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Thermal and spectroscopic analysis of inclusion complex of spironolactone prepared by evaporation and hot melt methods

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Abstract Solvent and melt techniques were used to obtain molecular dispersion of the poorly soluble spironolactone (SPIR) model drug enhancing its dissolution rate. DSC study of the interaction between SPIR and hydroxypropyl-β-cyclodextrin confirmed the need for molecular dispersion if their complexation is required. Solvent-free twin-screw extrusion was suitable for forming inclusion complex significantly below the melting temperature of the SPIR. According to DSC, Raman and XRPD results fine dispersion of both components was achieved in a hydrophilic polymer. The molecules of the active ingredient are separated from each other in the polymer and the lack of the lattice energy causes faster dissolution.

Keywords Amorphous · Extrusion · Inclusion complex · Solid solution

Introduction

The permanent maintenance of the amorphous state of the active ingredients became one of the most important challenges both in the original and generic drug development. A large amount of the recently synthesised and biologically effective molecules have very poor water solubility (Biopharmaceutical Classification System II). The different interactions between these molecules modify their physical properties (melting point, crystal habit, heat capacity etc.), including the dissolution rate. The dissolution is the fastest in the absence of the bonds forming the ordered structure of crystallites. Therefore, the formation of stable amorphous drugs is favourable in many cases. Drugs synthesised in amorphous form are, however, sensitive to humidity, heat or mechanical stresses, which can initiate the re-crystallisation of a glassy amorphous structure during storage or traditional granulation processes. This instability means the limiting factor for these methods, therefore techniques have been elaborated for forming amorphous drug structure in presence of stabilizing components, e.g. in course of granulation.

If the homogenous dispersion of the separated drug molecules can be obtained in a glassy matrix the association of the molecules and the interaction between them is inhibited. The rate of dissolution from such a drug–matrix system in the gastric fluids can be designed considering the Noyes–Whitney equation [1]

$$\frac{\mathrm{d}X}{\mathrm{d}t} = \frac{AD}{h}\left(C_\mathrm{s} - \frac{X_\mathrm{d}}{V}\right) \tag{1}$$

$\frac{\mathrm{d}X}{\mathrm{d}t}$ means the dissolution rate, A refers to the active surface of the particle available for dissolution, D is the diffusion constant, h is the thickness of the boundary diffusion layer, c_s is the saturation solubility concentration of the drug, V is the volume of the solvent, X_d is the amount of the already dissolved drug. The largest specific surface (A) can be achieved when the molecules of the drug are separated and dispersed in a hydrophyl polymer matrix, by which, after

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the dissolution of the delivering excipient, oversaturated solution of the active ingredient can be obtained [2–5].

Different types of cyclodextrins are widely applied not only to improve the stability, and the bioavailability but also the solubility and the dissolution rate. The semi-synthesised derivatives provide better solubility characteristics, furthermore, the inclusion capacity, determined basically by the cavity size, can be modified by the substituents. Versatile release profile can be obtained by the application of cyclodextrins. In the case of immediate drug liberalisation, achieved by a complex, the enhancement is ascribed to the increase of the solubility and wettability of drugs via the formation of the inclusion complex [6, 7].

Different drying and melting methods are also capable of promoting the molecular dispersion of drugs in solid state. Spray and freeze drying are techniques promising to carry out a controllable delivering system but their productivity is lower than other processes of pharmaceutical technology. Recently, melt extrusion proved to be efficient method for controlling the structure at high productivity. For example, it was shown that well-selected design of the system with correctly chosen parameters can ensure amorphous state of the active ingredient in a rapid continuous process [3, 8–11].

Berbenni et al. studied the thermal analytical aspects of the polymorphism of spironolactone (SPIR). They carried out recrystallization from different solvents (acetone, methanol, ethyl acetate) and found that the solvent, moreover, the heating rate influence the thermal behaviour of the selected active ingredient. It was also stated that the melting of this diuretic is accompanied by decomposition which decreases the melting temperature and the enthalpy, respectively [12, 13]. Thus, the processing of SPIR below its melting temperature should be avoided.

Among the different cyclodextrins the β variation and its derivatives are most widely used. Their thermal analytical profile can be divided into the following parts: water loss, thermal degradation and ignition. Depending on the way of preparation and level of hydration the β-cyclodextrin can exist in different crystalline forms that can transform into each other [14].

One of the most significant sign of the association of the drug with the cyclodextrin is the reduction or even the loss of crystallinity of the active ingredient due to the separation of its molecules. Differential scanning calorimetry was found sensitive enough for detecting the slight modification of the crystalline phase as a function of increasing concentration of the host oligosaccharide [15–17].

The aim of this work was to investigate the incorporation of SPIR into the inclusion complex and the loss of its crystalline structure consequently. This poorly water soluble antihypertension drug indicates intense flexibility, which promotes the assembling of the molecules thus it is supposed to show polymorphic changes that can be detected in the course of DSC studies. Hydroxypropyl-β-cyclodextrin (HPCD) is reported to be the strongest host molecule for the SPIR [18]. Due to the interactions of the active pharmaceutical ingredient (API) with the host molecule its restricted mobility does not support the formation of crystalline structure thus the dissolution concentration and rate in water are altered as well. Soliman et al. prepared inclusion complex of SPIR and HPCD by spray and freeze drying, respectively, by applying different solvents [18, 19]. Solvent-free kneading method for forming inclusion complexes was partially successful in the case of nifedipine and bifonazole [17, 20], while the interaction built up entirely between salbutamol laurate and 2-hydroxypropyl-β-cyclodextrin [21]. Fukuda et al. has attended to carry out cyclodextrin/drug adduct via solvent-free melt-extrusion process, however, in that case the heat sensistve API had to be in molten state during the process because the used single screw device, the excipients and the selected parameters did not allow the performance of the complexation at low temperature [9].

We decided to elaborate circumstances by which the extrusion temperature can be keept below the melting point of SPIR utilising the intense homogenisation effect of a twin-screw extruder and properly selected polymer matrix. DSC investigation was selected in order to characterise the solvent evaporated adducts containing different molar ratio of the drug and the excipient moreover to evaluate the influence of the preparation method on the host/guest interaction. The applicability of DSC method for evaluating the interaction occuring in the extrusion process was also a question to be clarified. The DSC analyses were planned to be supported by Raman-spectrometry too.

Experimental

Materials

Spironolactone (SPIR) ($M = 416$, $C_{24}H_{32}O_4S$, Gedeon Richter Plc.) aldosterone antagonist, HPCD (average molecular weight: $\sim$1400, München Wacker Chemie GMBH) and maize starch (Roquette) were the kindly gift of Gedeon Richter Plc. The sorbitol was purchased from Phoenix Magi Ltd (Fig. 1).

Preparation of solvent co-evaporated samples

Spironolactone (SPIR) was dissolved by acetone and then the aqueous solution of HPCD was poured to it. The clean liquid mixtures of the materials containing different molar ratio of them (Table 1.) were vacuum evaporated by Heidolph Laborata 4000 apparatus at 50 °C and 30 rpm, subsequently.

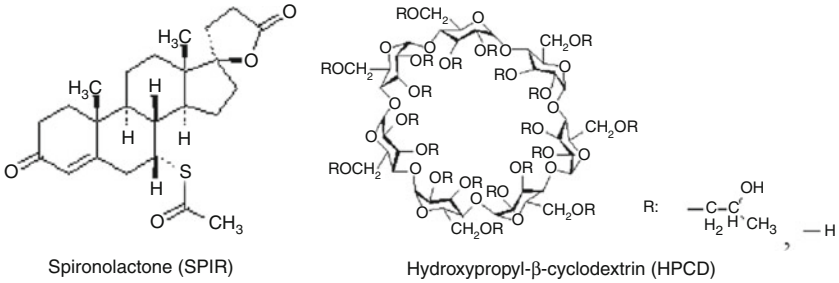

Fig. 1 The chemical structure of SPIR and HPCD

Table 1 The molar ratio of the solvent co-evaporated samples

Molar ratio/SPIR:HPCD/	Name of the sample
2:1	Comp 2/1
1:1	Comp 1/1
1:1.5	Comp 1/1.5
1:2	Comp 1/2

Comp 1/1 was also carried out as a physical mixture. At this case API and the oligosaccharide were grounded simultaneously in a mortar for 2 min.

Preparation of the aqueous suspensions

Comp 1/1 and Comp 1/2 were also evaporated from aqueous suspension. First, the SPIR then the HPCD was added to water. In the case of Comp 1/1 the evaporation started immediately while the Comp 1/2 sample was stirred for 15 min before the distillation.

Twin-screw extrusion method

The laboratory-scale experiments were carried out by a HAAKE MiniLab twin-screw extruder. The apparatus possesses co-rotating screws with conical shape. The blend of the powders containing 5% (w/w) SPIR, 25.2% HPCD (w/w), 5% (w/w) sorbitol, 64.8% (w/w) maize starch was prepared in a mortar. Maximum 1.5 g water was then added to the 7 g of sample. Subsequently the blend was fed into the hopper of the twin-screw extruder. The screw rate was 5, 20 and 50 rpm, respectively. According to the plasticizing properties of the maize starch, the temperature was set at 90 °C which is significantly lower than the melting temperature of the SPIR.

Differential scanning calorimetry

The thermal properties of SPIR and the influence of the interaction with HPCD on them were investigated using a Setaram DSC 92 apparatus. The evaluation of samples of ~10 mg was performed under nitrogen atmosphere in the temperature range of ambient temperature and 300 °C with heating rate of 10 °C/min.

Raman spectrometry

Raman-spectrometric analyses were carried out by a LabRam type confocal Raman microscope (Jobin–Yvon). 10, 50 and 100 fold magnification was applied and the excitation source was frequency doubled Nd-YAG laser emitting at 532 nm.

X-ray powder diffraction studies

The change of the crystalline structure of the drug was characterised also by PANanalytical X'pert Pro MPD type XRPD device equipped with X'celeratos detector.

Results and discussion

Spironolactone (SPIR), due to its sterane core, is a quite flexible molecule, which affects its polymorphism as published recently [12]. Nicolaï et al. [22] has found that different batches of SPIR included the mixture of the two orthorhombic phase of the active ingredient in different ratios. Before investigating its interaction with other components the stability of the drug itself was analysed.

Analysis of the drug

Although this aldosterone antagonist is considered as a quite stable material slight changes were detected in our laboratory during its storage. The question to be answered was the following: whether these changes are polymorphic or other origin. The melting point of the drug after production was determined at 486.1 K, furthermore, its decomposition did not start immediately after the melting process. In contrast, the SPIR stored at ambient temperature for a few months (without any change in its chemical composition) melted at significantly lower temperature moreover the decomposition occurred instantly after the melting finished (Fig. 2).

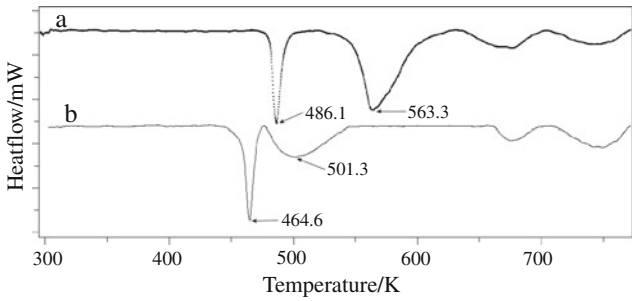

Fig. 2 The polymorphous instability of SPIR: DSC thermogram of the original (*a*) and stored drugs (*b*)

Berbenni et al. [12] crystallised the SPIR from different solvents and the samples were characterised by DSC. According to their results it could be supposed that our stored substance is somewhat similar to their crystal yield from acetone.

In order to make the picture more clear further methods such as X-ray powder diffraction (XRPD) and Raman spectrometry were involved in the analysis.

In contrast to the DSC results, neither the XRPD nor the Raman-microspectral investigation could detect real transformation as shown in Fig. 3. Slight difference of intensity can be observed at 15 and 20 2θ degrees of the diffractograms and negligible changes of a few minor Raman bands of the drug indicate only some alteration of the crystalline structure. Crystal-defects could probably form during the long storage of SPIR assisted by absorption of softening molecules from the atmosphere. As a consequence, alteration of the molecular mobility and the secondary bonds could occur resulting in the reduced melting temperature.

In order to clarify whether the observed slight morphological change influences the subsequent experiments or it can be neglected DSC cell was used for performing controlled recrystallization and melting experiments on amorphized SPIR. At first, the substances were dissolved entirely in sufficient amount of acetone/water mixture then the solvent was vacuum evaporated as fast as possible. The plots of recrystallization and melting of drug samples are shown in Fig. 4.

The exothermic peaks at 435.4 and 439.2 K indicate the solid phase crystallization of the amorphous sample, which is followed by the melting endothermic peaks (Fig. 4). According to the DSC results, the preparation method removed the morphological differences and resulted in very similar crystallites in cases of both original and stored drugs. Similarly, in the subsequent experiments, when the drug was dissolved in a solvent the results proved to be just about the same for both original and stored SPIR (Table 2).

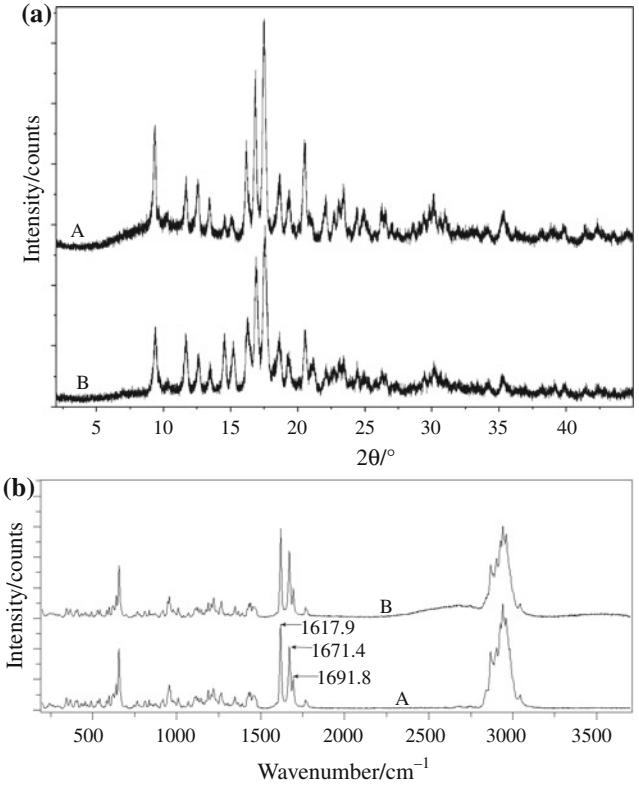

Fig. 3 X-ray powder diffractogram (**a**) and the Raman-spectra (**b**) of the original (*A*) and the stored drugs (*B*), respectively

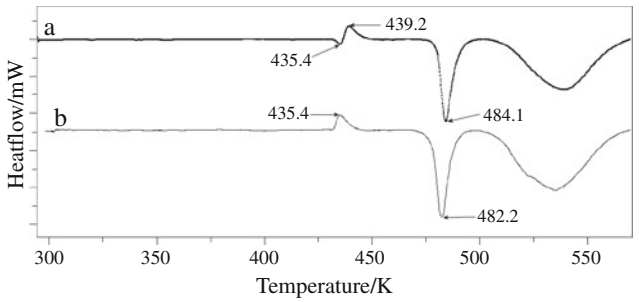

Fig. 4 DSC results of SPIR amorphized form of the original (*a*) and stored drug (*b*)

Analysis of drug–cyclodextrin systems

Drug–cyclodextrin systems were prepared at first in conventional aqueous-suspension process as described in the 'Experimental' section. Table 1 summarises the molar ratio of the solvent co-evaporated binary adducts. The DSC curves of the prepared complexes are shown in Fig. 5.

According to the Fig. 5, the increasing amount of cyclodextrin reduced the ratio of the crystalline phase of the SPIR, while from the ratio of Comp 1/1.5 the crystallinity is completely missing.

Table 2 DSC data of the original and the stored drug

Sample	T_{onset}/K	T_{max}/K	Melting enthalpy/J g^{-1}
Original drug	480.9	486.1	38.2
Stored drug	458.8	464.6	31.4
Amorphized original drug	480.1	484.1	41.5
Amorphized stored drug	477.9	482.2	43.2

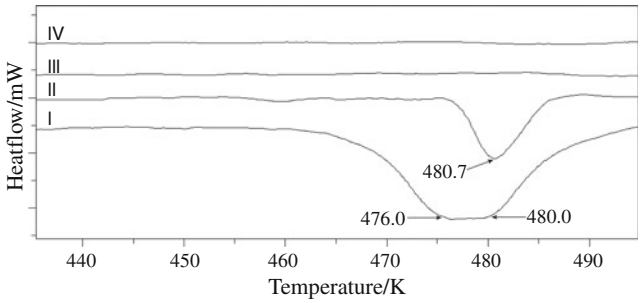

Fig. 5 The DSC thermograms of the solvent co-evaporated adducts: Comp 2/1 (*I*), Comp 1/1 (*II*), Comp 1/1.5 (*III*), Comp1/2 (*IV*)

Significant modification could be observed by the studies of Raman spectrometry, the results of which are shown in Fig. 6.

The molecules of the SPIR are entirely separated from each other, due to the host/guest interaction, when complex is formed. According to the Raman signals they cannot assemble any crystalline forms. The stable structure of crystalline drug indicates sharp, well-defined vibrational range (caused by the short distance between the molecules). When the interaction is increased the sign of the ketone group (1670 cm^{-1}) of the "A" ring is widened and shifted towards lower wave-numbers. The weak sign at 1689 cm^{-1} (thyoacetyl side group) is broadened too and merge with the range of the mentioned ketone group.

The results of calorimetric analysis of different types of SPIR/HPCD systems are compared in Fig. 7.

Figure 7a shows the DSC curve of a simple mixture of SPIR and HPCD, prepared in a mortar, where the heat

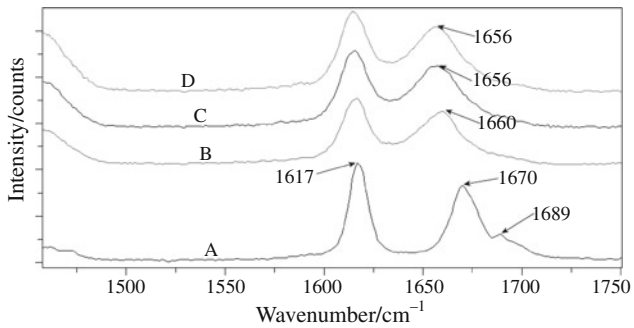

Fig. 6 The Raman spectra of SPIR (*a*), Comp 1/1 (*b*), Comp 1/1.5 (*c*), Comp 1/2 (*d*)

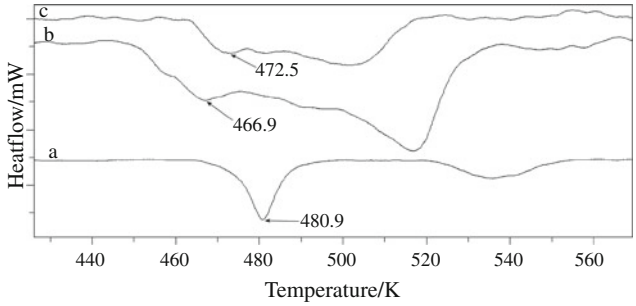

Fig. 7 DSC analysis of the solubilisation effect of the HPCD: the physical mixture of Comp 1/1 (*a*), evaporated aqueous suspension of Comp 1/1 (*b*) and Comp 1/2 (*c*)

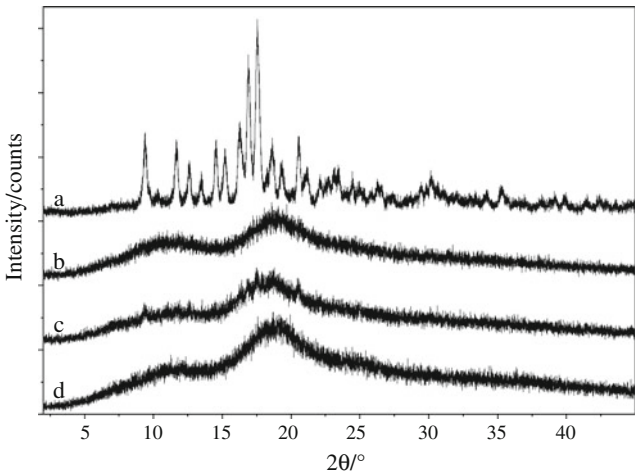

Fig. 8 XRPD results of the SPIR (*a*), HPCD (*b*), the evaporated aqueous suspension of Comp 1/1 (*c*) and Comp 1/2 (*d*), respectively

originating from the mixing energy is not enough for creating interaction between the HPCD molecules and the active ingredient in solid state. However, the oligosaccharide dissolved in the water is able to start the solubilisation (Fig. 7b, c). In the case of Comp 1/1, the melting peak and the decomposition signal can be distinguished from each other (Fig. 7b), while the sample containing increased amount of HPCD (Comp 1/2 in Fig. 7c) shows extended endothermic range, which refers to the developed host–guest interaction.

According to the XRPD studies, shown in Fig. 8, the adduct of 1:1 M ratio of the components contains detectable quantity of crystalline drug that is reduced below the detection limit when the concentration of the cyclodextrin is increasing.

Analysis of melt blended drug

Based on the discussed results, it can be concluded that intensive dispersion and appropriate ratio of the SPIR and HPCD leads to amorphous complex. Melt extrusion was

expected as an efficient solvent-free method for achieving fine distribution of both the active ingredient and the cyclodextrin in a selected polymer matrix. The shear stress, caused by the screw rotation, and the elevated temperature resulted in homogenous drug dispersion even below the melting point of the SPIR. (It is supposed that at even lower extrusion temperature the maize starch, plasticized by water and sorbitol, could not swell enough.)

According to the Raman and DSC results, shown in Fig. 9, the drug particles become, as a result of the extrusion process, so finely dispersed in the hydrophilic matrix that the inclusion complex with HPCD molecules could perfectly form.

Shear force, set by the applied screw speed (rpm), was supposed to alter the dispersion grade and, consequently, the interaction between SPIR and HPCD. This phenomena was investigated by DSC and Raman spectrometry.

It is clearly perceptible that the screw rate has significant influence on the incorporation of drug into HPCD. In the case of the lowest rotation speed (5 rpm), the shear force was insufficient to separate the SPIR particles thus large aggregates remained that impeded the interaction with the HPCD molecules. The stronger the shear force the finer the distribution, however, increasing the screw speed for achieving higher shear rate is not always advantageous because the residence time influences the incorporation too. At 50 rpm, the conveying of the mass was found too fast therefore the cyclodextrin molecules had not enough time for building up the complete interaction with the active ingredient. Based on the results shown in Fig. 10, screw speed of 20 rpm was found to be optimal screw rate generating efficient shear force and long enough residence time for achieving fine dispersion of the SPIR and formation of the complete inclusion complex.

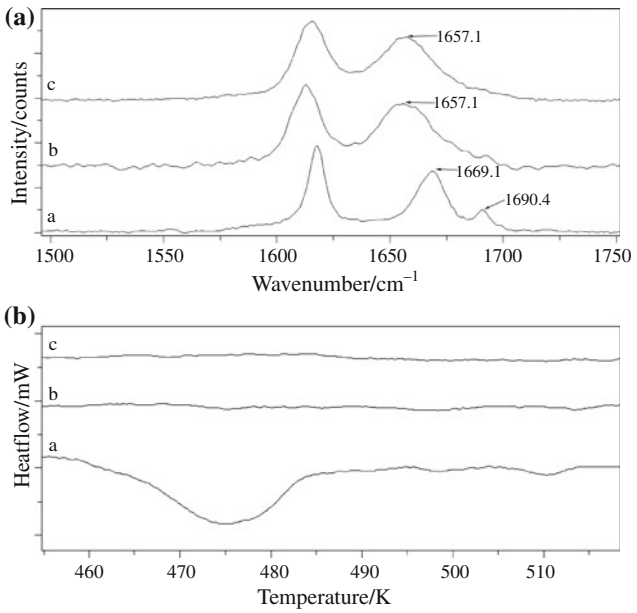

Fig. 9 Raman (**A**) and DSC (**B**) investigation of the interaction between SPIR and HPCD in the physical blend with the components of the extrudate (*a*), in the melt blended extrudate (*b*) and in the solvent co-evaporated Comp 1/1.5 sample (*c*)

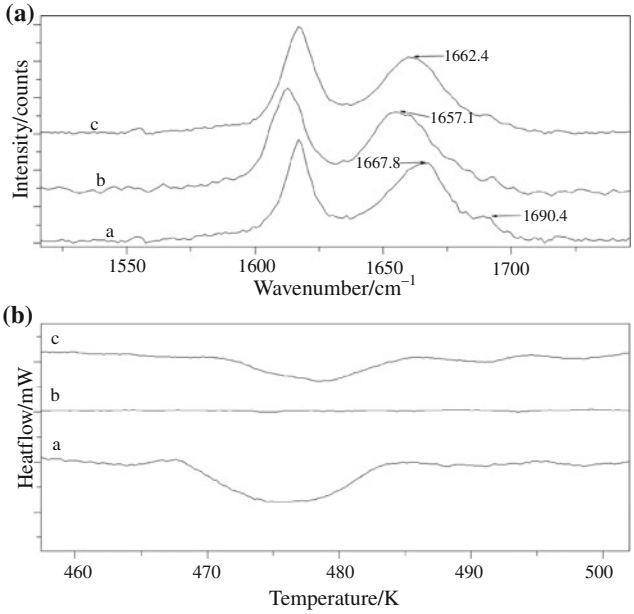

Fig. 10 The Raman (**A**) and the DSC (**B**) study of the influence of the screw rate on the interaction: 5 rpm (*a*), 20 rpm (*b*), 50 rpm (*c*)

Conclusions

The onward process of the synthesis of stable amorphous drugs is sometimes quite difficult, however, there are capable ways of pharmaceutical technology for decomposing the crystalline structure and restrain the recrystallisation permanently. Such methods were studied using SPIR as poorly dissoluble model drug. Difference of the melting points between the original and the stored drug was estimated by DSC, which, however, proved to be not associated to chemical modification or change of the crystalline structure of SPIR according to spectroscopic and XRPD results. Instead, it is suggested to be ascribed to increased molecular mobility and reduced number of secondary bonds as a consequence of the adsorbed plasticising molecules from the atmosphere. The subsequent amorphization and recrystallization process removed the starting differences and produced the same melting characteristics in cases of both drug substances.

Addition of HPCD in increasing concentration reduces the ratio of the crystalline phase of the SPIR furthermore at a critical molar ratio the melting peak of the active ingredient disappears from the DSC thermogram indicating complete interaction of the drug molecules with the oligosaccharide.

Preparation of the complex required molecular dispersion of both materials, while in the cases of physical mixture and aqueous suspension endothermic peak, corresponding to the melting point of the drug, could be detected.

Solvent-free melt extrusion was applied in order to obtain the requested fine distribution of SPIR and HPCD at a temperature far below the melting temperature of drug substance. The DSC, Raman microspectrometry and XRPD results confirmed the complete formation of the inclusion/association complex. The screw rate, rising the shear force and consequently the dispersion ratio, influences the interaction, which is controlled also by the residence time as the formation of the complex requires long enough mixing process.

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