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Comparison of high sensitivity micro differential scanning calorimetry with X-ray powder diffractometry and FTIR spectroscopy for the characterization of pharmaceutically relevant non-crystalline materials

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In this study, high sensitivity micro differential scanning calorimetry (MDSC) in the scanning of dynamic mode was compared to X-ray powder diffractometry (XRPD) for quantifying amorphous nifedipine in mixtures crystalline nifedipine. This technique was also compared with FTIR for quantifying polymorph A of chloramphenicol palmitate (CAP) and poly DL-lactide-co-glycolide) (PLGA) in pharmaceutical formulations. The limit of determination (LOD) achieved by MDSC were 0.06% compared to 5% for XRPD quantification of amorphous nifedipine and 0.02% compared to 7% for IR quanitfication of polymorph A of CAP. As little as 0.165 mg PLGA could be measured in excipients mixtures. Desirable linearity and repeatability were established in all cases.

1. Introduction

Polymorphism can influence every aspect of the solid-state properties of a drug. Different crystalline forms can exhibit different dissolutions kinetics, which, play a major, if not over-riding, role in determining the bioavailability of the drug substance (Bernstein 2002; Hancock and Parks 2000). The existence of drugs and excipients in multiple physical forms (e.g., non-crystalline, polymorphs, isomers) provides pharmaceutical scientists with an opportunity to select the preferred form(s) of the material used in a formulation. However, quantifying these compounds is very important to ensure the quality of dosage forms prepared with these crystal forms.

Many analytical methods have been developed to characterize polymorphs (Byrn et al. 1999). X-ray powder diffraction (XRPD) is perhaps the most extensively used for the characterization and quantification of crystal forms (Byrn et al. 1999; Giron and Piechon 1990; Tanninen and Yliruusi 1992; Keller et al. 2003; Bergese et al. 2003). In major pharmacopeias, Fourier transfer infrared spectroscopy (FTIR) is also used to quantify the crystal forms of chloramphenicol palmitate in dosage forms (USP 2000; BP 1993). This technique has also been used for the characterization of terfenadine crystallized from solvents (Leitao et al. 2003). Thermal analysis and in particular differential scanning calorimetry is often used for quantifying crystal forms (Bryn 1999; Clas et al. 1999).

One thermal analysis technique, microcalorimetry, both in the isothermal and scanning (dynamic) modes, has been used to determine the amorphous content of drug powders and in particular, the polymorphic transformation of glassy materials under varied conditions of temperature and humidity (Brigger et al. 1994; Ahmed and Rawlins 1996; Yonemochi et al. 1997; Buckton and Darcy 1999; Kawakami et al. 2002; Keymolen et al. 2003). Royall et al. (2001) reported the strengths and limitations of micro-thermal analysis as a means for identifying different physical forms of the same substance within a single sample. They found that using different scanning rates (2–20 °C/s) did not demonstrate significant changes to the temperatures of transition and crater size for the drug indomethacin. Studies on partically crystallized samples clearly showed that the technique was able to differentiate between the amorphous and crystalline forms of this drug. The technique may therefore have considerable potential as a means of identifying distinct thermal events in a single sample of a mixtures.

However, few reports are available that comprehensively describe the use of micro differential scanning calorimetry (MDSC) for the quantification of not only the amorphous content, but for determining amorphous and crystalline content in mixtures of crystal forms of low levels of non-crystalline excipients in pharmaceutical formulations. This study reports the comparative results for the quantification of amorphous nifedipine in mixtures with crystalline nifedipine and ternary mixtures with commonly used pharmaceutical excipients using dynamic MDSC and XRPD. In addition, the quantification of chloramphenicol (CAP) polymorphs FTIR and dynamic MDSC and measuring low levels of a non-crystalline excipient poly (DL-lactide-co-glycolide) by dynamic MDSC are also shown.

2. Investigations, results and discussion

2.1. Characterization of the crystal forms

XRPD results, Figs. 1 and 2 showed that amorphous nifedipine and the crystal forms of CAP was successfully prepared. Amorphous nifedipine transformed into crystalline

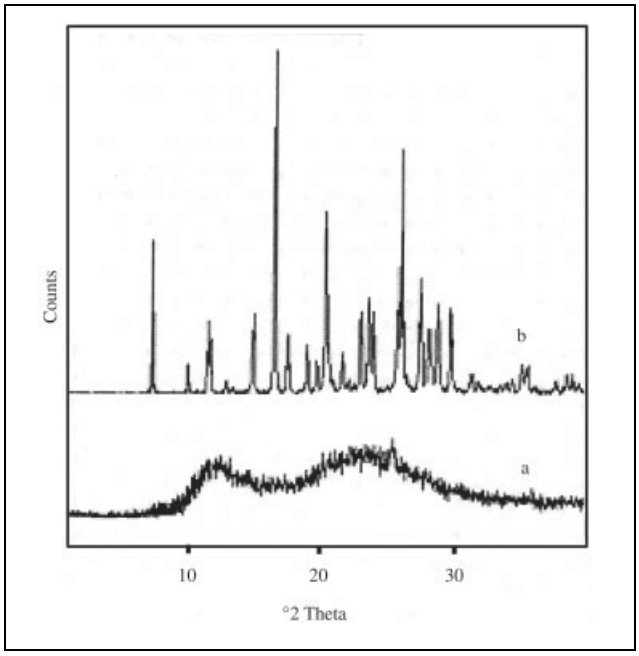

Fig. 1: XRPD patterns of (a) freshly prepared amorphous nifedipine, (b) crystalline nifedipine (raw material)

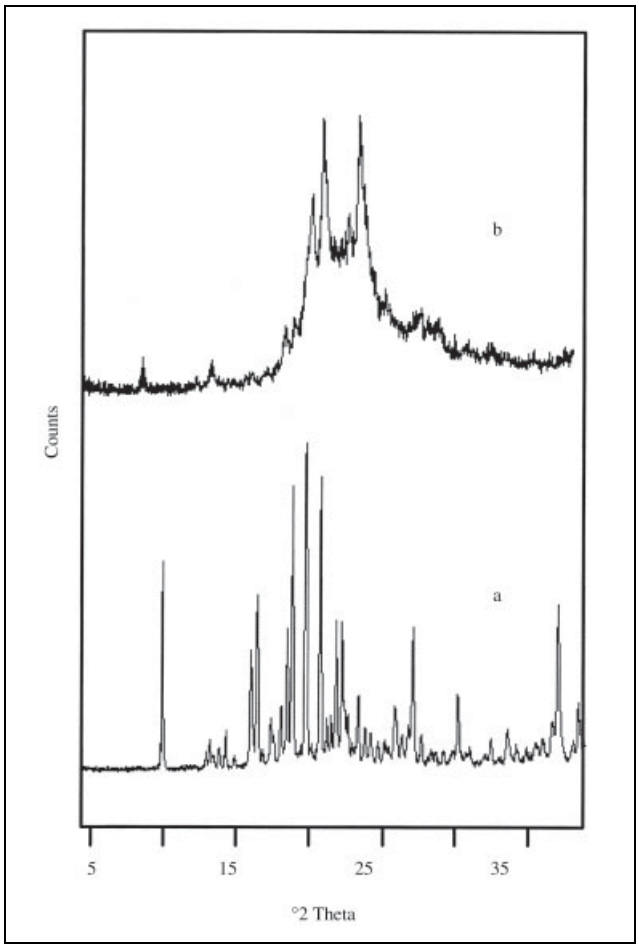

Fig. 2: XRPD patterns of CAP (a) polymorph A, (b) polymorph B

nifedipine after being heat to 119 °C as shown by the results of DSC analysis shown in Fig. 3. These results corresponded with the characteristic values reported earlier for the crystal forms of these drugs (Song and De Villiers 2004; Caira et al. 2003).

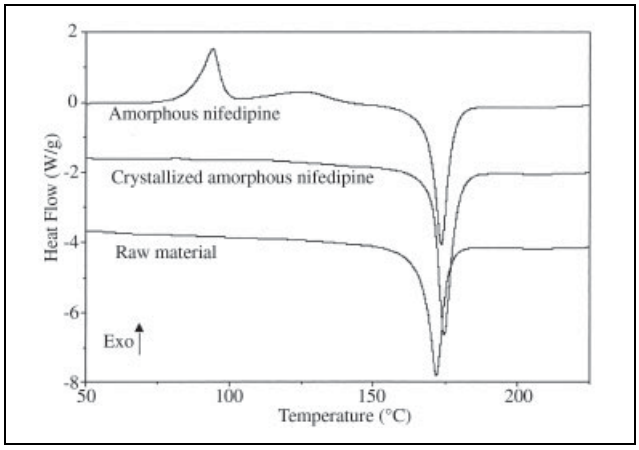

Fig. 3: DSC thermograms of nifedipine raw material, amorphous nifedipine and recrystallized amorphous nifedipine

2.2. MDSC versus XRPD for quantifying amorphous nifedipine

In Fig. 4 a typical dynamic MDSC trace of amorphous nifedipine showing the glass transition (T_g) at 45–50 °C and the recrystallization peak at 89 °C is given. Due to considerable variation in the peak temperature of the T_g it was decided to use the recrystallization peak to quantify amorphous nifedipine content. In addition, it was found that the sample preparation method influence the determination of the height of the crystallization peak by MDSC. The height of the crystallization peak for the same amount and composition of amorphous and crystalline nifedipine sample followed the order, shown in Table 1. When the crystalline form was placed on top of the amorphous form without mixing the peak height was the greatest with the peak only slightly smaller when the amorphous form was placed on top of the crystalline form wihout mixing. When the amorphous and crystalline forms were mixed by shaking by hand or mechanically (Turbula mixer at 60 rpm for 5 min) the peak heights were smaller but the peak height for the crystallization of the amorphous nifedipine in mixtures prepared by mechanical mixing showed the least variation ($p < 0.05$). This showed that when determining the amount of amorphous nifedipine in mixtures also containing the crystalline forms the results will depend on the distribution of the amorphous form in the mixture. Results did not depend on the heat-

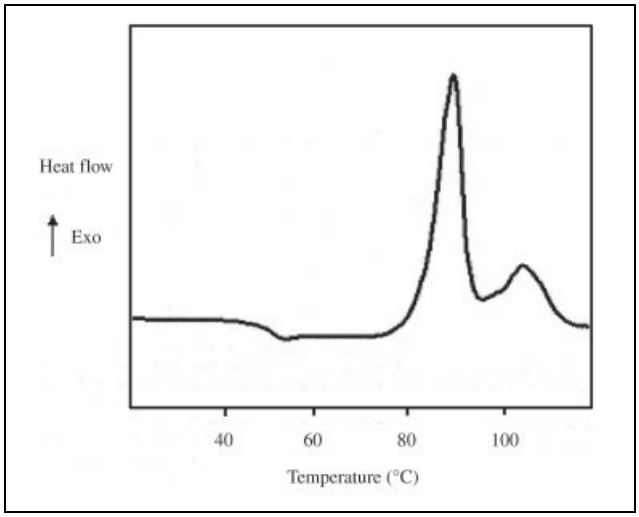

Fig. 4: A typical dynamic MDSC trace of amorphous nifedipine

Table 1: Effect of sample preparation method on the height of crystallization peak of amorphours nifedipine (n = 3)

Sample preparation method	Peak height (mW)
Crystalline form on top amorphous form without mixing	0.808 ± 0.103
Amorphous form on top of crystalline form without mixing	0.708 ± 0.077
Amorphous form between two layers of crystalline form	0.508 ± 0.049
Amorphous and crystalline forms mixed by shaking by hand	0.312 ± 0.117
Amorphous and crystalline forms mixed in Turbula® mixer	0.324 ± 0.036

ing rate (0.3–1 K/min) employed when recording the MDSC traces.

The limits of detection and quantification for the detection of amorphous nifedipine using the heat of melting measured by dynamic MDSC were found to be 0.063 and 0.180 mg in the absence of crystalline nifedipine (Table 2). This showed that this technique was more sensitive that XRPD because as shown in Fig. 5 crystalline nifedipine was only detected at a level of about 5% when present in mixtures with amorphous nifedipine. In contrast it was not possible to detect small amounts, 1–10%, of amorphous nifedipine mixed with crystalline by XRPD. Because of overlapping peaks it is not always possible to use the heat of melting. To avoid this problem the peak height (mW) measured by MDSC was also used. There was a linear relationship between the amount of amorphous nifedipine and peak height measured by MDSC. Linearity, $y = 0.9397x + 0.625$, $R^2 = 0.995$, in the presence of crystalline form, and $y = 2.686x - 2.7775$, $R^2 = 0.997$, without the crystalline form, where y is the peak height in mW and x ist the concentration (%) of amorphous nifedipine in the sample. Although not as sensitive and the heat of melting, using this method it was still possible to between 0.15 and 0.35 mg or amorphous nifedipine in the presence of 100 mg crystalline nifedipine, depending on the method used to prepare the mixtures, the lowest amount being detected in mechanically mixed mixtures.

The MDSC measured limits of detection and quanitfication were lower than 0.5% when the sample size was 100 mg. This was 10 times smaller than the LOD determined by XRPD. In ternary mixtures containing amorphous and crystalline nifedipine combined with either anhydrous dibasic calcium phosphate or microcrystalline cellulose, the height of the crystallization peak was not significantly different from that measured without the excipients. This held truth for all excipients as long as thermal events associated with the excipients did not interfere with the crystallization peak of the amorphous nifedipine. This demonstrated that amorphous nifedipine could be quantified accuratly by MDSC without being influenced by excipients.

Table 2: Relationship between the heat of melting/crystallization and limit of detection and quantification

Material	Heat of Melting/crystallization (J/mg)	LOD (mg)	LOQ (mg)
Nifedipine	0.0376	0.063	0.180
CAP A	0.236	0.020	0.055
PLGA	0.0058	0.165	0.340

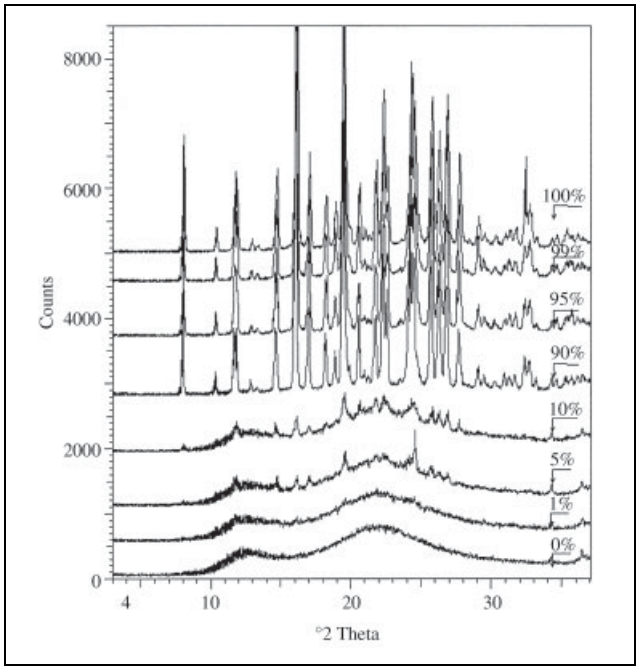

Fig. 5: XRPD patterns of mixtures containing from 0–100% crystalline nifedipine mixed with amorphous nifedipine

2.3. MDSC versus FTIR for quantifying CAP polymorph A

Chloramphenicol palmitate was used as a model drug because it is perhaps the classic example of the dependence

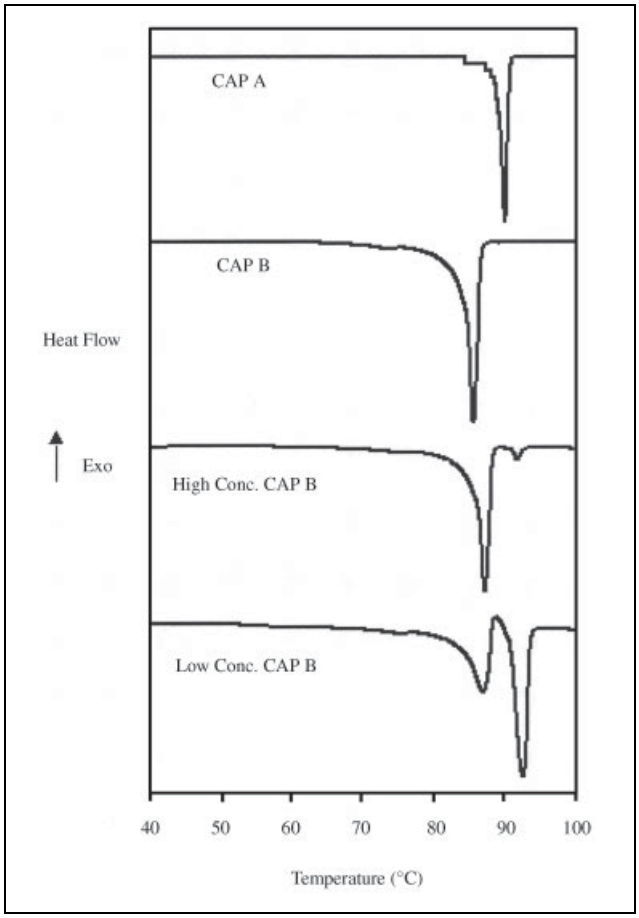

Fig. 6: Dynamic MDSC traces of CAP A, CAP B, and mixture of CAP A and B (ramped at 0.3 K/min)

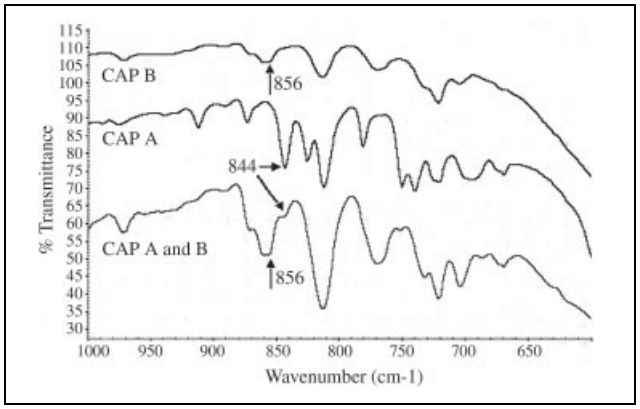

Fig. 7: FTIR spectra of CAP A and B and a mixture of the two forms

of bioavailability of on polymorphic form and also it represents a drug for which there is pharmacopeial specification with regard to the amount of the inactive crystal form present in pharmaceutical dosage forms. There are three polymorphic forms (A, B and C) in addition to an amorphous form. Polymorph A is the most stable, but only polymorph B and the amorphous forms are biologically active. The USP requires that the amount of polymorph A should not be more than 10% in a suspension (USP 2000). In Table 2 the LOD and LOQ for the measurement of CAP polymorph A by dynamic MDSC is listed.

However, in this study the height of the melting peak of polymorph A obtained by dynamic MDSC was also used because at high concentration of polymorph A, its melting peaks (93 °C) was not well separated from that of polymorph B (88 °C) (Fig. 6). There was a linear relationship between the amount of polymorph A and peak height measured by MDSC ($y = 0.7109x + 1.6537$, $R^2 = 0.993$). When using FTIR to quantify the amount of polymorph A, Fig. 7, the characteristic peaks at 844 cm^{-1} for polymorph A, and 856 cm^{-1} for polymorph B were used (USP 2000; BP 1993). The limit of detection limit for polymorph B using FTIR analysis was 7%. When MDSC was used to quantify polymorph A in the presence of polymorph B or polymorph B mixed with anhydrous dibasic calcium phosphate or microcrystalline cellulose, the LOD was 20 μg (0.02% for 100 mg sample) and the LOQ was 55 μg (0.04% for 100 mg sample). These results are important because it was impossible to determine the content of polymorph A in the excipient mixtures using IR due to the spectroscopic properties on the excipients.

2.4. MDSC for quanitfying PLGA in excipient mixtures

PLGA is an amorphous polymeric excipient and it is impossible to quantify it in pharmaceutical mixtures using XRPD. In addition, due to ist complicated FTIR pattern is also not feasible to use this technique to quantify this excipient. A typical dynamic MDSC trace of the melting of PLGA at 45 °C is shown in Fig. 8. There was a linear relationship between the weight of PLGA and the heat of melting ($y = 0.0058x + 0.0007$, $R^2 = 0.9964$, where y is the heat of melting and x is the weight of PLGA). The LOD and LOQ for the detection of PLGA in excipient mixtures were found to be 0.165 and 0.340 mg, respectively.

2.5. Conclusions

The results of this study showed that dynamic MDSC is a useful technique for the quantification of small amounts of

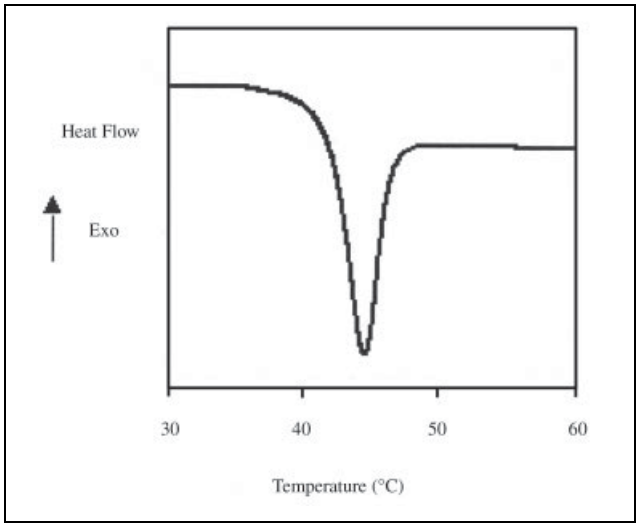

Fig. 8: A typical dynamic MDSC trace of PLGA at a heating rate of 1 K/min showing its melting peak

pharmaceutical relevant materials in pharmaceutical formulations. Compared to XRPD and IR, dynamic MDSC proved more sensitive and convenient in the characterization of these materials. For the three materials used in this study the measured LOD and LOQ showed that the higher the heat of melting, the smaller the LOD and LOQ (Table 2). Thus, if the thermal behavior of drugs or excipients is known, MDSC can be used for the quantification of amorphous drugs or excipients in pharmaceutical mixtures or products, which is not feasible using XRPD or IR. Although the limitation of temperature range is a disadvantage of dynamic MDSC, as this technique develops, equipment with larger temperature ranges should satisfy the purpose of most studies.

3. Experimental

3.1. Materials

Crystalline nifedipine was purchased from Spectrum Chemical Company (Lot No. RF 0565, New Brunswick, NJ,) was used as such. Amorphous nifedipine was prepared by melting nifedipine raw material on a piece of aluminium foil at 180 °C, keeping isothermal for 5 min and then cooling at room temperature. The glassy material was pulverized gently with a mortar and pestle and screened with a standard test sieve with openings of 180 μm. The portion with particle size below 180 μm was used. Chloramphenicol palmitate (CAP) powder (Lot No. PR0617, Spectrum Chemical Corp., NJ, USA) was used to prepare CAP polymorphs. Polymorph A was prepared by dissolving 5 g of CAP powder in 10 ml chloroform by heating. The solution was set aside for undisturbed crystallization and the precipitated solids collected by filtration after six hours then dried in a vacuum oven. Polymorph B was prepared by dissolving 5 g of CAP powder in 50 ml anhydrous ethanol by heating. The solution was cooled to room temperature and then poured into 1000 ml water under vigorous stirring. The mix was allowed to stand overnight. The precipitated solids were collected by filtration and then dried in a vacuum oven. Poly (DL-lactide-co-glycolide) (PLGA, Resomer® RG 503, Lot No. 271442, Boehringer Ingelheim, Pittsberg, VA, USA), anhydrous dibasic calcium phosphate (Emcompress®, USP grade, Lot No. 2041X, Mendell, NY, USA), and microcrystalline cellulose (Avicel® PH-103, NF grade, Lot No. 3010, FMC Corp., DE, USA) were used as received.

3.2. Characterization of crystal forms

The successful preparation of amorphous nifedipine was established using a differential scanning calorimeter (DSC 2920, TA Instrument, Inc., DE, USA) at a heating rate of 20 K/min and X-ray powder diffraction analysis (XRPD). XRPD measurement were obtained with a Bruker D8 Advance diffractometer (Bruker, Germany). The measurement conditions were: target, Cu; voltage, 40 kV; current, 30 mA; divergence slit, 2 mm; anti-scatter slit, 0.6 mm; receiving slit, 0.2 mm; monochromator; detector slit, 0.1 mm; scanning speed, 2 °C/min (step size 0.025 °C, step time, 1.0 s).

Approximately 200 mg samples were weighed into aluminium sample holders, taking care not to introduce a preferential orientation of crystals.

The limit of detection of amorphous nifedipine presented in crystalline nifedipine samples was determined from the XRPD of known mixtures.

3.3. Micro differential scanning calorimetry (MDSC)

The amount of amorphous nifedipine presented in crystalline nifedipine samples was determined by dynamic high sensitivity microcalorimetry (MicroDSC III, Setaram, France) at 0.5 K/min from −20 to 120 °C using the standard batch vessel. The detection limit on the micro DSC III is much better (by a factor of ten) than that of conventional DSC (powder compensated, thermocouple-detector) at scanning rates al low as 0.001 K/min. This means that variations in the calorimetric signal below one microwatt can be detected. Before each analysis, dry nitrogen was purged into the sample vessel for 20 min to remove moisture. The height of the crystallization peak of amorphous nifedipine during the heating process was used as the signal for quantification. The limit of detection (LOD) and quantification (LOQ) of pure amorphous nifedipine and amorphous nifedipine in crystalline nifedipine sample and ternary mixtures was determined by finding the amount of amorphous nifedipine that had a crystallization peak with a height 3 and 12 times that of the baseline fluctuation, respectively (Swartz and Krull 1997).

The effect of sample size on the height of the crystallization peak of amorphous nifedipine was determined by measuring the peak height of the same amount of amorphous nifedipine combined wiht increasing amounts of the crystalline form (50, 100, and 150 mg). The effect of the sample preparation method on peak height was studied by mixing amorphous nifedipine and crystalline nifedipine in a Turbula® mixer at 27 rpm for 10 min, mixing in the batch vessel by shaking with hand, loading amorphous nifedipine on top of crystalline nifedipine without mixing and *vice versa*, and loading amorphous dibasic nifedipine between two layers of crystalline nifedipine without mixing.

For quantifying amorphous nifedipine in unknown mixtures, a standard curve of the concentration of amorphous nifedipine in 100 mg samples versus the height of crystallization peak was prepared. Feasibility of quantifying amorphous nifedipine in quatenary mixtures (amorphous and crystalline, anhydrous calcium phosphate, and microcrystalline cellulose) was also evaluated.

The concentration of polymorph A in CAP powder mixtures was determined by heating the sample in a batch vessel at 0.3 K/min from 20 to 110 °C. The limit of detection and quantification was determined using the same method as described for nifedipine. A standard curve of the concentration of polymorph A (0–20%) versus the height of the melting peak of polymorph A was also prepared. The concentration of polymorph A in CAP powder was also determined using FTIR spectroscopy (Impact 400D FT-IR, Thermo Nicolet Corporation, USA) as described in the USP (2000). The minimum concentration of polymorph A that can be detected in a mixture of polymorphs A and B was taken as the limit of detection.

PLGA was used as a model polymer to be quantified in a mixture of excipients including anhydrous dibasic calcium phosphate and microcrystalline cellulose. Pure PLGA or mixtures of PLGA, anhydrous dibasic calcium phosphate and microcrystalline cellulose were heated from 15 to 80 °C at 1.0 K/min under the flow of dry nitrogen. The area under the melting peak was used for quantifying PLGA. LOD and LOQ werde determined using the same method as described for nifedipine.

3.4. Statistical analysis

All statistical evaluations were performed using SAS 6.12 (SAS Institute Inc., Cary NC, USA).

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