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Increased dissolution and physical stability of micronized nifedipine particles encapsulated with a biocompatible polymer and surfactants in a wet ball milling process

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Suspensions of nifedipine, a practically water-insoluble drug, were prepared in the presence of a biocompatible polymer, polyvinylpyrrolidone (PVP, K value 17), and three surfactants, sodium lauryl sulfate (SLS, anionic), cetyltrimethylammonium bromide (CETAB, cationic), polysorbate 80 (Tween 80, nonionic), by wet milling in ceramic ball mills. Nifedipine powders encapsulated with PVP and the surfactants were recovered from the suspensions after milling and evaluated for changes in particle size, morphology, sedimentation rate in aqueous suspensions, crystal form, and dissolution. Particle size analysis indicated that milling of suspensions in solutions of PVP and surfactants is an efficient method for reducing the particle size of nifedipine to below 10 μm. Furthermore, DSC and XPS analysis indicated that during milling the nifedipine crystals were coated with the PVP or surfactants and that milling with PVP stabilized the nifedipine crystal form during milling while nifedipine was gradually amorphisized when milled in a quaternary nifedipine/PVP/SLS/CETAB system. The decrease in particle size caused a significant decrease in sedimentation rate and increased the dissolution rate of nifedipine in simulated gastric fluid when compared to milled nifedipine and powder mixtures of the drug and the excipients.

1. Introduction

Few materials used in the manufacturing of pharmaceuticals exist in the optimum size, and most materials must be reduced in size at some stages during the production of a dosage form. Milling is the mechanical process for reducing the particle size of solids. Fine milling, particles smaller than 50 μm, is often achieved by ball milling (Parrott 1976; Brain 1994). A ball mill consists of a horizontally rotating hollow vessel of cylindrical shape with the length slightly longer than the diameter. The mill is partially filled with balls of pebbles, which act as the grinding medium. At the optimum speed, the balls roll and cascade over one another to provide an attrition action while the balls are also carried up the side of the mill and fall freely onto the material with an impact action. Milling is therefore a combination of impact and attrition depending on the speed of the mill.

The critical speed of a ball mill is the speed at which the balls just begin to centrifuge with the mill because at or above this speed no significant size reduction occurs. Normally ball mills are operated at 60–85% of the critical speed and in general lower speeds are for finer grinding. An empirical rule for the optimum speed of a ball mill is given by $n = 57 - 40 \log D$, where n is the speed in revolutions per minute and D is the inside diameter of the mill. For a given speed, smaller balls will give a slower but finer grinding (Voller 1983).

Ball mills are operated either wet or dry. Normally with wet milling particles smaller than 50 μm can be produced from slurries containing 30–60% solids (Staniforth 2002). From the viewpoint of power consumption, wet grinding is more efficient than dry grinding. To produce even finer particles small amounts of grinding aids can be added to the mill (Apte et al. 2003). However, the use of grinding aids for pharmaceutical products is limited by the physiological and toxicological restrictions on medicinal products. For example, some dispersing agents have been added to wet milling where the addition of as little as 0.1% of a surface active agent may increase the production rate of a mall mill 20 to 40% (Parrott 1976). Grinding aids that have been used for grinding drugs include sodium chloride for dexamethasone, amorphous magnesium aluminosilicate for ketoprofen, indomethacin, naproxen and progesterone, sodium lauryl sulfate for ursodeoxycholic acid, polyvinyl pyrrolidone for sulfathiazole, chitin and chitosan for griseofulvin, phenobarbital, prednisolone, flufenamic acid, and indomethacin, and amorphous silicon dioxides for prednisone, digoxin and griseofulvin (Apte et al. 2003; Gupta et al. 2003; Chung et al.

2002; Boldyrev at al. 1994; Yang et al. 1979). However, although all these adjustments can increase the efficiency of wet milling, flocculation restricts the lower limit to approximately 10 μm. Another drawback of milling pharmaceuticals is the change in crystal form upon milling (Sato et al. 1997; Itoh et al. 2003; Gupta et al. 2003).

In one study by Sato et al. (1997) the pharmaceutical properties of ground mixtures of nifedipine with casein, magnesium silicate, and cellulose acetate phthalate in a vibrational ball mill was reported. The X-ray powder diffraction patterns and DSC data suggested that nifedipine was present in its amorphous form in these ground mixtures. Grinding increased the wettability, the solubility in water and bioavailability after oral administration of nifedipine. However, the authors did not look at the stability of the amorphous drug particles. This study added to results reported earlier that showed roll mixing of nifedipine with PVP produced amorphous drug with increased dissolution (Nozawa et al. 1986). The biggest drawback of this approach is that amorphous nifedipine is not stable (Caira et al. 2003).

In another study, Itoh et al. (2003) reported that when the poorly water-soluble drug nifedipine was ground in a dry process with polyvinylpyrrolidone (PVP) and sodium dodecyl sulfate (SDS) different crystallinity, predominantly amorphous, behavior was shown in the ternary drug/PVP/SDS system. However, when the ternary drug/PVP/SDS ground mixture was added to distilled water, crystalline nanoparticles, which were 200 nm or less in size, were formed that had excellent stability. Zeta potential measurement suggested that the nanoparticles had a structure where SDS was adsorbed onto the particles that were formed by the adsorption of PVP on the surface of drug crystals. The stable existence of crystalline nanoparticles was attributable to the inhibition of aggregation caused by the adsorption of PVP and SDS on the surface of drug crystals and to the electrostatic repulsion due to the negative charge of SDS on a shell of nanoparticles. However, the yield of nanoparticles produced by this process was low (500 μg/ml).

In an effort to increase the yield of stable, crystalline, micronized nifedipine particles this study evaluated wet ball milling in the presence of polyvinylpyrrolidone, which was used to either stabilize or wet the drug solids, and a series of ionic and nonionic surfactants, cetyltrimethyl ammonium bromide (CETAB), sodium lauryl sulfate (SLS), and polysorbate 80 (Tween 80), which were introduced as wetting agents to improve the milling efficiency. Evaluation of the reduction in particle-size, the coating process, the physical stability of micronized drug crystals, and the dissolution rates of the nifedipine ball-milled products were used to determine the efficiency of the milling process.

2. Investigations, results and discussion

2.1. Milling process

In this study two ceramic ball mills, 16 cm diameter with 20 cm length (optimum speed 68 rpm) and 8 cm diameter with 10 cm length (optimum speed 80 rpm) respectively were used. The ceramic round balls used in the large ball mill had a diameter of 3.5 cm and in the small ball mill 1.5 cm. Each ball mill was filled to about 30% with grinding media. The large ball mill was operated at optimum speed and the small mill at the optimum speed and 40 rpm (50% of the optimum speed). Aqueous suspensions of nifedipine powder, mean volume particle size 76.5 μm, with and without PVP and/or surfactants, were milled for up to 48 h.

2.2. Changes in particle size caused by milling

To test the applicability of the chosen speeds of the ball mills, the decrease in particle size of nifedipine when milled in the small mill at 40 rpm and 80 rpm was measured. The optimum speed for this mill was calculated to be 80 rpm; 40 rpm represents 50% and 80 rpm represents 100% of the optimum speed. The results show that for the 0.5% nifedipine suspensions used in this study the increase in speed did not significantly change the rate at which and the mean size obtained after milling for 48 h. Secondly, to determine the effect of the size of the ball mill on the milling process, the decrease in nifedipine particle size when milled in a small (500 ml) versus a large (4000 ml) amount was determined. The results show that when these mills were rotated at 100% of the optimum speed there was not a significant difference in the particle size obtained after milling for 48 h. The mean volume particle sizes of nifedipine after milling in the small and larger ball mill were 46.6 ± 1.5 and 48.3 ± 15.4 μm, respectively. For the combination of nifedipine and PVP or surfactant, Tween 80 or SLS or CETAB, the mean volume particle size in small and large mill are 4.2 ± 1.2 and 7.8 ± 6.6, 4.4 ± 0.98 and 2.7 ± 1.6, 4.2 ± 0.87 and 2.1 ± 1.1, 2.7 ± 0.7 and 2.4 ± 1.1 respectively. Based on these results, further experiments were performed in the small mill rotated at 80 rpm.

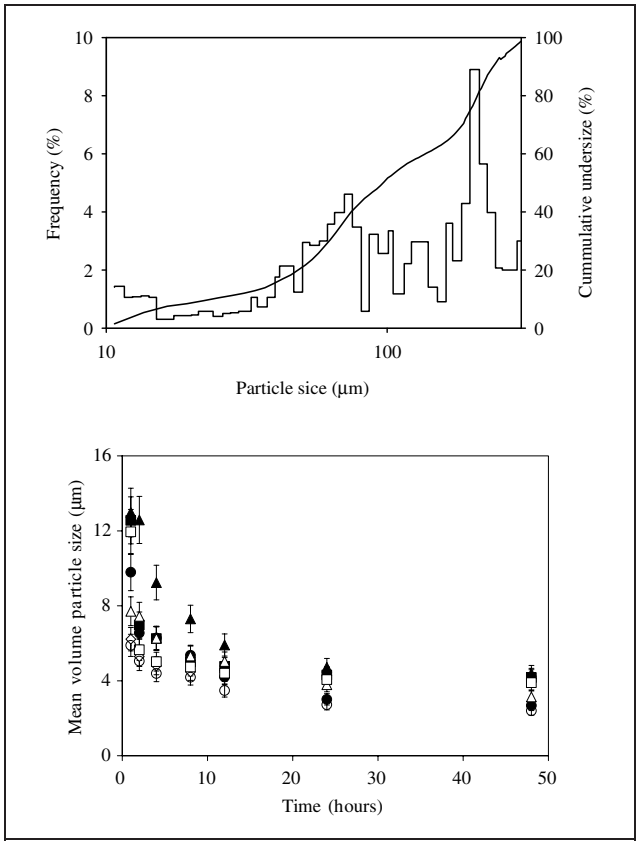

Fig. 1: Particle size distribution of nifedipine (top) without milling, (bottom) changes in particle size upon milling for 1–48 h in the presence of PVP and surfactants
◇ Nifedipine + PVP; ▲ Nifedipine + Tween 80; ■ Nifedipine + SLS; ● Nifedipine + CETAB; △ Nifedipine + PVP + Tween 80; □ Nifedipine + PVP + SLS; ○ Nifedipine + PVP + CETAB

The nifedipine powder used in this study consisted of crystalline particles with a mean volume particle size of 76.5 ± 6.6 μm. The particle size distribution of this powder (Fig. 1) was multi-modal with a mixture of large particles (100–200 μm) and very small particles (5–20 μm). Milling a 0.5% suspension in water of this water-insoluble powder for up to 48 h in the small ball mill at 80 rpm only reduced the mean particle size to 50 μm and changed the particle size distribution to bi-modal distribution around two means of 20 and 70 μm.

The addition of 1% PVP to this suspension significantly reduced the particle size because after 48 h, the mean volume size was 4.2 μm as shown in Fig. 1. The milling was very efficient because even after an hour the mean size was already reduced to about 7 μm. The decrease in particle size followed first order kinetics with a rate constant of 3.8 h^{-1} (Table 1). Milling in the presence of SLS, CETAB and Tween 80 (0.5%) also significantly reduced the particle size by a first-order process. The speed by which the particle size was reduced in the presence of these surfactants (Table 1) was slower (mean 2.37 h^{-1}) than when PVP was added. The combination of PVP and the surfactants sped up the particle size reduction process for CETAB and Tween 80 but not for SLS. These results showed that the addition of the grinding aids significantly increased the efficiency of the wet milling process and that there was not a significant difference in the effect on the particle size with regard to the addition of a single grinding aid versus combinations of PVP and the surfactants. In addition, although in this study milling was performed for up to 48 h, results showed that there was not a significant difference in the mean size obtained after 24 h compared to 48 h.

Comparison of the particle size distribution obtained after 48 h for nifedipine milled in the presence of the surfactants or combinations of PVP and surfactants showed that for SLS there was not a significant difference in the mean size. In addition, both processes produced uni-modal size distributions with very few particles below 1 μm. These results are in contrast with the results reported by Itoh et al. (2003) that showed for a dry milling process nifedipine particles below 1 μm was produced when the milled powder was suspended in water. When Tween 80 was added the particle size distribution was uni-modal after milling for 48 h but when PVP and Tween 80 was added a bi-modal distribution was produced with a significant number of particles below 5 μm. This effect was even more pronounced when CETAB or PVP plus CETAB was added to the suspensions because bimodal distributions with significant numbers of particles below 2 μm was observed. In this study, it was not possible to explore the properties of the small particles because the particle-sizing instrument used in this study has a lower size limit of

Table 1: Kinetic parameters describing the first-order decrease in the particle size of nifedipine during wet ball milling in the small ball mill

Product	Initial size, b (μm)	Final size, a (μm)	Rate, c (h^{-1})	R^2
Nifedipine	76.30	48.94	0.67	0.917
Nifedipine + PVP	71.92	4.58	3.76	0.999
Nifedipine + Tween 80	69.36	7.09	2.32	0.990
Nifedipine + SLS	71.42	5.07	2.22	0.999
Nifedipine + CETAB	71.92	4.57	2.57	0.997
Nifedipine + PVP + Tween 80	71.38	5.12	3.25	0.997
Nifedipine + PVP + SLS	72.02	4.48	2.25	0.999
Nifedipine + PVP + CETAB	72.82	3.68	3.44	0.999

Data obtained by fitting a first-order decay equation, $y = a + be^{-cx}$, using Table Curve 2 D v.4 (Systat Software, Inc., Point Richmond, CA, USA)

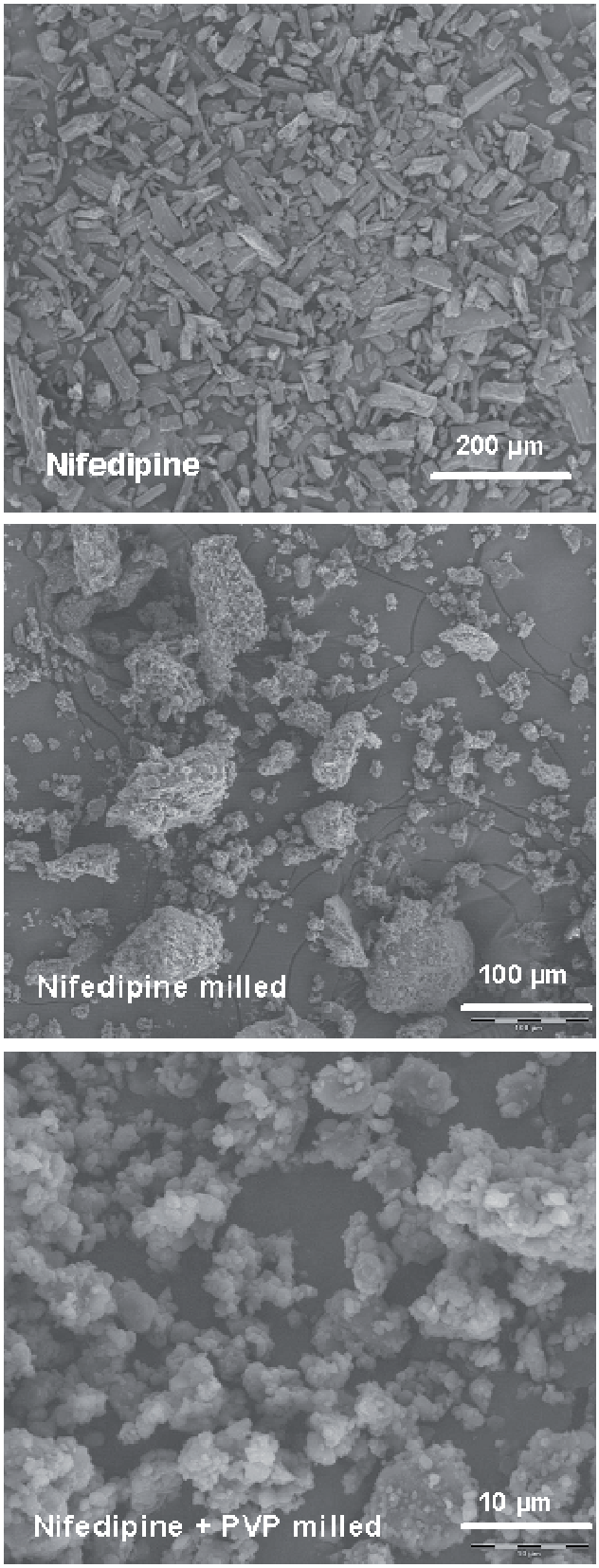

Fig. 2: SEM pictures of un-milled and 0.5% nifedipine suspensions milled for 48 h in the small ball mill at 80 rpm without and with adding 1% PVP

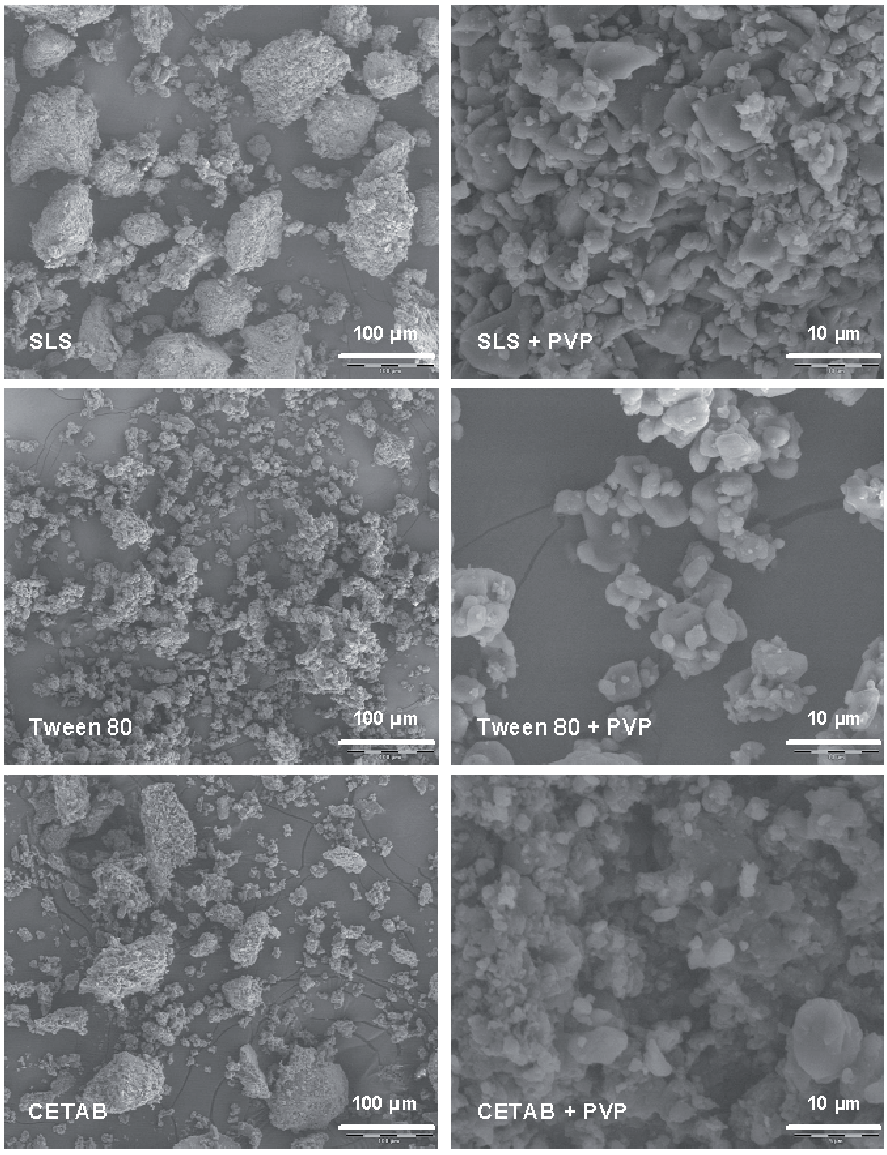

Fig. 3:
SEM pictures of 0.5% nifedipine suspensions milled for 48 h in the small ball mill at 80 rpm without and with adding 0.5% surfactant and 1% PVP

0.5 µm. Not withstanding this limitation it seemed as though it was possible with the addition of Tween 80, and especially CETAB combined with PVP, to produce significant populations of very small nifedipine particle, <5 µm, by a simple ball milling process.

Particle size reduction by milling was also confirmed by SEM evaluation. As shown in Fig. 2 the nifedipine raw material consisted of acicular crystals of large size. Milling reduced the particle size but without the addition of grinding aids such as PVP or surfactants, the particles were still large. The addition of the surfactants or combinations of the surfactants and PVP significantly reduced the particle size as shown in Fig. 3. As the size decreased, the cohesiveness of the powders increased and when removed from the suspensions and dried the particles aggregated to form loose aggregates. Aggregation was more pronounced when the strong negatively changed SLS was added compared to CETAB and Tween 80.

2.3. Physical and chemical stability of nifedipine after milling

A drawback of milling pharmaceuticals is the change in crystal form upon milling (Sato et al. 1997; Itoh et al. 2003; Gupta et al. 2003). It is known that nifedipine can form an amorphous product when crystallized under the right conditions or when rapidly cooled from the melt (Caira et al. 2003). For this reason crystal form changes during milling were followed using differential scanning calorimetric (DSC) and x-ray powder diffraction (XRPD) analysis. The DSC thermogram of the nifedipine raw material showed a melting point of 174 °C with no desolvation endotherms present, indicating that it is Modification I, a true polymorph (Caira et al. 2003). The heat required for the melting process was 98 J/g. Upon milling in the small mill there was no change in the crystal form as confirmed by DSC results and the XRPD patterns shown in Fig. 4 (a) and (b). However, after milling in the large mill the nifedipine melting peak shifted, because the an onset was at 162 °C, with the melting peak at 166 °C and a heat of melting of 78 J/g. This corresponded to the melting of nifedipine Modification II (mp. 161–168 °C) as reported by Burger and Koller (1996) and Caira et al. (2003).

When nifedipine was milled with CETAB or the combination of PVP and CETAB the amorphous content of the milled product increased as indicated by the transformation peak observed in the DSC thermograms of these products in the temperature range 95–120 °C (Fig. 5). In this temperature range after the glass transition at around 90 °C nifedipine recrystallized between 100–120 °C into

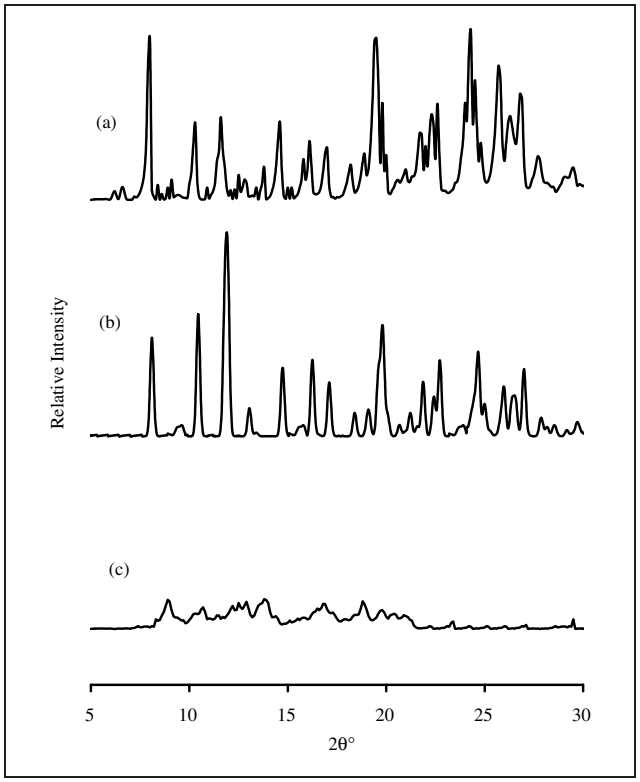

Fig. 4: XRPD patterns of (a) ball milled nifedipine, (b) nifedipine raw material and (c) nifedipine milled with PVP + SLS + CETAB

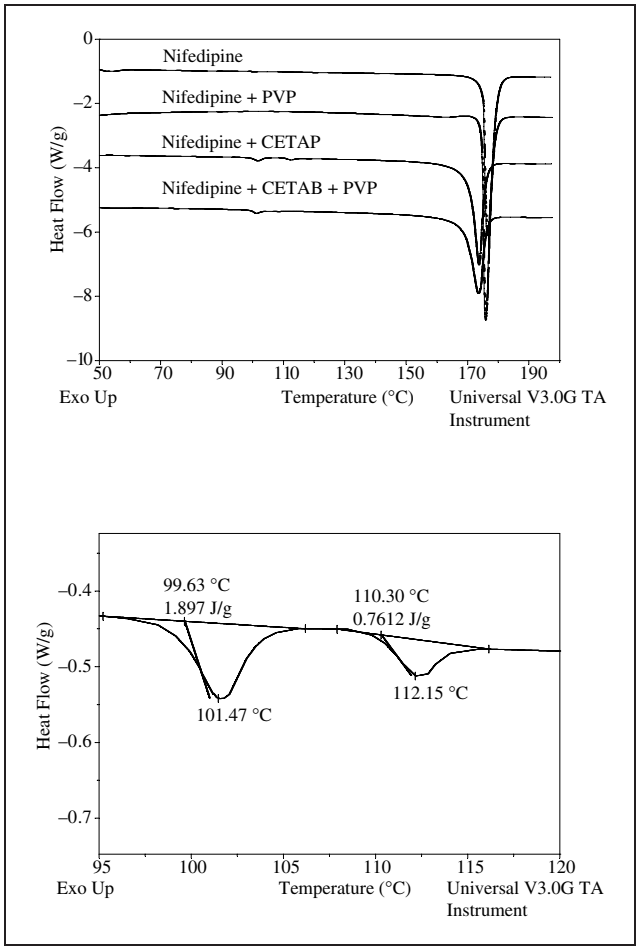

Fig. 5: Top: Changes in DSC thermograms of nifedipine after ball milling with CETAB. Bottom: Transformation of non-crystalline nifedipine ball milled with CETAB and PVP added

the stable Modification I that again melts at 174 °C. The amorphinization of nifedipine during milling was limited and was not detectable by XRPD. However, when nifedipine was milled in the presence of a combination of PVP, SLS and CETAB, both in the small and large mill, the melting peak of nifedipine at 170–174 °C completely disappeared and the product was x-ray amorphous as shown in Fig. 4(c).

Nifedipine remained chemically stable during the milling process because HPLC analysis using a stability indicating method (Devarakonda et al. 2004) did not show any decomposition of nifedipine after milling. For the product produced by milling nifedipine in the presence of a combination of PVP, SLS and CETAB the amount of intact nifedipine recovered after milling was between 88 and 98%. This low recovery was not due to decomposition because no decomposition products were detected in the HPLC chromatograms. The samples might have increased amounts of PVP and the surfactants included in the milled product. Upon milling in the aqueous suspensions, the solubility of nifedipine in the suspension medium did increase because it was determined, based on the amount of nifedipine powder recovered, that the amount of nifedipine that went into solution upon milling ranged from 0.1% in the absence to 2% in the presence of the surfactants.

2.4. Polymer and surfactant coating during milling

The powder samples were processed using x-ray photoelectron spectroscopy (XPS). The sample preparation included grinding the powder samples onto double sided clear tape, which were then placed onto carbon tape that had been previously adhered to the sample rod. A survey

Table 2: XPS elemental analysis of ball-milled nifedipine powders

Product	Measurement	Atom							
		O 1s	N–(CH)	N–(O_2)	N–(O)	C 1s	S 2p	Na 1s	Br 3d
Nifedipine + PVP	Peak position (eV)	532.8	399.8	405.7	–	284.9	171.6	1072.6	–
	Atomic conc. (%)	22.6	3.9	3.4	0	70.1	0	0	0
Nifedipine + SLS	Peak position (eV)	532.4	399.6	405.5	–	284.9	168.9	1071.4	–
	Atomic conc. (%)	19.8	2.3	1.7	0	70.6	3.4	2.3	0
Nifedipine + CETAB	Peak position (eV)	532.8	399.8	405.7	402.2	284.9	168.8	1071.4	67.8
	Atomic conc. (%)	18.6	3.5	2.7	1.4	72.7	0	0.2	1.1
Nifedipine + PVP + SLS	Peak position (eV)	532.8	399.7	405.6	–	284.9	169.19	1070.4	–
	Atomic conc. (%)	20.1	3.6	2.6	0	73.6	0	0.1	0
Nifedipine + PVP + CETAB	Peak position (eV)	532.8	399.8	405.7	–	284.9	166.4	1072.4	–
	Atomic conc. (%)	22.2	4.0	3.1	0	79.7	0	0.1	0

Relative atomic percents represent normalized atomic compositions

scan at 160 eV was collected for each powder (Fig. 6). High sensitivity scans at 80 eV for oxygen, carbon, sulfur, bromine, nitrogen, and sodium were collected. The atomic concentrations are listed in Table 2. There were two to three species of nitrogen found in the high sensitivity scan. This was expected from the molecular structures of nifedipine, SLS, CETAB, and PVP. The nitrogen species included NO_2, NO, and NCH.

In terms of the PVP or surfactants adsorbing to the nifedipine particles during milling the following observations were made from the XPS results shown in Fig. 6. For all the samples milled with PVP, PVP was detected on the surface of the drug particles (Table 2). SLS and CETAB were present on the surface of the particles produced by milling in the surfactant solutions. However, both SLS and CETAB were not present on the surface of the powder milled in solutions containing PVP and the surfactants. The absorbance of SLS and CETAB to the nifedipine crystals in the absence but not in the presence of PVP was confirmed by elemental analysis with the EDAX Si(Li) Energy Dispersive Spectrometer (EDS) attached to the SEM. This analysis showed that there was about 0.5% bromine presenting on the surface of nifedipine milled with CETAB and 0.4% sulfur on the surface of nifedipine milled with SLS. These elements were not detected on the surface of the other samples.

Changes in the zeta potential of the nifedipine particles during milling also confirmed the adhesion of the grinding aids to the surfaces of the drug particles. Nifedipine after wet-milling has a surface charge of -24.2 ± 5.9 mV. When milled with SLS the negative surface charge is increased to -33.1 ± 5.8 mV while in the presence of CETAB the surface charge is reversed to 54.9 ± 4.8 mV. The addition of PVP neutralized the nifedipine particle surfaces to a zeta potential of 12.9 ± 1.8 mV. Tween 80 did not significantly change the surface charge, -24.4 ± 5.4 mV. When the PVP was combined with the surfactants the surface charge more closely followed that of PVP alone rather than that of the surfactants because for PVP + SLS the surface charge was -2.5 ± 4.5 mV; for PVP + CETAB, 20.7 ± 3.4 mV; and PVP + Tween 80, 4.3 ± 7.8 mV. These results closely followed the XPS and EDAX surveys, showing that the charged surfactants, CETAB and SLS, strongly sorbed to the nifedipine crystals but that PVP either preferentially adsorbed to the crystals and thereby prevents the sorption of the surfactants or that the surfactants sorbed first and then are coated with the PVP.

Micro-DSC analysis has shown changes in the heat of adsorption when solutions of PVP, SLS, Tween 80 or CETAB were added to nifedipine or nifedipine particles coated with PVP. The heat measured when water was added to nifedipine particles was 2.1 mJ. This was an endothermic heat change. When water was added to nifedipine pre-coated with PVP the heat of sorption increased to 6.3 mJ. This indicated a stronger interaction between water and the now hydrophilic nifedipine particles compared to the hydrophobic uncoated nifedipine crystals. When a PVP solution instead of water was added to the nifedipine crystals, an exothermic heat change of -1.7 mJ was measured. It was assumed that the interaction between PVP and the drug particles is associated with a loss of chain conformational entropy of the polymer during the formation of charge/charge interaction between the PVP macromolecules and negatively charged drug particles (Ball et al. 2002). The loss of configurational entropy of the polymer chains is compen-

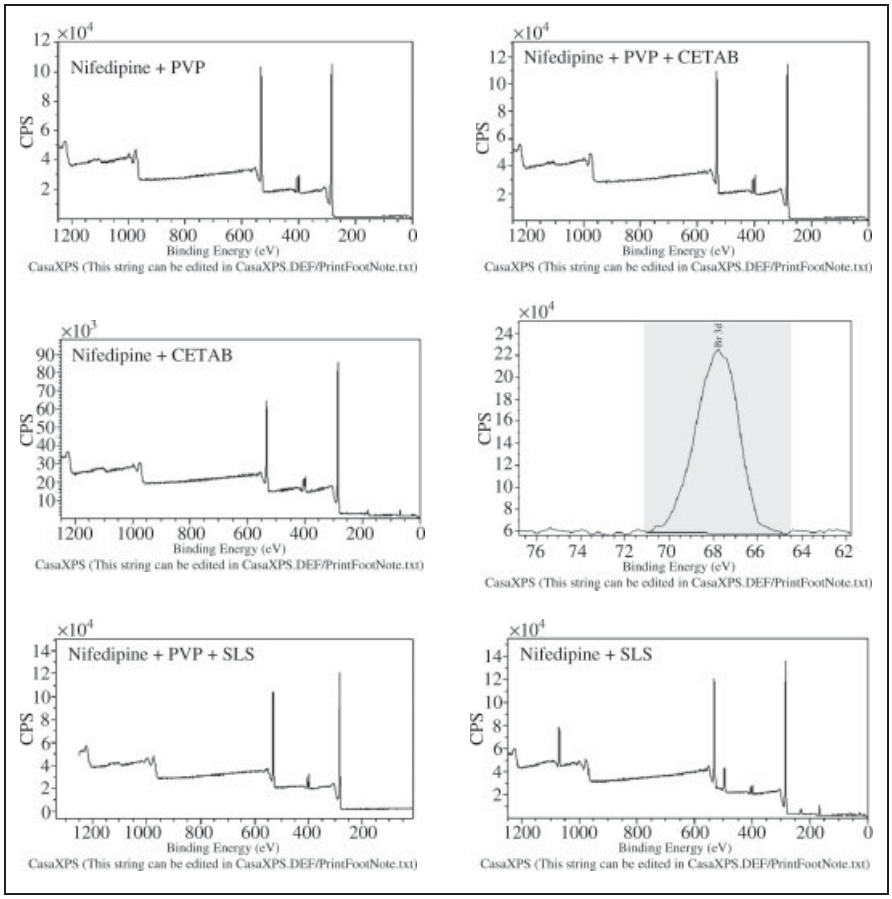

Fig. 6:
XPS survey scans with pass energy 160 eV of nifedipine powders ball milled with PVP and combinations of PVP and SLS or CETAB. XPS high resolution scan of Br 3d is also shown

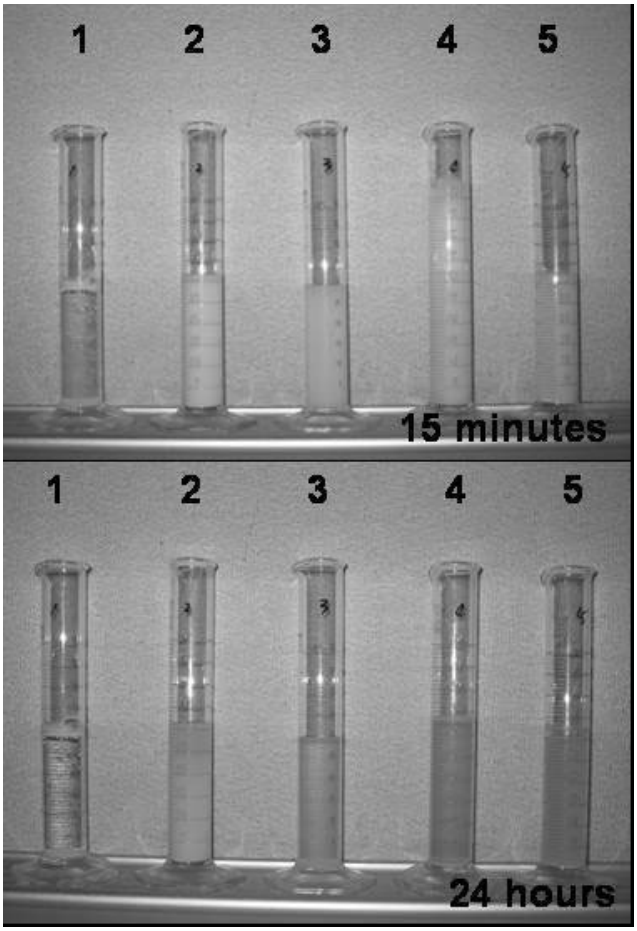

Fig. 7: Photographs of the suspensions showing sedimentation: (1) nifedipine ball-milled; (2) milled with PVP; (3) milled with CETAB; (4) SLS; (5) Tween 80

sated for by a gain in entropy associated with the release of the counterions producing a positive enthalpy. The interaction between the Tween 80 (−1.3 mJ) and SLS (−3.8 mJ) and the drug particles was also exothermic. CETAB interacted with the drug particles with an exothermic heat of sorption of 2.7 mJ. When solutions of the surfactants were added to PVP coated particles CETAB interacted with the particles producing a heat of sorption of −4 mJ and SLS −7.9 mJ. The interaction between the charged surfactants and the nifedipine particles or PVP coated crystals was stronger than that between PVP or Tween 80 and the drug particles (Bezan et al. 1996; Wang and Tam 2004).

2.5. Sedimentation of ball-milled nifedipine suspensions

After 15 min the nifedipine ball-milled suspension (0.5% w/v) became transparent while nifedipine fine particles in the ball-milled suspensions containing combination of 0.5% nifedipine and 1.0% w/v PVP with or without the introduction of 0.5% w/v of the surfactants, CETAB, SLS or Tween 80, were still dispersed (Fig. 7). After 12 and 24 h, ternary suspensions containing nifedipine, PVP, and surfactant were still well dispersed and the slight sediment was easily redispersed. It implies that in suspensions prepared with nifedipine particles ball-milled in the presence of PVP and the ionic or nonionic water-soluble surfactants CETAB, SLS, and Tween 80 the sedimentation rate of nifedipine can be significantly decreased. In addition, suspensions containing PVP and the surfactants were easily redispersed by mild shaking but the nifedipine suspension tended to form a cake that was hard to redisperse.

The sedimentation results are in good agreement with the particle size results for these ball-milled products because suspensions with smaller particles are less prone to sedimentation. In addition the addition of PVP and/or the charged surfactants also helped because after adsorption of the compounds to the surface of the particles the uniformly charged positive or negative charged particles repel each other decreasing the possibility of flocculation and thus sedimentation. Overall, suspensions milled with PVP and either the cationic (CETAB) or anionic (SLS) surfactants were the least susceptible to sedimentation and were the easiest to redisperse.

2.6. Dissolution of ball-milled nifedipine powders

To examine the effects of ball-milling and the addition of PVP and surfactants on the dissolution profiles of nifedipine ball-milled products, the dissolution rate of the different milled products were measured in 0.1 M HCl. The slowest dissolution was shown by the nifedipine ball-milled powder without the addition of suspension aids followed by nifedipine milled with PVP or Tween 80 (see Fig. 8).

According to the dissolution curves shown in Fig. 8, the best dissolution profile was observed for nifedipine ball-milled in the presence of PVP and CETAB with the highest amount released of 80% after 180 min while the second highest amount of drug went in solution (60% after 120 min) from the quaternary formulation containing nifedipine, SLS, Tween 80 and PVP. Slower dissolution profiles were measured for the other formulations including nifedipine + CETAB, nifedipine + SLS, nifedipine + SLS + PVP, nifedipine + Tween 80 + PVP, nifedipine + CETAB + SLS + PVP, and nifedipine + CETAB + Tween 80 + PVP. There was no significant difference in the dissolution behavior of these mixtures. In the simulated gastric fluid the highest amount of drug that dis-

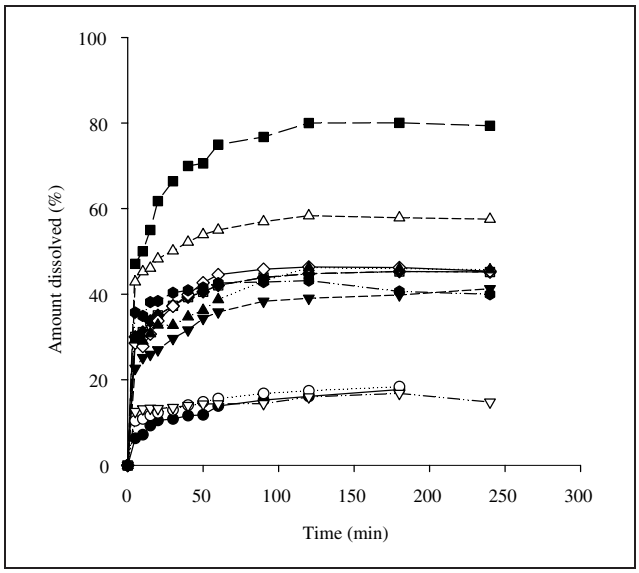

Fig. 8: Dissolution profiles of ball-milled products in simulated gastric fluid pH 1
——●—— Nifedipine ball-milled; ····○···· PVP; - - -▼- - - SLS; -··-▽-··-Tween 80; – –■– – PVP + CETAB; – · -□- · – CETAB; – –◆– – PVP + SLS; ——◇—— PVP + Tween 80; ····▲···· PVP + CETAB + SLS; – –△– – PVP + SLS + Tween 80; –··–●–··–PVP + CETAB + Tween 80

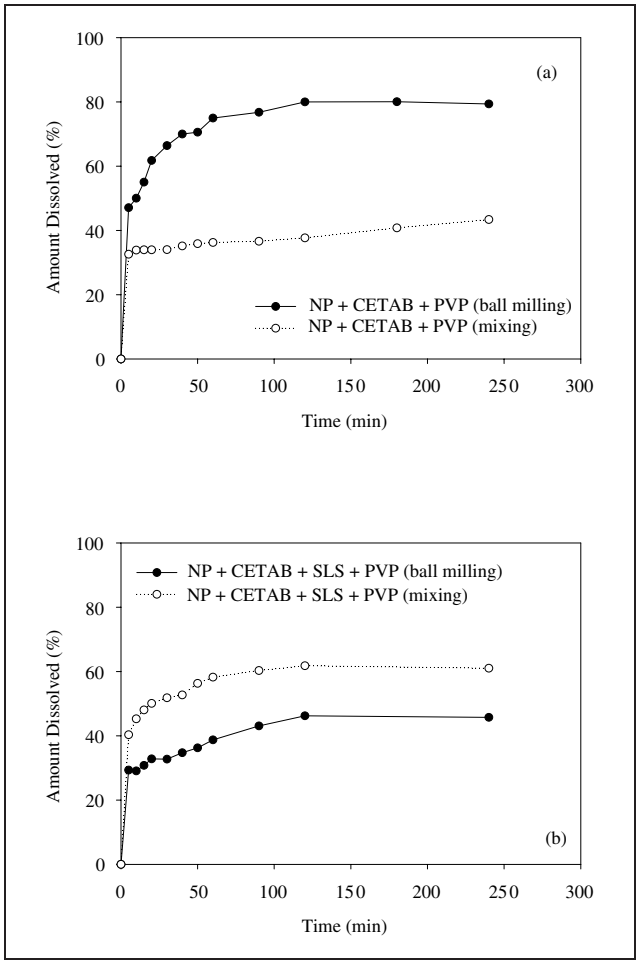

Fig. 9: Dissolution profiles of mixed versus ball-milled nifedipine: (a) mixed or milled with CETAB + PVP; (b) mixed or milled with CETAB + PVP + SLS

solved was only 80% of the 20 mg nifedipine added. However, this amount was 4 times more than the pure milled drug. The increase in dissolution of the nifedipine milled in the presence of the grinding aids could be a combination of smaller particles produced by co-grinding and the surface-active properties of the surfactants that increase the wettability of the hydrophobic nifedipine particles. Once added to the simulated gastric fluid, the water soluble PVP and surfactants dissolved in the aqueous fluid, completely dispersing the fine nifedipine particles in the dissolution medium. This ensured that the maximum surface area is exposed to the dissolution medium.

This action was due to the intimate mixing of the PVP and surfactants with the nifedipine in the ball mill because the dissolution profiles of nifedipine ball-milled products are much better than that of products produced by powder mixing of the same composition of drug: suspension aids ratios. Both the amount dissolved and the speed at which the drug went into solution was higher for the drug + SLS or CETAB or drug + PVP + SLS or CETAB products recovered after wet milling. Fig. 9 (a) shows that in the formulation of nifedipine, CETAB, and PVP the dissolution rate of nifedipine from the ball-milled products was significantly faster and greater than from the powder mixture.

However, for the nifedipine + PVP + CETAB + SLS mixtures, the dissolution from powder mixtures was higher than that from the ball-milled products as seen in Fig. 9(b). In this case, as seen from DSC and XRPD results wet milling caused complete amorphinization of nifedipine with very little PVP and surfactants adhering to the surface of the milled product. On the other hand, a relatively large amount of PVP and surfactants that could aid in the dissolution process were contained in the mixed products. This showed that the interaction between nifedipine and the excipients in the quaternary milled product had a detrimental effect on the dissolution rate of the drug although the drugs was in the amorphous form.

2.7. Conclusions

Overall the results of this study showed that wet ball milling in the presence of the biocompatible polymer PVP and the surfactants SLS, CETAB and Tween 80 is an efficient mechanical method to reduce the particle size of nifedipine, reduce the sedimentation rate of suspensions and increase the dissolution of the drug. This was caused by the formation of PVP or surfactant layers around the drug particles during milling. Also, the PVP and surfactants seem to stabilize the nifedipine crystals during milling.

3. Experimental

3.1. Materials

Nifedipine (Lot No. RF0565, Spectrum Chemicals, Gardena, CA, USA), sodium lauryl sulfate (SLS, anionic surfactant, Lot No. KS026, Spectrum Chemical), cetyltrimethylammonium bromide (CETAB, cationic surfactant, Lot No. 122K0036, Sigma-Aldrich, St. Louis, MO, USA), polysorbate 80 (Tween 80, nonionic surfactant, Lot No. KU010, Spectrum Chemicals), and polyvinylpyrrolidone (PVP, K value 17, GAF, Wayne, NJ, USA) were used in the study.

3.2. Preparation of ball-milled powders

A series of nifedipine aqueous suspensions containing nifedipine (0.5%), PVP (1.0%), and one or two of the surfactants (0.5%) were prepared in ball mills rotated for up to 48 h. The two ceramic ball mills, with diameters of 8 and 16 cm, were rotated with a Erweka AR400 variable speed drive (Erweka, Heusenstamm, Germany). The nifedipine powders were recovered form the suspensions by centrifugation and dried at 30 °C for 24 h. Since nifedipine is light sensitive, all operations were performed and the recovered powders stored in the dark.

3.3. Morphological and size characterization of ball-milled particles

A Galai-Cis-1 particle size analyser (Galai, Ltd., Migdal Ha'Emek, Israel) was used to measure the particle size distribution in suspension of the nifedipine powders recovered after ball milling. Particle sizing on this instrument is done by a dual discipline analysis, integrating laser diffraction and image analysis. Samples suspended in liquid paraffin were placed in small cuvettes and fitted into the analyser. A small magnetic stirrer inside the cuvette prevented sedimentation of the particles during the measurement. The acquired data were used to construct particle size distribution graphs and to compute the mean volume particle size $D[4,3] = \Sigma d^4/\Sigma d^3$ where d is the diameter of individual particles.

A Philips XL 30 scanning electron microscope (Philips, Netherlands) was used to obtain photomicrographs of the micronized nifedipine particles. Samples were mounted on a metal stub with an adhesive and coated under vacuum with carbon (Emscope TB500 sputter-coater, Emscope Laboratories, Ashford, UK) before being coated with a thin gold-palladium film (Eiko Engineering Ion Coater IB-2, Eiko Engineering, Ibaraki, Japan).

3.4. Characterization of nifedipine crystal form changes during milling

DSC traces were recorded with a DSC 2920 modulated DSC (TA Instruments, New Castle, DE, USA). The DSC was calibrated for temperature and enthalpy using the melting temperature of highly pure indium standard. Samples weighing 3–5 mg were heated in crimped aluminum cells at a rate of 10–20 K/min under nitrogen gas flow of 35 ml/min.

X-ray powder diffraction (XRPD) patterns were obtained at room temperature on a Bruker D8 Advance diffractometer (Bruker, Rheinstetten, Germany). The isothermal 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°/min (step size 0.025°, step time, 1.0 s). Approximately 300 mg

samples were weighed into aluminum sample holders, taking care to avoid introducing preferred orientation of the crystallites. The XRPD diffractograms of the samples were compared with regard to peak position and relative intensity, peak shifting and the presence or lack of peaks in certain angular regions.

3.5. Nifedipine analysis

Nifedipine was analyzed by HPLC (AS 1000 autosampler and P2000 pump, Thermo Separation Products, Waltham, MA) equipped with a multiple wavelength UV detector (UV 3000 detector) set at a wavelength of detection $\lambda_{max} = 254$ nm. Chromatographic separation was performed using a C_{18} column (Synegi, 4 μm particles, 150×4.6 mm, Phenonenex, Torrance, CA, USA). The mobile phase was methanol : water (2 : 1 v/v); flow rate 0.7 mL/min; injection volume 20 μl. The retention time for nifedipine was 4.4 min, the limit of detection was 1.0 ng/ml. Results were the mean of three analyses, and the solutions were protected from light to prevent photo degradation of nifedipine. The HPLC method used in this study complied with specifications for nifedipine analysis as required by the USP XXIV (2000).

3.6. Establishment of polymeric and surfactant coating

To ensure the charge and charge changes after milling, the zeta-potential of the suspended particles were measured and the reported results represents the mean of 10 measurements determined with a Zeta-plus photon correlation spectroscopy and microelectrophoresis instrument (Brookhaven Instruments, Holtsville, NY, USA). To further study the adhesion of the PVP and/or surfactant to the nifedipine particle during milling the heat involved when solutions of the grinding aids were added to nifedipine crystals at 25 °C was measured with a micro differential scanning calorimeter (Micro DSC III, Setaram, Caluire, France). The instrument was operated in the isothermal mode using 1 ml batch mixing vessels. Drug particles were placed in the bottom of the mixing vessel while the PVP, surfactant, or PVP-surfactant solution was added to the top reservoir. Once the instrument was equilibrated at 25 °C, the plunger was pushed down allowing the electrolyte solution to be exposed to the drug particles. The heat measured, once corrected for the heat involved in mixing and the addition of solvent without grinding aids, represent the heat of adhesion of the grinding aid molecules to the nifedipine particle surfaces.

In addition to these studies, x-ray photoelectron spectroscopy (XPS) analyses were performed on the milled particles using a Kratos Analytical Axis Ultra instrument (Chestnut Ridge, NY, USA). X-ray source was a monochromatic aluminum (1486.6 eV) and powered by 280 W. Survey and high-resolution spectra were collected at a takeoff angle of 90° with respect to the sample plane. The analysis for these powder samples required the charge neutralization to be turned on at low energy. All spectra were referenced for C−C in carbon 1 s peak at 285 eV. Survey spectra were collected from 0 to 1200 eV with pass energy of 160 eV, high sensitivity scans for the CETAB and PVP coated particles were collected at a pass energy 80 eV, and high-resolution spectra were collected for each detected element (C, O, N, S, BR) with pass energy of 20 eV. The sample, powder form, was mounted on silicon wafers containing double sided carbon tape. Each silicon wafer was then placed on a sample holder and was mounted into the XPS transfer arm.

XPS results were confirmed by quantitative Energy Dispersive Spectrometer (EDS) analysis using a Philips XL30 SEM capable of chemical analysis with a detectable element range from Boron to Uranium was used and qualitative X-ray Mapping (XRM) (FEI Electron Optics, Eindhoven, The Netherlands). Detail about trace elements present on the surface of the nifedipine particles was determined by this method but elemental analysis with the EDAX Si(Li) Energy Dispersive Spectrometer (EDS) combined with a EDAX multi-channel analyzer was limited because the spatial resolution for compositional analysis is, in general, is about 1 micrometer diameter for relatively flat specimens.

3.7. Sedimentation rate

Suspensions of the ball-milled products were prepared and stored in 10 ml graduate cylinders. The suspensions were left untouched and height of the sediments measured over time up to 24 h after preparation.

3.8. Dissolution studies

The dissolution rates of dried nifedipine powders (equivalent to 20 mg drug) recovered from the ball-milled suspensions were measured in 900 mL simulated gastric fluid (without pepsin, pH 1) at 37 °C. A USP Apparatus 2, Dissolution Tester (Vanderkamp 600, Van Kel Industries, NJ, USA) with standard 900 ml vessels and paddles rotated at 100 rpm was used to measure the dissolution of nifedipine. To obtain the amount dissolved the UV-absorbance of samples take from the dissolution flask was measured at 237 nm (Multispec-1501 UV spectrophotometer, Shimadzu, Kyoto, Japan). The dissolution results of the ball-milled products with and without grinding aids were compared. In addition, the dissolution of powder mixtures of the drug and PVP, and/or surfactants was also measured.

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