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# Advantages of celecoxib nanosuspension formulation and transformation into tablets

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ABSTRACT

Drugs with low aqueous solubility and high permeability (BCS class II) present a high proportion of all drugs. This study examines the critical issues regarding engineering of a nanosuspension tailored to increase drug dissolution rate and its transformation into dry powder suitable for tabletting. Nanosuspensions of celecoxib, a selective COX-2 inhibitor with low water solubility, were produced by the emulsion-diffusion method using three different stabilizers (Tween® 80, PVP K-30 and SDS) and characterized by particle size analysis, dissolution testing, scanning electron microscopy imaging, differential scanning calorimetry and X-ray powder diffraction. Spray-dried nanosuspension was blended with microcrystalline cellulose, and compressed to tablets, and their tensile strength, porosity and elastic recovery of tablets were investigated. The selection of solvent and stabilizers is critical, firstly to achieve controlled crystallization and size, and secondly to increase the wettability of the hydrophobic drug. The crystalline nano-sized celecoxib alone or in tablets showed a dramatic increase of dissolution rate and extent compared to micronized. SEM images showed that the nanoparticle morphology was influenced by the choice of stabilizers. Celecoxib nanosuspension stabilized with PVP K-30 and SDS showed advantages over Tween® 80 due to sticking of the dried product and unexpected changes observed on DSC curves. Markedly lower compaction forces are needed for nano-sized compared to micro-sized celecoxib to produce tablets of equal tensile strength.



## 1. Introduction

Poor water solubility of active pharmaceutical ingredients is an industry wide problem in drug discovery and development. It is estimated that 40% of all newly developed drug compounds are poorly soluble or "insoluble" in water, and up to 50% of orally administered drug compounds present formulation problems related to high lipophilicity. In recent years, nanoparticle engineering process has been seen as a promising approach for the enhancement of drug solubility (Wong et al., 2006; Rabinow, 2004; Patravale et al., 2004). Unlike micronization, nanonization often increases the solubility as well as the dissolution rate (Kesisoglou et al., 2007). According to the Noyes–Whitney equation only the dissolution rate increases with surface area (Dressmann and Reppas, 2000). The Ostwald–Freundlich and Kelvin equations show that this no long applies below a particle diameter of approximately 1 μm, preferably less then 0.1 μm, where the extreme curvature of the particles leads to an increase in dissolution pressure and hence solubility (Műller and Keck, 2004; Hornyak et al., 2008; Colombo et al., 2009). In principle the formulation of nano-sized particles could be applied to all drug compounds belonging to biopharmaceutical classification system (BCS) classes II and IV to increase their solubility and hence partition into the gastrointestinal barrier (Dubey, 2006). Variability of absorption could also be diminished by their tendency to stick to the gastrointestinal wall enabling rapid replenishment of the absorbed drug (Műller and Peters, 1998; Dressmann and Reppas, 2000; Műller and Keck, 2004). Thus, nanoparticle technology could play a major role in the successful development and marketability of a poorly water soluble drug compounds.

Nanosuspensions in aqueous or non-aqueous vehicles can be produced by bottom-up (e.g. precipitation) or top-down (e.g. wet milling) processes (Rabinow, 2004; Patravale et al., 2004; Douroumis and Fahr, 2006). High pressure homogenizers such as the piston gap homogenizer have proved to be a highly successful technology in nanosuspension formation. This process is based on the cavitation forces produced when the dispersion/emulsion is forced through a very thin gap (Műller and Peters, 1998; Hu et al., 2004; Keck and Müller, 2006). The particle size of the nanosuspension depends on pressure, the number of cycles and, in the case of suspension, the hardness of the drug particles (Keck and Müller, 2006). In the solvent diffusion technique a solvent-in-water emulsion with partially water-miscible solvents is prepared with the drug dissolved in the dispersed phase. The selection of solvent and the stabilizer is critical to obtain drug particles in the nanometre

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range. In general solvents with high water miscibility and stabilizers able to form stable emulsions are preferred (Kocbek et al., 2006; Quintanar-Guerrero et al., 2005). After solvent diffusion particle formation is extremely fast due to high supersaturation, which in turn leads to fast nucleation growth (Peukert et al., 2004).

Despite the progress of the knowledge in this field, nanosuspensions have the drawback of instability caused by nucleation and particle growth. In the absence of a stabilizer, the high surface energy of nano-sized particles can induce aggregation—a phenomenon known as Ostwald ripening (Patravale et al., 2004). Stabilizers need to wet, i.e. accumulate at the interface of the drug particles to provide steric or ionic barriers. The type and amount of stabilizer has a pronounced effect on the physical stability and *in vivo* behaviour of nanosuspensions. A mixture of stabilizers is sometimes required to obtain a stable nanosuspension. The drug-to-stabilizer ratio in the formulation may vary from 1:20 to 20:1 and should be investigated for each specific case. Stabilizers that have been explored so far include cellulose derivatives, poloxamers, polysorbates, lecithins and povidones (Kocbek et al., 2006).

With these principles in mind the poorly soluble drug celecoxib was formulated as nanosuspension. Celecoxib as a selective cyclooxygenase 2 (COX-2) inhibitor is widely used at the treatment of osteoarthritis, rheumatoid arthritis and acute pain. Several preclinical and clinical studies explored the therapeutic benefit of combining specific COX-2 inhibitors with chemotherapeutic agents and have improvement in cancer treatment outcome (van Wijngaarden et al., 2007). Celecoxib is weakly acidic with a pKa of 11.1 and is classified as a BCS class II substance, since it has low aqueous solubility (~5 μg/ml) and good permeability. Low solubility contributes to high variability in absorption after oral administration. Bioavailability of celecoxib in polyethylene glycol/saline solution after oral dosing in dogs is ~70% and only 30% when given in capsules. Inter-individual variability of celecoxib plasma profiles in humans is also significantly high. Increasing the solubility and dissolution rate of celecoxib has the potential to improve its overall oral bioavailability (Paulson et al., 2001).

Additionally, celecoxib exists as long needle-shaped crystals that have undesirable properties such as cohesiveness, low bulk density and compressibility. They therefore tend to separate out and agglomerate leading to poor blend uniformity and formation of a monolithic mass upon compression in the tablet die, making successful tabletting very problematical (Chawla et al., 2003).

Despite the fact that numerous publications report about celecoxib pharmacological effects, reports of technologies with conclusive evidence for the formulation of celecoxib in dosage forms with adequate solubility are very rare. Chawla et al. (2003) showed that problems associated with its poor aqueous solubility and non-ideal physical properties could be improved by utilizing alternate solid forms. Beta-cyclodextrin inclusion complexes have recently been described achieving higher celecoxib dissolution rate than physical mixture and pure drug (Rawat and Jain, 2004). Celecoxib solubilization in a self-emulsifying drug delivery system showed promise (Subramanian et al., 2004) and microemulsions were reported as an excellent solubilization vehicle (Garti et al., 2006; Homar et al., 2007). Furthermore, the influence of polymers on the bioavailability of microencapsulated celecoxib and loading in polymeric nanoparticles by encapsulator with a vibrating nozzle device was investigated (Homar et al., 2007; Zvonar et al., 2009). Another interesting approach is also mechanochemical activation of the drug which does not require the use of solvents whose elimination from the activated product can be difficult and expensive (Grassi et al., 2007; Colombo et al., 2009).

In this study two celecoxib nanosuspensions with various stabilizers by the emulsion-diffusion method and intensive homogenization were prepared. In the first formulation Tween® 80 was used as stabilizer and in the second the combination of polyvinylpyrrolidone (PVP K-30) and sodium dodecyl sulfate (SDS). Nano-sized celecoxib was characterized by particle size analysis, dissolution test, scanning electron microscopy (SEM) imaging, differential scanning calorimetry (DSC), and X-ray powder diffractometry. The PVP/SDS nanosuspension was spray dried and compressed to tablets with microcrystalline cellulose. Finally the effect of nano-sized celecoxib on dissolution rate, the tensile strength, porosity and elastic recovery of tablets were investigated. Micronized celecoxib served as a reference in all tests.

## 2. Materials and methods

### 2.1. Materials

Celecoxib: Matrix Laboratories Limited (Sinnar, India); SDS, acetonitrile, and ethylacetate: Merck KgaA (Darmstadt, Germany); Tween® 80: Fluka (Buchs SG, Switzerland); polyvinylpyrrolidone K-30: BASF (Germany); microcrystalline cellulose (MCC, Avicel PH 101): FMC (USA). All other chemicals were of analytical grade used as received. Water was purified by reverse osmosis.

### 2.2. Determination of celecoxib solubility

Excess of celecoxib was added to 50 ml of water and different media (varying pH and % of SDS), stirred on a magnetic stirrer at 25 °C for 24 h and put in the ultrasound bath for 30 min. Samples were ultracentrifuged and the amount of dissolved celecoxib was analyzed by HPLC as described below.

### 2.3. Preparation of celecoxib nanosuspension

Nanosuspensions were produced by the emulsion-diffusion (solvent exchange) method (Kocbek et al., 2006; Quintanar-Guerrero et al., 2005). Celecoxib dissolved in ethylacetate was added to aqueous solutions of stabilizers and homogenized. Tween® 80 and a combination of PVP K-30 and SDS (1:1 by weight) were used as stabilizers.

*Stabilization with Tween® 80*: 300 mg of celecoxib dissolved in 4 ml ethyl acetate was poured into 20 ml of 0.75% aqueous solution of Tween® 80 and stirred for 5 min at 13,500 rpm with Ultra Turrax T25 (UT; Janke & Kunkel, IKA Labortechnik, Germany). The resulting emulsion was diluted with 45 ml of water and further homogenized by high pressure homogenization (HPH; APV-2000, Invensys, Denmark) at 600/60 (first/second HPH valve) bars for 15 min to form nano-sized celecoxib particles.

*Stabilization with PVP K-30/SDS*: 800 mg of celecoxib dissolved in 10 ml ethyl acetate was poured into 50 ml 1.6% (w/w) aqueous solution of SDS/PVP K-30 (1:1) and stirred with UT for 2 min at 20,000 rpm. The emulsion was further homogenized by HPH at 700/70 bars for 5 min, diluted with 250 ml of water and further homogenized at the same pressure for 10 min to eliminate the organic solvent by diffusion and to form celecoxib nano-sized particles.

### 2.4. Spray drying of nanosuspension

A spray dryer Büchi B290 (Flawil, Switzerland) was used to convert nanosuspensions to dry powder. The drying was applied to all nanosuspension batches but for further production only PVP/SDS nanosuspensions was appropriate.

Prior to spray drying the excess of PVP and SDS was removed by ultracentrifugation at 15,000 rpm for 15 min (Sorvall RC5C, Thermo Fisher Scientific Inc., Waltham, USA) and the supernatant carefully removed. The sediment was redispersed in 40 ml water and spray

dried at inlet air temperature 160 °C. The aspirator and pump were set to 100% and 15% respectively.

### 2.5. Characterization of nanoparticles

#### 2.5.1. Particle size analysis

The particle size distribution was measured using a laser diffractometer Matersizer X equipped with a small sample dispersion unit (Malvern Instruments, Worcestershire, UK) diluting with water saturated with celecoxib as appropriate. Laser diffraction measures the volume based diameter distribution in the size range 0.1–2000 μm. Particle-size distribution typically includes $d(v, 0.1)$, $d(v, 0.5)$ and $d(v, 0.9)$, which represent the percentage of particles below given size (μm).

#### 2.5.2. Dissolution studies

Dissolution behaviour of celecoxib in nanosuspension or in tablets was studied by a paddle method using dissolution system Erweka DT6 (Heusentamm, Germany) with different media as shown in Table 1 at stirring speed 50 rpm at 37 ± 0.5 °C.

Celecoxib Tween® 80 nanosuspension with known amount of celecoxib (1.0–1.5 mg) was transferred to 900 ml phosphate buffer (pH 6.8) dissolution medium. Samples were withdrawn at intervals, immediately filtered through 0.2 μm PTFE (polytetrafluoroethylene) filters (Minisart SRP 25, Sartorius AG, Goettingen, Germany) to minimize drug adsorption and precipitation, and immediately analyzed using HPLC by the method below. For comparison a suspension of micronized celecoxib with the same amount of Tween® 80 was used. The dissolution rate of pure micronized celecoxib in powder form was also measured.

Celecoxib was quantified using a HPLC system Agilent 1100 series (Hewlet Packard, Waldbron, Germany) equipped with a Nucleosil C18 column (250 mm × 4.0 mm 5 μm Hypersil ODS, Thermo, USA) at 25 °C. Mobile phase acetonitrile and water (60:40 v/v) was used at a constant flow rate of 1 ml/min and the eluent was monitored at 238 nm. The response was linear between 10 and 150 mg/L. All samples were analyzed in triplicate.

Tablets with 100 mg PVP/SDS nanosuspension were compressed with microcrystalline cellulose. The dissolution medium was tailored to maintain sink conditions throughout the dissolution testing and consisted of 900 ml sodium phosphate buffer with pH 10 and 1% SDS at 37 ± °C. The dissolution study was also carried out in 900 ml sodium phosphate buffer with pH 6.8 and 0.5% SDS at 37 ± 0.5 °C. At predetermined time intervals 4 ml samples were withdrawn and analyzed by HPLC.

#### 2.5.3. Scanning electron microscopy

The morphological examination of celecoxib nanoparticles and tablets was performed using a scanning electron microscope (SEM, Supra 35 VP-24-13, Carl Zeiss, Germany) operated at an accelerating voltage of 1 kV and a secondary detector. Samples were deposited on a double-sided carbon tape (diameter 12 mm, Oxon, Oxford instruments, UK) for analysis.

**Table 1**
Particle size in particular volume fraction of raw celocoxib and formulated nanoparticles, stabilized with Tween® 80 or PVP/SDS are presented. For the PVP/SDS nanosuspension particle size before and after spray drying process was determined, $N = 3$.

| Particle size (μm) | $d(v, 0.1)$ | $d(v, 0.5)$ | $d(v, 0.9)$ |
|---|---|---|---|
| Raw celecoxib | 1.70 | 4.10 | 11.24 |
| Tween® 80 nanosuspension | 0.14 | 0.32 | 0.70 |
| SDS/PVP nanosuspension *before* spray drying | 0.14 | 0.36 | 1.29 |
| SDS/PVP nanosuspension *after* spray drying | 0.13 | 0.36 | 1.84 |

#### 2.5.4. Differential scanning calorimetry (DSC)

A differential scanning calorimeter Mettler Toledo, Inc. (Columbus, OH, USA) was calibrated using indium standard. The samples were placed in non-hermetically sealed aluminium pans and heated from 25 to 180 °C at 10 °C/min and nitrogen purge 20 ml/min. The output was evaluated by StarE 9.10 software.

### 2.6. X-ray powder diffraction

X-ray diffraction measurements were carried out on samples using a Philips PW 1710 diffractometer (Philips Electronic Instruments Inc., Mahwah, NJ) with Cu-K$\alpha_1$ radiation ($l$ = 1.5418 Å) at 40 kV and 30 mA. Data was collected from 2 to 70° with 0.04° steps.

### 2.7. Tabletting of spray-dried nanosuspension

Microcrystalline cellulose (MCC) was used as filler to compress tablets containing celecoxib nano-sized particles stabilized with PVP/SDS after ultracentrifugation and spray drying. The celecoxib and MCC in the ratio 1:3 were carefully mixed using a pestle and mortar. 2% of magnesium stearate was added to all compositions. The mixture was compacted using a hydraulic tabletting apparatus Killian SP 300 (Köln, Germany) equipped with MGC plus processing unit (Hottinger Baldwin Messtechnik GmbH Darmstadt, Germany) allowing us determination of the compaction forces for individual tablets.

Circular flat-faced tablets were prepared using 12.0 mm diameter punches. For each tablet 400 mg of powder mixture was weighed into the die and compressed manually. The distance between the upper and the lower punch were carefully varied to evaluate the behaviour of mixtures during tabletting and the compaction forces of the upper punch were monitored. The tablets were weighed and their dimensions measured by micrometer (MIB Messzeuge GmbH, Germany).

### 2.8. Porosity, tensile strength and elastic recovery of the tablets

#### 2.8.1. Porosity

The true density, $\rho_t$, of the tablets was determined in triplicate by a helium pycnometer Micromeritics (AccuPyc 1330, USA). The porosity, $\varepsilon$, of the tablets was calculated by:

$$\varepsilon = 100\left(1 - \frac{\rho_c}{\rho_t}\right) \quad (1)$$

where, $\rho_c$ is the density of the tablet calculated as a (mass/volume). The mean of ten measurements was used.

#### 2.8.2. Tensile strength

The tensile strength of the tablets was calculated from

$$\mathrm{TS} = \frac{2F}{\pi dh} \quad (2)$$

where, $F$ is the crushing force, $d$ is diameter and $h$ is the thickness of the tablet (Sonnergaard, 2006). Tensile strength is the stress at which a material breaks or permanently deforms. It is an intensive property independent of the size of the tested tablet. It was measured diametrically using tablet hardness tester Vanderkamp (VanKel Edison, USA).

#### 2.8.3. Elastic recovery

This quantifies the degree of expansion after removal of the compression force. It was calculated from

$$\mathrm{ER} = \frac{h_{\mathrm{punch}} - h_{\mathrm{tablet}}}{h_{\mathrm{punch}}} \times 100 \quad (3)$$

where, $h_{punch}$ is the distance between punches (upper and lower) and $h_{tablet}$ the height of the tablet measured 1 day after compression.

## 3. Results and discussion

Our research in the field of celecoxib nanosuspension formulation is focused on the emulsion-diffusion method, in which the surface energy of the system increases during the process of crystallization. Small particles, which spontaneously aggregate to decrease the surface energy, were stabilized by a layer of surfactant or/and protective polymer. The selection of partially water-miscible solvent was critical to obtain nanoparticles. In general, solvents with high water miscibility and stabilizers able to form stable emulsions are preferred. Ethyl acetate was chosen from four candidate solvents as the most promising for production of celecoxib nanosuspension. Celecoxib is highly soluble in ethyl acetate which is partially miscible (1 part in 15) with water. If precipitation of celecoxib was carried out in pure water without stabilizer a fast crystal growth was observed. Three stabilizers (Tween® 80, PVP K-30 and SDS) were tested for their stabilization potential. Tween® 80 was chosen to prevent aggregation of nano-sized particles, and the rationale for using combination of SDS and PVP was based on combined electrostatic and steric stabilization. The combination of PVP and SDS was used for stabilization of nanosuspensions as was examined for drug/PVP/SDS ternary ground mixtures (Pongpeerapat et al., 2004).

### 3.1. Particle size of celecoxib nanosuspension

As described in Section 2.3 an emulsion of very small droplets of ethyl acetate with dissolved celecoxib was made by high pressure homogenization. Dilution with water resulted in rapid diffusion of ethyl acetate from the droplets precipitating celecoxib as nanoparticles. The characteristics of nanoparticles are shown in Table 1. Raw celecoxib particle size ranges from 1.7 to 11.2 μm, the size of the nanoparticles is approximately 10 times smaller (0.13 to 1.84 μm). All nanosuspensions were stable over 6 weeks at room temperature. Sedimentation of some material occurred after a few weeks but the sediment was easily redispersed by gentle shaking or short exposure of sample to ultrasound. Similar approach for preparation of griseofulvin nanoparticles precipitation from triacetin-in-water was published by Trotta et al. (2003).

### 3.2. The effect of particle size on celecoxib dissolution

While nano-sized celecoxib have been successfully produced, the question arises whether it has faster dissolution rate. Preliminary the solubility of celecoxib was determined in media with different pH and SDS concentration (Table 2) to assure sink condition for determination of dissolution profile.

**Table 2**
Solubility of celecoxib in medium with different pH and SDS concentration, $N = 3$.

| Solubility of celecoxib in different media | | |
|---|---|---|
| % SDS | pH | $c$ (mg/L) |
| 0 | 6.8 | 5.6 |
| 0.5 | 1.2 | 145.5 |
| 0.5 | 4.5 | 202.4 |
| 0.5 | 6.8 | 202.8 |
| 1.0 | 1.2 | 236.8 |
| 1.0 | 4.5 | 282.6 |
| 1.0 | 6.8 | 300.2 |
| 1.0 | 10.0 | 453.5 |

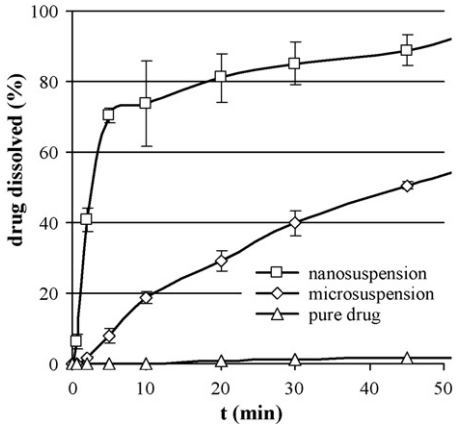


**Fig. 1.** The dissolution profiles of nano-sized celecoxib stabilized with Tween® 80 (–□–), the suspension of micronized celecoxib with the same amount of Tween® 80 (–◇–) and the pure celecoxib (–△–). The results were obtained with paddle dissolution test (900 ml of phosphate buffer, pH 6.8, 50 rpm, 37 °C, without surfactant in medium). Withdrawn samples were filtered through PTFE membrane filter with 0.2 μm pore size. Data points are means ± SD for separate triplicate samples in two parallel sampling. Error bars may be obscured by symbols.

As shown in Fig. 1 nano-sized celecoxib showed a dramatic increase of rate and extent of dissolution compared to micronized celecoxib with the same amount of Tween® 80 and much larger compared to celecoxib without Tween® 80. The slope of dissolution profile is especially different for nanoparticles in the initial stage (first 10 min). After that time the slope of dissolution profile was quite similar to micronized celecoxib. Dissolution rate of nano-sized celecoxib with Tween® 80 was maintained at a higher level throughout the experiment compared to micronized celecoxib. The nanoparticles exhibited higher surface-to-volume ratio enabling hydration over a larger surface area, which offered the potential for increased drug dissolution.

The dissolution of micronized celecoxib in water was barely observable. The slow dissolution can be partly attributed to its hydrophobicity as evidenced by poor wetting of the powder surface. This causes the particles to aggregate rather than disperse. Surfactants are well-known for the enhancement of wettability of drug, and thus their frequent use in poorly soluble formulations. Poor wettability of micronized celecoxib without Tween® 80 in aqueous medium was also observed from the dissolution experiments. Surfactants such as Tween® 80 enhance wettability and improve the *in vitro* dissolution of micronized celecoxib. Dissolved celecoxib amount after 45 min for nano- and micro-sized suspensions with Tween® 80 and pure celecoxib were 1.02, 0.79 and 0.03 mg/L, respectively.

### 3.3. The effect of filters on dissolution results

The rapidity of dissolution of nano-sized particles requests careful performance of dissolution tests. Modifying dissolution media enabling sink condition can cover the effect of particle diminution on the dissolution rate. Another critical process is the removal of un-dissolved nanoparticles from dissolution vessel withdrawn with the sample for determination of dissolved amount. Before HPLC analysis filtration through membrane filters or ultracentrifugation are commonly used. The problem with ultracentrifugation is that the smallest particles are very slow to sediment and further dissolution can occur over this time. So the dissolution rate cannot be determined in real time using ultracentrifugation for particles removal. The critical points in filtration are the pore size and adsorption of dissolved drug on filter membrane. Results of close examination of the different filter types and their pore size used for filtering samples are shown in Table 3. For each case volumes

**Table 3**
Effect of pore size and filter material on celecoxib filtrate concentration. 1 ml or 4 ml of filtrate was discarded before HPLC analysis. $N = 3$.

| Media | Waste | Water | | 0.1% SDS in water | | 0.5% SDS in water | | 1% SDS in water | |
|---|---|---|---|---|---|---|---|---|---|
| | | 1 ml | 4 ml | 1 ml | 4 ml | 1 ml | 4 ml | 1 ml | 4 ml |
| Filter | Pore size (μm) | Conc. (mg/L) | Conc. (mg/L) | Conc. (mg/L) | Conc. (mg/L) | Conc. (mg/L) | Conc. (mg/L) | Conc. (mg/L) | Conc. (mg/L) |
| Without filtering | / | 3.14 | 3.14 | 3.66 | 3.66 | 2.03 | 2.03 | 2.46 | 2.46 |
| PTFE[a] (SRP[b]) | 0.45 | 0.18 | 0.69 | 0.37 | 1.90 | 2.02 | 2.02 | 2.33 | 2.35 |
| PTFE[a] (SRP[b]) | 0.2 | 0.00 | 0.26 | 0.03 | 1.37 | 1.98 | 2.01 | 2.30 | 2.32 |
| cellulose acetate (CE[b]) | 0.2 | 0.00 | 0.00 | 0.00 | 0.00 | 1.78 | 1.92 | 2.16 | 2.26 |
| Cellulose acetate | 0.2 | 0.00 | 0.00 | 0.00 | 0.01 | 1.35 | 1.43 | 2.44 | 2.45 |

[a] PTFE—polytetrafluoroethylene.
[b] CE and SRP are Sartorius Minisart brands.

of 1 and 4 ml were rejected before analysis to estimate the effect of adsorption.

It can be seen that all filters strongly adsorb celecoxib especially when water was used as dissolution media. The effect of adsorption was reduced by increasing surfactant concentration. Furthermore, it is seen that higher concentrations were determined if more sample was filtered before it was injected in HPLC. Regarding the obtained results for further work PTFE filters (SRP 25, 0.2 μm, Sartorius, Goettingen, Germany) were used.

### 3.4. Spray drying of liquid nanosuspension

Spray drying is generally preferred over lyophilisation by pharmaceutical industry to transform liquid nanosuspension to a dry product, since it is less time and energy consuming. We observed that drying of the nanosuspension stabilized with Tween® 80 was problematic due to the sticking of the product to the wall of the drying chamber. This was not observed when nanosuspension was stabilized with PVP/SDS.

The ultracentrifuged PVP/SDS nanosuspension (prepared with 800 mg of celecoxib) was collected and redispersed in water to final volume of 40 ml and spray dried (Table 1). The size distributions $d$ ($v$, 0.1) and $d$ ($v$, 0.5) values were unaffected but $d$ ($v$, 0.9) increased after drying as consequence of aggregation as shown in Fig. 2c and d. The yield of spray drying was higher than 75%.

### 3.5. SEM studies

In order to characterize the morphology of raw and nano-sized celecoxib particles SEM imaging was performed. SEM images shown in Fig. 2 reveal distinct differences in the morphologies of samples. Lower magnification (Fig. 2a) shows that raw micronized celecoxib to be predominantly needle-shaped crystals with sizes between 2 and 100 μm. Particles of nano-sized celecoxib with Tween® 80 were collected on a 0.2 μm filter for SEM studies at higher magnification (Fig. 2b). The images showed some particles with size below 200 nm together with larger plates. PVP/SDS nanoparticles taken after ultracentrifugation and spray drying were thin plates (Figs. 2c and d) aggregated into spheres. All images revealed sub-micrometer particles in at least one dimension but almost all are very thin, estimated to 50 nm. The images correlate with the assessment of the particle size presented in Table 1. X-ray powder diffraction analysis of nano- and micronized celecoxib particles revealed the same crystalline state (data not shown).

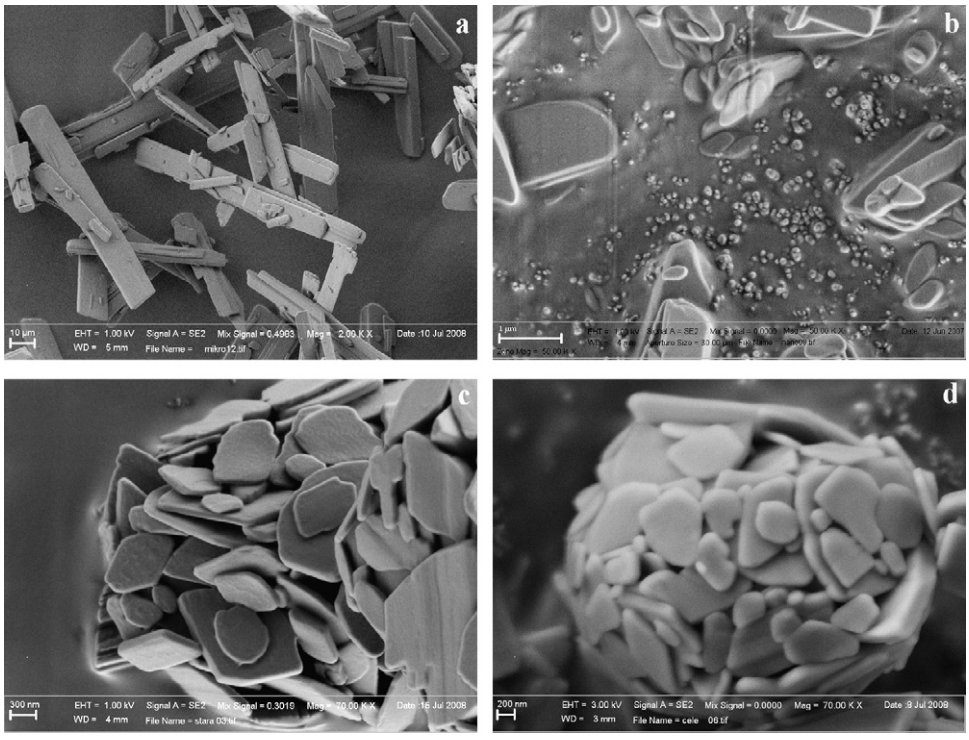


**Fig. 2.** SEM images of celecoxib particles: (a) raw celecoxib (size bar 10 μm); (b) Tween® 80 nanosuspension (size bar 1 μm); (c and d) spray-dried nanosuspension stabilized with PVP/SDS (size bars 300, 200 nm respectively).

### 3.6. Thermal behaviour of celecoxib

Additionally, the thermal behaviour of celecoxib was studied in order to screen its differences in solid state. DSC curves are shown in Fig. 3. Raw celecoxib (Fig. 3a) shows a single endothermic peak with single melting point ($T_{onset}$) at 162.03 °C. This agrees with published results. If polymorphs and pseudopolymorphs had been present the endothermic peak would shift to lower temperatures. Amorphous celecoxib has higher solubility and lower melting point than crystalline (Chawla et al., 2003). This apparent advantage is outweighed by its instability during storage and use. The amorphous form has a higher energy state, due to lack of long range order, shown as molecular mobility leading to crystal formation. Crystalline nano-sized celecoxib has both desirable properties of stability and extremely rapid dissolution.

The physical mixture of raw celecoxib with Tween® 80 exhibited a large endothermic peak at $T_{max}$ 146.53 °C (Fig. 3b). A similar endothermic peak was also detected on the DSC curve of nanosuspension with Tween® 80 ($T_{max}$ 141.25 °C) (Fig. 3b), where additionally a narrow endothermic peak occurred at 69.61 °C. The large peaks may indicate drug–surfactant interaction or the dissolution of celecoxib in Tween® 80 at high temperature. The peak at 69.61 °C for the nanosuspension could be the elastic relaxation caused by the thermal history of the sample. In this case the peak should not appear if the sample is cooled and re-heated. Although the 69.61 °C peak was absent, another two peaks appeared at 66.22 and 52.32 °C (Fig. 3b).

No corresponding events were observed for nanoparticles produced with PVP and SDS (Fig. 3c). Nanosuspension stabilized with PVP and SDS was chosen for further tabletting studies because of this absence of drug–stabilizer interaction.

DSC indicated that both celecoxib nanoparticles stabilized with PVP/SDS (Fig. 3c) and micronized particles were crystalline and no amorphous form had been created during their formation. The melting point of nanosuspension stabilized with PVP/SDS after ultracentrifugation was 159.29 °C which is 2.5 °C lower than raw celecoxib (Fig. 3c). Lower $T_{onset}$ could indicate a different crystalline state or the presence of PVP and SDS remaining bound to the nanoparticle surface. No glass transitions were noticed on the DSC curve that would indicate amorphous structure, and cooling and re-heating resulted in no peak shifts. The formation of a different state is therefore unlikely suggesting that the shift in the $T_{onset}$ is the consequence of a small amount of PVP and SDS bounded to celecoxib nanoparticles.

Since MCC was used in the tabletting process, DSC curves of MCC with micro- and nano-sized celecoxib were also recorded (Fig. 3d). These did not show any significant interaction. The very wide and small endothermic event below 100 °C probably indicates water evaporation from MCC as it disappeared after cooling and re-heating.

### 3.7. Tablets with nano-sized celecoxib

The compressibility and compactibility of dry powder blend are critical factors in tablet production. Compressibility is the ability to deform under pressure and compactibility describes the ability to form coherent tablets (Sonnergaard, 2006). It is known that for most powdered pharmaceuticals, compaction of smaller particles results in harder tablets because of the high area available for binding (Sun and Grant, 2001). In our study tablets of approximately the same hardness (70 ± 2N) were prepared. Results showed that much lower compaction force was needed for dried nanosuspension–MCC blend than for micronized celecoxib–MCC.

To remove the dependence of the tablet size to the hardness the tensile strength was determined and presented against compaction pressure (Fig. 4). The tensile strength of a tablet is

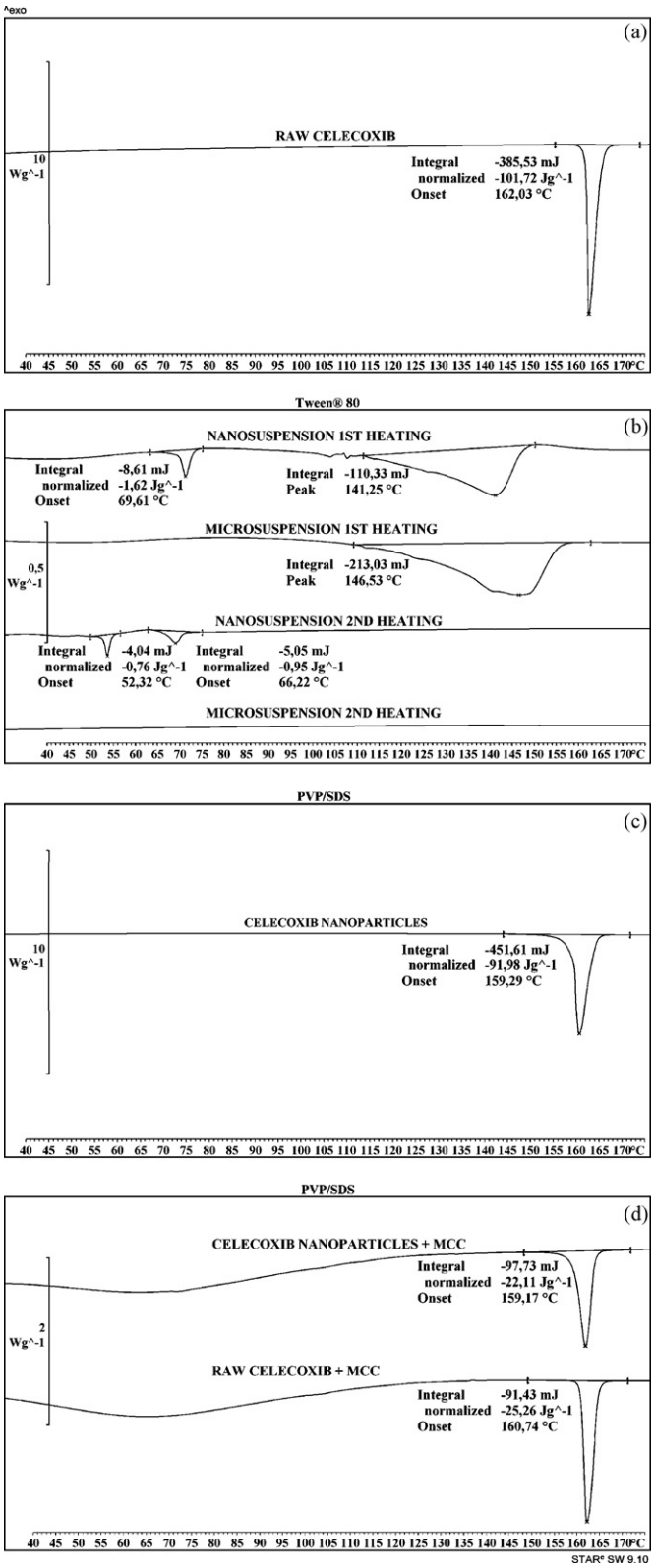


**Fig. 3.** DSC curves: (a) raw celecoxib; (b) nano-sized celecoxib stabilized with Tween® 80 and suspension of raw celecoxib with the same amount of Tween® 80; (c) dry nano-sized celecoxib particles stabilized with PVP/SDS; (d) physical mixture of nano-sized and raw celecoxib with MCC in mass ratio 1:3.

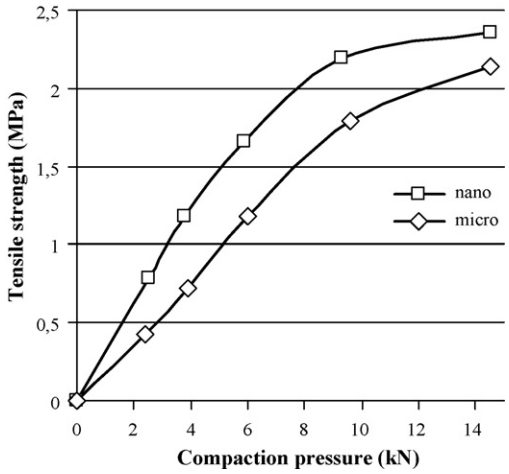


**Fig. 4.** Relationship between tensile strength and compaction pressure for MCC tablets containing nano-sized celecoxib stabilized with PVP/SDS (–□– nano) and micronized particles of celecoxib (–◊– micro). Ratio MCC to celecoxib was 3:1.

independent of tablet size and is a measure of the bonding strength between particles within the tablet. The mechanical strength depends on particle size and shape which determine number of contact points between particle surfaces generated during compaction. Tensile strength increased sharply at low compaction pressure and its slope decreases after 10kN of compaction pressure for both tablets. At a given compaction force the tensile strength is greater for tablets with nanoparticles, indicating stronger interparticular interactions. As shown in Figs. 2c, d and on 5c, d the nano-sized celecoxib exists as thin plates with a high surface-to-volume ratio enabling numerous contact points for bonding. For quantitative relationship between compaction pressure and mechanical (tensile) strength different methods and mathematical models were developed (Sonnergaard, 2006). A simple linear relationship between compaction pressure and tensile strength up to 310 MPa was found for lactose monohydrate tablets (Ellison et al., 2008). In our case we have observed almost linear relationship between compaction pressure and tensile strength up to approximately 2 MPa.

Elastic recovery was higher in the case of tablets compacted with micronized celecoxib than nano-sized (Table 4). This indicates, once again, that nanoparticles interact with MCC filler stronger than microparticles. Tablets with micronized celecoxib need to be compacted with higher forces to reach the same hardness as tablets with nanoparticles. Higher forces result in greater deformation and more elastic behaviour of tablets after the compacting force is removed.

### 3.8. Surface morphology of tablets

SEMs of the tablet surfaces are shown in Fig. 5. Fig. 5a shows tablet compressed from MCC alone to enable its identification in the images with celecoxib (Fig. 5b–d). Tablet containing micronized celecoxib showed large, partly fractured crystals enclosed in MCC (Fig. 5b). Nano-sized celecoxib appeared as fine particles with irregular distribution amongst deformed MCC (Figs. 5c and d).

**Table 4**
Diameter, height, porosity and elastic recovery of the tablets composed of celecoxib and MCC in ratio 1:3 with hardness 70 ± 2N is shown.

| Tablet parameters tablets with MCC and, etc. | Diameter (mm) | Height (mm) | Porosity (%) | Elastic recovery (%) |
|---|---|---|---|---|
| Raw celecoxib | 12.04 | 2.64 | 16.12 | 55.3 |
| Nano-sized celecoxib | 12.04 | 2.99 | 26.10 | 35.9 |
| Only MCC | 12.04 | 3.33 | 32.16 | 24.9 |

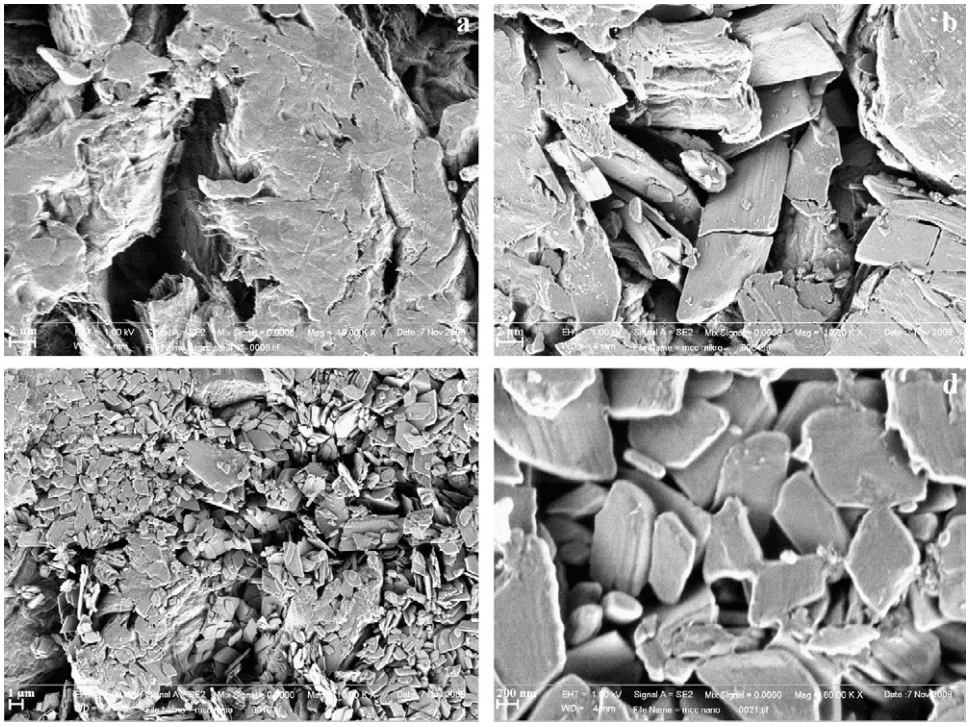


**Fig. 5.** SEM images of tablet surfaces. (a) MCC; (b) MCC with micronized celecoxib; (c and d) MCC with nano-sized celecoxib. Magnification 10 KX for (a–c), 80 KX for (d). Size bars represent 2 μm (a and b), 1 μm (c) and 200 nm (d). Tablets consist of celecoxib and MCC in ratio 1:3 with tablet hardness 70 ± 2N.

### 3.9. Dissolution of celecoxib in tablets

Incorporated amount of drug in the tablets was 100 mg which is the same as pharmacological dose of marketed capsules. Dissolution studies are very challenging issue because of the low solubility of celecoxib at physiological conditions. The paddle apparatus is intended to operate under sink conditions normally considered to occur in dissolution medium at least 3 times the saturation volume. Regarding published literature data different dissolution medium were used to determine the dissolution rate of pharmaceutical preparations with celecoxib. The dissolution media contained different solubilizers, mostly SDS in different concentrations (0.5–2%) and different pHs (Babu et al., 2002; Homar et al., 2007).

Our dissolution studies have been performed in media with different pH and SDS concentration (Table 2). Regarding celecoxib amount in tablet (100 mg) and its solubility 453.5 mg in phosphate buffer medium with pH 10 and 1% SDS this medium was used to ensure sink conditions during the dissolution rate testing. The experiment revealed higher dissolution rate for tablets containing dried nanosuspension compared to those containing micronized drug (Fig. 6a). The amount of dissolved celecoxib after 120 min for tablets with nanoparticles and micronized particles were 96 and 84 mg/L respectively.

Another medium with pH 6.8 and 0.5% SDS was used under the same conditions to compare the dissolution rate of celecoxib tablets at more physiological pH. In that case the difference between dissolution rates of tablets with nano- and micro-sized particles was even higher (Fig. 6b) compared to Fig. 6a results. The concentrations after 120 min for tablets with nanoparticles and micronized particles were 78 and 69 mg/L respectively. According to this data it is expected that differences between tablets with nano- and micro-sized particles will even increase at more acidic conditions and the relevance of particle diminution will be even more valuable. On the basis of DSC curves we can also conclude that the improvement of celecoxib dissolution rate was mainly caused by an increased surface-to-volume ratio and wettability. Celecoxib nanoparticles with size distribution as reported in Table 1 are obviously not small enough to determine an appreciable solubility increase.

Irrespective to pH, statistically significant differences were observed for both dissolution testing which indicated that the nanoparticles in tablets compared to microparticles larger available amount of celecoxib for absorption due to the faster drug dissolution. In such situation *in vivo* celecoxib will be absorbed very rapidly increasing the probability of achieving an effective concentration in plasma.

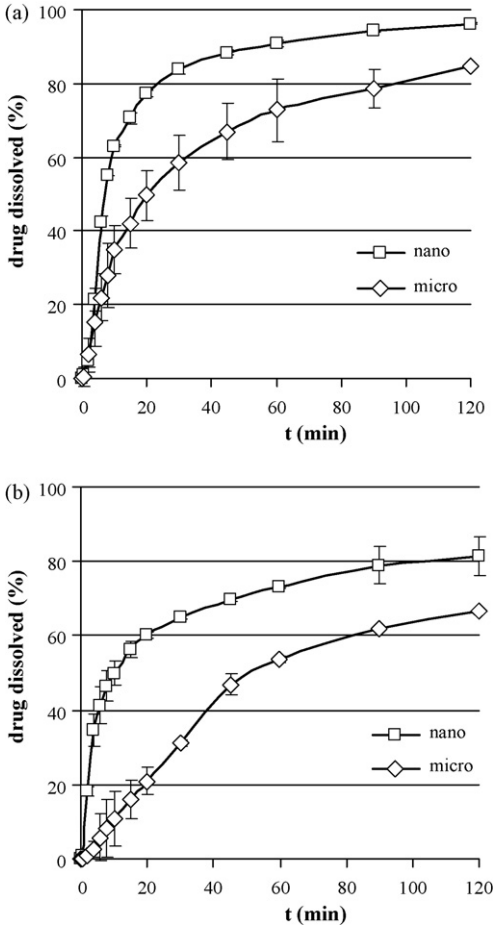


**Fig. 6.** The dissolution profiles of tablets with nano-sized (–□– nano) or micronized celecoxib particles (–◊– micro). Tablets were composed by celecoxib and MCC in ratio 1:3, with approximately the same hardness (70 ± 2N) and disintegrated in less than 10 min. The results were obtained with paddle dissolution test: 50 rpm and 37 °C, 900 ml of phosphate buffer (a) pH 10, 1.0% SDS; and (b) pH 6.8, 0.5% SDS. Data points are means ± SD for separate triplicate samples in two parallel sampling. Error bars may be obscured by symbols.

## 4. Conclusions

Dry nanoparticles of poorly water soluble drugs can be prepared using an emulsion-diffusion method with surfactant or hydrophilic polymer stabilizer followed by spray drying. Careful selection of homogenization procedure and stabilizer are critical, firstly to achieve stabilization during controlled crystallization and secondly to increase the wettability of hydrophobic drug in dissolution medium. Nano-sized celecoxib dissolved much more rapidly than micronized. SEM images reveal distinct differences in the morphological structure of nanoparticles influenced by the stabilizers. Markedly lower compaction forces are needed to compress tablets with nano-sized compared to micro-sized celecoxib powder to produce tablets of equal tensile strength. Clearly, these findings indicated the suitability of formulation procedure for preparation of nano-sized poorly water soluble drug with significant improvement of the *in vitro* dissolution rate, and thus possibly improve their oral bioavailability.

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