι) and the molecular relaxation time distribution parameter (β), were calculated. The calculation methods and equations are described in detail in the literature [19,20]. Triplicate measurements were conducted. Bulk samples were stored in an oven at 10 °C above T_g for 5 min, and then characterized by XRPD and microscopy to confirm that the amorphous state and particle size of samples were unchanged.

2.2.5. High-performance liquid chromatography (HPLC)

HPLC analysis was carried out with a Shimadzu Prominence system (Shimadzu, Tokyo, Japan) using an Econosphere 3 μm C18, 150 × 4.6 mm column (Alltech, Deerfield, USA) and a UV detector. An isocratic solvent system consisting of 40% (v/v) water and 60% (v/v) acetonitrile was used at a flow rate of 1.0 mL/min. Both mobile phases contained 0.05% (v/v) trifluoroacetic acid. The detection wavelength was set at 238 nm. Drug content was determined by calculating the peak area at 4.83 min.

2.2.6. Stability study

The stability of QC in both particle size ranges and CM was studied at 25 and 55 °C under dry conditions (over silica gel) up to 42 days. Samples were removed for analysis at predetermined time points. The physical stability was analyzed with XRPD and DRIFTS, while chemical stability was studied with HPLC.

2.2.7. Solubility measurements

Twenty milligrams of the differently prepared amorphous samples were added to 10 ml of phosphate buffer (pH 6.8) in triplicate. Samples were rotated at 20 rpm in a water bath (25 °C) for 48 h. Samples were withdrawn and then filtered with a syringe filter unit (0.22 μm) and suitably diluted. The concentrations of simvastatin were then determined by UV-spectrophotometry (UV-1601PC, Shimadzu, Kyoto, Japan) at the wavelength of 238 nm. Undissolved samples were recovered after 2 and 48 h during the test by filtration and, after vacuum drying, investigated by XRPD to detect crystallinity.

2.2.8. Statistical analysis

Statistical analysis (double sided *t* test) was performed using Minitab software (Minitab, State College PA, USA).

3. Results

3.1. Formation of amorphous simvastatin

Both methods, quench-cooling and cryo-milling, were able to produce pure amorphous simvastatin as confirmed by a halo in the XRPD pattern and by PLM showing no birefringence. For QC-small, 5 min ball milling was used for particle size reduction. Even though long-term milling can induce solid-state transformations [21], the short milling time to reduce the particle size of QC used here is not likely to cause significant changes in the physicochemical properties of the amorphous material.

3.2. Solubility

The solubility testing showed increased solubility for the amorphous form compared to crystalline drug (Fig. 2). The solubility of crystalline simvastatin was 15.1 ± 0.7 mg/L, which agreed well with the literature [22]. Quench-cooling of the melt resulted in an approximately 2-fold increase in solubility after 48 h (29.9 ± 1.1 mg/L for QC-big and 30.5 ± 2.0 mg/L for QC-small) in comparison with crystals. However, the reduction in the particle size of QC did not increase the solubility significantly ($P > 0.05$). Increased solubility was also observed for CM (25.4 ± 2.2 mg/L) but was significantly lower than that of QC ($P < 0.05$). By measuring undissolved samples recovered at 2 h using XRPD, it was found that that CM had already started exhibiting several small crystalline peaks. However, only a halo was observed for both QC-small and QC-big suggesting that they remained amorphous at the 2 h time point. The rapid recrystallization of CM might be the explanation for its lower solubility than QC. At 48 h, crystalline peaks were found in all recovered samples indicating recrystallization. Regardless of the recrystallization, the concentration of initially amorphous samples remained higher than the solubility of crystalline simvastatin.

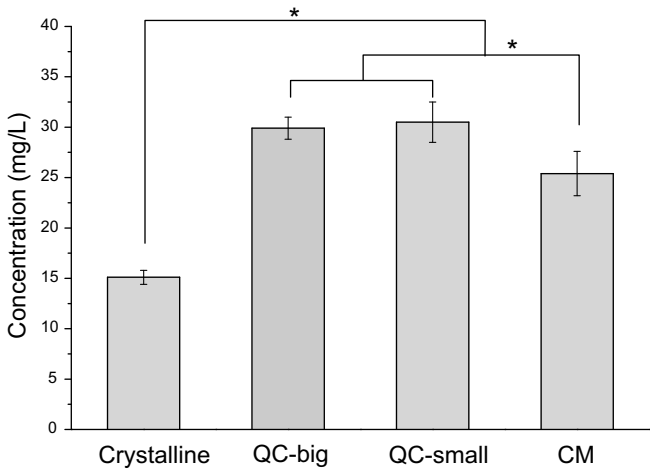

Fig. 2. Solubility of crystalline and differently prepared amorphous forms of simvastatin. Significant difference between samples is labelled with an asterisk ($n = 3$).

3.3. Physical stability of amorphous simvastatin

T_g values of QC-big (25.2 °C), QC-small (25.5 °C) and CM (24.7 °C) were not significantly different ($P < 0.05$). All amorphous samples were stored at 25 and 55 °C, which were around T_g and 30 °C above T_g, respectively, under dry conditions. Recrystallization was monitored by XRPD and DRIFTS.

3.3.1. XRPD

Fig. 3 shows the diffractograms of QC-big, QC-small and CM stored at 25 °C for different time periods. Neither QC-big nor QC-small showed any sign of recrystallization during the storage period (Fig. 3a and b). They remained amorphous after 42 days of storage showing the characteristic XRPD halo. However, CM was less stable, and a few crystalline peaks could be detected already after 4 h of storage (Fig. 3c). With increasing storage time, more crystalline peaks appeared and the intensities of peaks increased. CM was almost completely recrystallized after 4 days.

Different recrystallization behavior between QC-big and QC-small was observed upon storage at 55 °C as shown in Fig. 4. QC-big remained completely amorphous without any crystallization after 1-day storage (Fig. 4a), while QC-small had already started to recrystallize (Fig. 4b). Only very small peaks at 7.5° 2θ, 17.1° 2θ and 17.6° 2θ were observed after 4–6 days which suggested that a small amount of QC-big had recrystallized. The intensities of these peaks increased very slowly with increasing storage time. After 42 days, QC-big was mostly amorphous showing only few very small peaks in the XRPD patterns. However, QC-small was less stable than QC-big during storage. It had already started to recrystallize after 1-day storage with many crystalline peaks present in the diffractograms. The intensities of crystalline peaks increased with increasing storage time, and the sample showed almost complete recrystallization after 6 days. QC-small therefore recrystallized at a faster rate than QC-big. When comparing the recrystallization behavior of QC-small and CM, which were prepared using different methods but within the same particle size range, a higher recrystallization rate was observed for CM. At 55 °C, CM started to crystallize immediately at the beginning of the storage, and it was almost completely recrystallized after 4 h according to the XRPD results. The positions of all peaks in the XRPD patterns of the recrystallized, initially amorphous samples

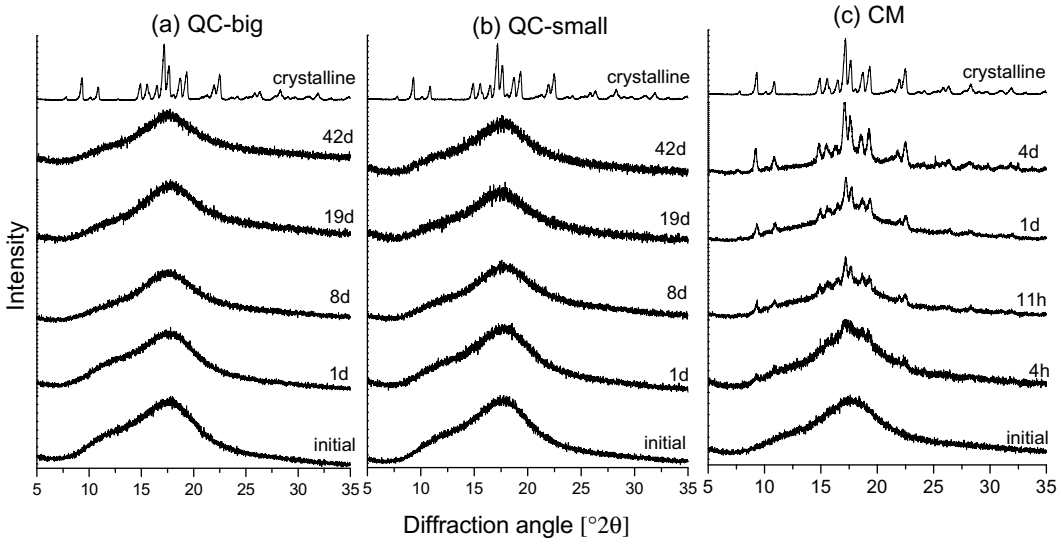

Fig. 3. XRPD of amorphous simvastatin stored at 25 °C. (a) QC-big, (b) QC-small, and (c) CM. The XRPD pattern of pure crystalline simvastatin is given for comparison (top).

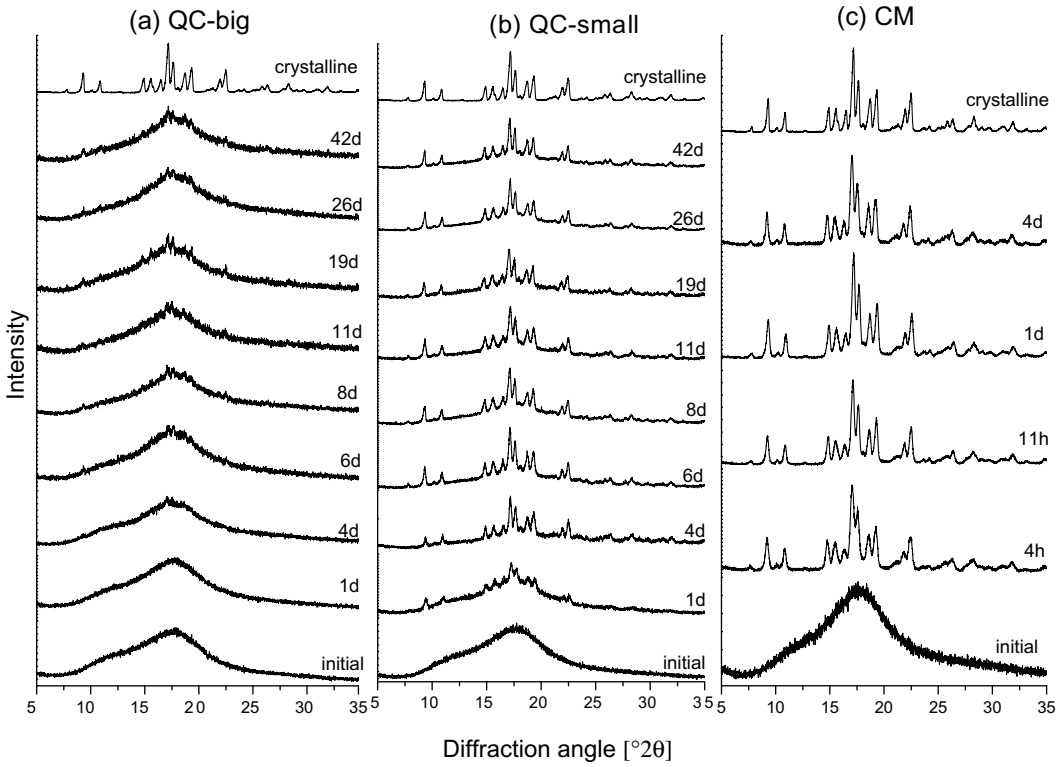

Fig. 4. XRPD of amorphous simvastatin stored at 55 °C. (a) QC-big, (b) QC-small, and (c) CM. The XRPD pattern of pure crystalline simvastatin is given for comparison (top).

were identical to those of pure crystalline simvastatin. However, the baseline of the recrystallized samples was shifted up slightly, and the peaks were also slightly broadened compared to those of pure crystalline sample. All amorphous forms of simvastatin showed a higher recrystallization tendency when stored at the higher temperature conditions.

3.3.2. DRIFTS

In the crystal structure, simvastatin molecules are linked by hydrogen bonds between the hydroxyl group and the ester group [23]. After transformation to the amorphous form, two regions in the spectra, namely the carbonyl functional group region (1800–1600 cm^{-1}) and the OH bond region (3650–3200 cm^{-1}) showed significant peak shifts and broadening (Fig. 5), which indicated that amorphization is associated with a change in intermolecular hydrogen bonding. Initial DRIFTS spectra of QC-big, QC-small and CM showed no obvious difference even though peak intensities varied.

The DRIFTS spectra of QC-big, QC-small and CM stored at 25 °C are shown in Fig. 5. For both QC samples (Fig. 5a and b), a change associated with the carbonyl functional group was observed with a shift of the peak maximum from 1716 to 1724 cm^{-1}. Moreover, a shoulder peak at 1738 cm^{-1} disappeared during storage. This was attributed to a change in the intermolecular hydrogen bonds dur-

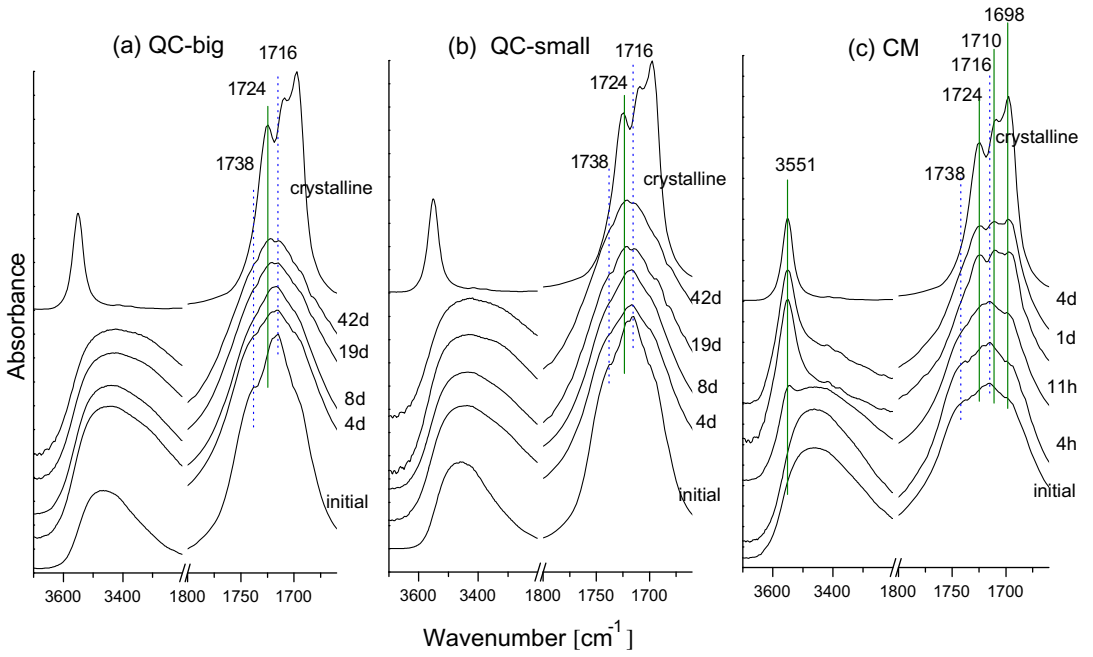

Fig. 5. DRIFTS spectra of amorphous simvastatin stored at 25 °C. (a) QC-big, (b) QC-small, and (c) CM. The spectrum of pure crystalline simvastatin is given for comparison (top).

ing storage and demonstrated the crystallization process of amorphous samples. These changes were also present in the spectra of CM (Fig. 5c). Additionally, the broad peak in the carbonyl group region split and two peaks appeared at 1710 and 1698 cm^{-1}, which agreed well with the spectrum of the crystalline form. Furthermore, an obvious change in the OH bond region was found in the spectra of CM samples. A small shoulder arose and then a peak developed at 3551 cm^{-1} with increasing storage time.

Similar to the XRPD findings, DRIFTS spectra of the samples stored at 55 °C showed a higher recrystallization rate than when stored at lower temperature (Fig. 6). For both QC samples (Fig. 6a and b), the peak shift of signals corresponding to the C=O group (1800–1600 cm^{-1}) was clearly seen on the spectra. Nevertheless, the appearance of a small peak at 3551 cm^{-1} was only observed in the spectra of QC-small (Fig. 6b), which suggested a higher extent of recrystallization than in QC-big where no peak at that region was observed in the spectra (Fig. 6a). Unlike in the spectra of QC samples, a sharp peak at 3551 cm^{-1} developed rapidly during storage in the spectra of CM (Fig. 6c). The change in the carbonyl group region was again obvious with splitting of the broad peak at around 1700 cm^{-1}. The spectra of CM after a 4-day storage at 55 °C resembled that of crystalline drug; however, differences in the relative peak intensities and baseline could be observed. DRIFTS clearly demonstrated the occurrence of much faster recrys-

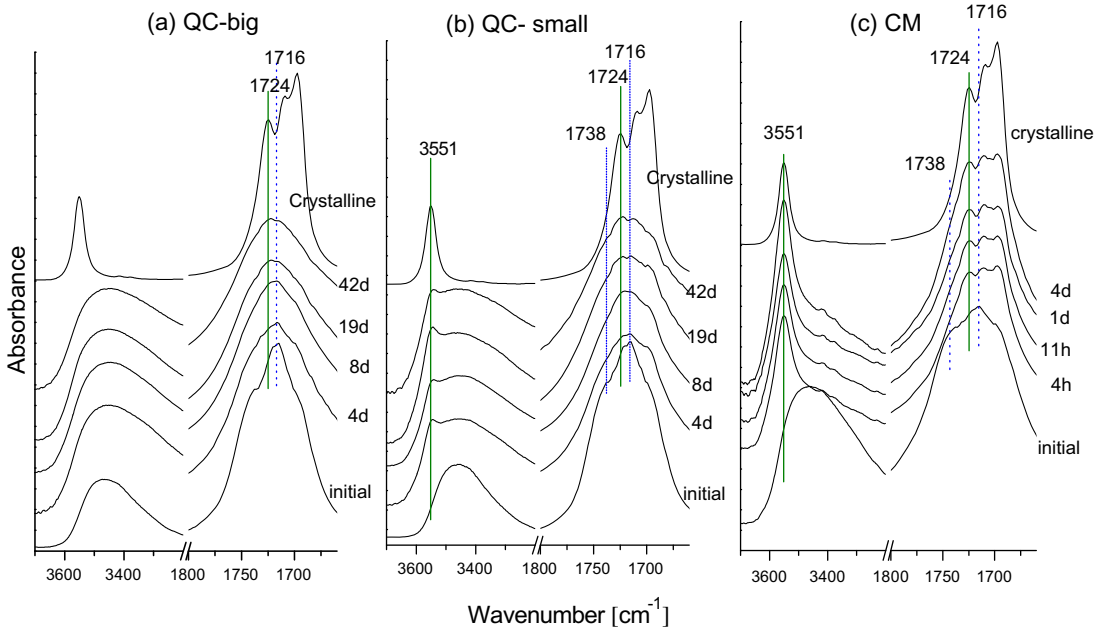

Fig. 6. DRIFTS spectra of amorphous simvastatin stored at 55 °C. (a) QC-big, (b) QC-small, and (c) CM. The spectrum of pure crystalline simvastatin is given for comparison (top).

Table 1
Thermal properties of amorphous simvastatin

Sample	T_g (°C)	ι (d)	β
QC-big	25.2 ± 3.3	578 ± 31.2	0.55 ± 0.04
QC-small	25.5 ± 2.5	532 ± 27.5	0.53 ± 0.03
CM	24.7 ± 2.7	15 ± 4.1	0.50 ± 0.07

Mean ± SD, $n = 3$.

tallization of CM than the QC samples. The better physical stability of QC-big compared to QC-small could also be seen in the DRIFTS spectra by the absence of the peak at 3551 cm^{-1} in the spectra of QC-big (Fig. 6a).

3.4. Enthalpy relaxation study

To understand the stability difference of amorphous simvastatin prepared by different methods, structural relaxation below T_g was assessed. After a 16-h aging at 0 °C, the samples showed no recrystallization according to XRPD. However, due to molecular mobility, the amorphous glass underwent a loss of enthalpy which drove it towards the equilibrium supercooled liquid state. The calculated mean relaxation time constants (ι) and the molecular relaxation time distribution parameters (β) for QC-big, QC-small and CM are listed in Table 1. It was found that there was no significant difference ($P > 0.05$) in the predicted mean relaxation time constants of QC-big and QC-small (578 ± 31.2 days and 532 ± 27.5 days, respectively), or in the molecular relaxation time distribution parameters ($P > 0.05$). The predicted mean relaxation time constant of CM (15 ± 4.1 days) was significantly smaller than those of QC samples ($P < 0.05$), while the molecular relaxation time distribution parameters were not significantly different ($P > 0.05$). Since molecular mobility is usually quantified using relaxation time constants, it indicated that QC samples had lower molecular mobility than CM which in turn can explain the difference in recrystallization tendency.

3.5. Chemical stability

The freshly prepared QC-big, QC-small and CM samples all showed a small amount of decomposition (2.8 ± 1.3%, 2.9 ± 2.0% and 1.7 ± 1.7%, respectively) with no significant difference between them ($P > 0.05$). After 42 days of storage at 25 °C, a significantly higher amount of decomposition was detected for QC-big and QC-small (8.5 ± 1.3% and 9.6 ± 0.7%, $P < 0.05$, respectively), but for CM the amount of decomposition was lower (2.8 ± 1.5%).

4. Discussion

The amorphous state is a high-energy unstable solid state. This results in enhanced solubility and bioavailability compared to the crystalline state, which depending on the polymorphic form is a metastable or stable low energy state. The solubility advantage for amorphous drugs compared to the corresponding crystalline forms has been reported to vary between 1.1- and 24-fold [24]. Amorphous simvastatin prepared by both quench-cooling and cryo-milling was found to show increased solubility compared to crystalline simvastatin. The solubility increased by 2- and 1.7-fold by quench-cooling and cryo-milling, respectively. The statistically significant difference between the solubility of QC and CM could be due to a more disordered molecular structure in QC than in CM as reported previously [2]. Moreover, due to different crystallization rates of the amorphous drug during solubility measurements, the measured solubility advantage would be expected to show certain variations. It was found that QC remained amorphous after 2 h of immersion in the buffer solution, while CM had already partly recrystallized. This further added to the solubility advantage of QC over CM. It was shown that no significant increase in solubility was obtained by reducing the particle size of QC samples from 150–180 μm to ⩽10 μm.

Different crystallization behavior of QC and CM was observed in the stability studies using XRPD and DRIFTS. QC recrystallized at a much slower rate than CM indicating that QC was a more physically stable amorphous state. The enthalpy relaxation study revealed lower molecular mobility existing in QC than in CM, which could be related to the difference in the molecular near order. It is possible that QC samples went through a substantial molecular disordering which, however, had not happened for cryo-milled samples, leading to a lower probability of nucleation [5].

Another factor contributing to the faster recrystallization of the milled samples could be that the mechanical activation leads to the formation of small crystallites that promote crystallization. It has been reported that ball milling can leave some of the crystalline raw material unconverted to the amorphous form [25]. Although XRPD is not capable to detect such small crystallinity in amorphous materials [26], these residual crystallites could play a role as nucleation seeds and thus facilitate the recrystallization process.

It is well known that storing amorphous materials at a temperature lower than T_g or adding polymers with a high T_g to an amorphous system can inhibit crystallization [13,20,22,27]. This study showed that controlling particle size could also influence the stability of quench-cooled amorphous simvastatin. Similar behavior has been reported for crystalline samples by Cardew et al., who reported that the rate of polymorphic transformation is dependent on the particle size [10]; the surface to volume ratio decreases as the crystal size increases, and this leads to a slower transformation. Analogous results have also been reported for amorphous indomethacin [11]. It has been suggested that crystallization is most likely to be initiated on the surface of amorphous substances [28]. Therefore, for the amorphous materials having a smaller particle size and thus increased specific surface area, the crystallization initiation could be expected to be faster. Although QC-big and QC-small exhibited different recrystallization rates, their molecular mobility was not significantly different as indicated by their mean relaxation constants. This is understandable because the two samples were prepared in the same process.

Both XRPD and DRIFTS proved to be useful techniques for monitoring the recrystallization process of amorphous simvastatin during storage. The obtained XRPD diffractograms and DRIFTS spectra showed a clear trend of recrystallization with increasing storage time. When samples were stored at 25 °C, XRPD showed no signs of recrystallization in QC samples; however, DRIFTS clearly showed a peak shift in the C=O band region which suggested changes in the intermolecular bonding, and further, the onset of crystallization. Therefore, DRIFTS was sensitive to detect low crystallinity existing in the samples.

The extent of decomposition in the freshly prepared amorphous materials agreed well with the findings for other drugs (e.g. 1.56 ± 0.13% for quench-cooled amorphous indomethacin [4]). After storage, the extent of decomposition of both QC and CM increased and the former showed lower chemical stability. It is believed that higher molecular mobility could accelerate chemical reactivity during storage [16]. Therefore, CM, exhibiting higher molecular mobility, was expected to show higher decomposition during storage than QC. However, the results showed that CM was more chemically stable. This can be explained by its lower physical stability. The fast crystallization of CM retarded its chemical degradation, since crystalline simvastatin is chemically stable.

This study confirmed a disadvantage of the use of XRPD in amorphous investigation as also recognized by other authors [26,29]. XRPD is a technique that essentially determines crystalline

properties, and the absence of diffraction peaks may then be interpreted as amorphousness. However, the existence of small crystallites in the amorphous materials as well as the initiation of recrystallization can often not be detected by XRPD. This raises some interesting questions. Not only can the preparative technique with which an amorphous form has been generated lead to different properties of this form (as shown in this study by the different properties of QC and CM samples), but what an analytical technique determines as "amorphous" depends on the technique itself. A combination of diffractometric techniques with other methods including thermal and spectroscopic techniques is necessary to determine the solid-state properties of non-crystalline solid materials.

5. Conclusions

QC-small showed no increase in solubility compared to QC-big, but it was found that the smaller particle size led to decreased physical stability. Thus, the results of this study suggest that controlling particle size is a way to enhance the stability of amorphous forms. To prevent recrystallization of the amorphous form, it is important to remove the fine particles.

Preparation methods were found to influence the solubility, as well as the physical and chemical stability of amorphous simvastatin. Cryo-milling resulted in an amorphous form with lower solubility and physical stability, but higher chemical stability than quench-cooling. CM was found to have a higher molecular mobility than QC, which caused lower physical stability during storage. The higher chemical stability of CM was explained by its lower physical stability. Both QC and CM showed lower chemical stability than the crystalline form. This study clearly demonstrated that different preparation methods result in amorphous forms with different properties. Therefore, the choice of the preparative and also the analytical method should be carefully considered during the development of amorphous drugs.

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