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Microcrystals for dissolution rate enhancement of poorly water-soluble drugs

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Abstract

Slight dissolution rates related to poor water-solubility are one of the well-known difficulties to be covered during the development of new drug substances. The poorly water-soluble drug ECU-01, a low molecular enzyme-inhibitor with anti-inflammatory properties for oral administration, shows a poor dissolution rate. This study is intended to enhance the drug dissolution rate by using microcrystals. The common way for micronization is the milling of previously formed larger crystals. However, milling shows several disadvantages as the newly created surfaces are thermodynamically activated due to the high energy input and not naturally grown. In this study microcrystals were not produced using any cutting up techniques, but only by association. Naturally grown microcrystals were prepared by a precipitation method in the presence of stabilizing agents (e.g. gelatin, chitosan, different types of cellulose ethers) followed by spray-drying of the formed dispersion. First the drug was dissolved in acetone and then precipitated by rapid pouring an aqueous solution of the stabilizer into the drug solution. Particularly, cellulose ethers were able to form stable and homogeneous dispersions of microcrystals (mean particle size = 1 μm) showing a tight particle size distribution. By spray-drying, the drug powder was obtained. The dissolution rate is significantly enhanced (common drug: 4% after 20 min/microcrystals 93% after 20 min) due to the large surface, which is hydrophilized by adsorbed stabilizers as shown by the decreased contact angle (65 and 30°, respectively).

Keywords: Micronization; Microcrystal; Dissolution rate enhancement; Poorly water-soluble drug

1. Introduction

The bioavailability of poorly water-soluble drug substances (like many newly developed pharmaceutically active molecules) is a well-known difficulty to be coped with during the development of new drug substances. For class II-drugs, according to the biopharmaceutics classification system (Löbenberg and Amidon, 2000), the dissolution rate is the limiting factor for the drug absorption rate. Also for class IV-drugs the dissolution rate can be the limiting factor. An enhancement in dissolution rate is important to attain suitable blood-levels of these drugs. Several methods are existent to achieve a higher solubility or a higher dissolution rate of a drug. If the drug substance molecule shows a basic or acid property, water-soluble salts can be formed. Because of the precipitation of the hydrophobic molecule from the solution previously formed during the passage of the GI-tract, the bioavailability of these salts can be lowered. Another chemical modification is the formation

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of prodrugs. Produgs have to be activated by enzymes. Thus, intact enzymes are necessary, and interindividual differences in metabolism and drug–food interactions can cause problems.

Galenical methods are more convenient to solve bioavailability problems. For example, the free energy of the system can be increased by the use of polymorphic crystals or amorphous forms, as realized in solid dispersions (Chiou and Riegelman, 1971). However, manufacturing difficulties, problems in scaling up, and stability problems limit their commercial use (Serajuddin, 1999). A recovery of the system into a thermodynamically stable form has to be prevented, thus a high amount of excipients (e.g. PEG or PVP) is necessary. The dissolution rate can also be optimized by special crystallization techniques, which influence the crystal habit and the crystal surface (Rasenack and Müller, 2002). A method for increasing the solubility of a drug is the solubilization. This method is used in liquid preparations for oral or i.v. drug delivery. Solubility can also be increased by forming a complex with cyclodextrins (Szejtli, 1993). However, cyclodextrin preparations have several disadvantages, as the drug-load is low and this method only works with drugs which fit into the cavity of the cyclodextrin and which have a high complex-forming constant. The molecular structure, the polarity, the size and the possibility for interactions with the cyclodextrin molecule are important factors determining the success of cyclodextrin-preparations.

A common method for increasing the dissolution rate is the forming of a high specific surface area by micronization. The process which is usually used to obtain small particles is the disruption of large crystals. Chaumeil (1998) describes the improvement in dissolution rate and in bioavailability by micronization of sparingly water-soluble drugs using jar mills and fluid energy mills. Cospite and Dominici (1989) describe a better clinical efficacy for micronized diosmin compared with the nonmicronized drug. Beside this advantage of micronized drugs, several disadvantages resulting from the preparation process are existent. The micronization process using mills is extremely inefficient (Parrott, 1990). Due to a high energy input, disruptions in the crystal lattice can cause physical or chemical instability. Disordered regions in the resulting product are thermodynamically unstable. Ticehurst et al. (2000) describe revatropate hydrobromide that was micronized in a jet mill. Disordered structures were detected and analyzed by dynamic vapor sorption analysis. Amorphous or disordered material will recrystallize, especially when water from the atmosphere is adsorbed. Because of a reduction of the glass transition temperature, the energy threshold to recrystallization is decreased (Elamin et al., 1995). The conversion of crystalline solid surfaces into partially amorphous solid surfaces leads to a “dynamic nature” of the micronized drug (Ward and Schultz, 1995). In addition to this, disordered structures in the material can also influence the performance in formulations (Buckton, 1997). Surface energy changes can also influence processing properties such as the powder flow. Micronized powders with a higher energetic surface (measured by inverse gas chromatography) show poorer flow properties (Feeley et al., 1998). Due to their high specific surface, micronized particles are often agglomerated. Because of fracture, electrostatic effects can occur. An increase in the electron donation of the surface of milled DL-propranolol can be detected using the IGC (York et al., 1998). A further disadvantage of jet milling processes is a broad size distribution (Müller et al., 1996). Another milling technique is the high pressure homogenization. Nanosuspensions can be produced using milling by high pressure homogenizers (Jacobs et al., 2000; Müller et al., 2001). The drug crystals, which have a starting size that is preferably as small as possible, are suspended in an aqueous surfactant solution. This dispersion is homogenized at pressures of approximately 1000 bar with up to 10 cycles, causing a high shear strain of the drug. These high pressures (power density $= 10^{13}$ W/cm^3, nearly the same as in a nuclear power station, Müller et al., 1999) cause changes in the crystal structure, the amorphous fraction in the particle increases. Ideally completely amorphous particles are obtained. As a disadvantage of all milling processes the product can be afflicted with impurities due to abrasion.

Beside these cutting up methods, some techniques for the preparation of small particles by precipitation processes are described in the scientific literature. Drug nanoparticles, which contain the drug in an amorphous form as in the case of hydrosols (Gaßmann et al., 1994), are obtained. So, colloidal suspensions are yielded, which are suitable formulations for the parenteral application of poorly water-soluble drugs.

The stabilizing agents used are for example poloxamer and modified gelatins. For stabilizing the hydrosol, it is spray-dried with excipients such as lactose or mannitol immediately after precipitation. According to this principle, colloidal dispersions of β-carotene are produced. Amorphous carotenoid nanoparticles contain high amounts of stabilizing agents such as gelatin and softening agents such as sugar. The amount of β-carotene is approximately 15% (Horn, 1989; Auweter et al., 1999). Micronized drugs can also be prepared using supercritical carbon dioxide (Steckel et al., 1997; Kerc et al., 1999). A disadvantage of this technique is the high machine expenditure.

The aim of this study was to prepare microcrystals of the poorly water-soluble (0.0003 g/100 ml) drug ECU-01, an anti-inflammatory drug in preclinical state of development, without the use of any milling methods in order to enhance the dissolution rate of the drug. The common crystals of ECU-01 have a mean particle size of 20 μm. In terms of the nearly cuboid-like form of ECU-01 (Fig. 1), their specific surface area is relatively low (0.19 m^2/g) in spite of the particle size. The drug substance can also exist in a polymorphic crystal form which has a needle-shaped appearance resulting in a high increase of the specific surface area (2.34 m^2/g). However, these crystals are thermodynamically unstable and galenically unacceptable due to their felted nature (data unpublished). Thus, to obtain crystals with an increased surface, the particle size has to be reduced. By the use of the precipitation method employed in this study small particles were obtained. The resulting product contains the drug in crystalline form, the drug-load is >90% (m/m). The preparation method is easy to handle and needs only a common equipment.

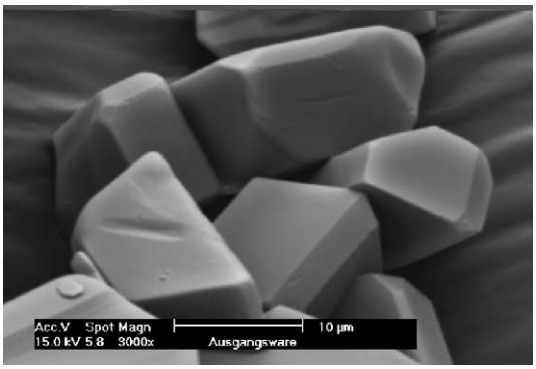

Fig. 1. Nonmicronized drug substance.

2. Materials and methods

2.1. Materials

The new drug substance ECU-01, a low molecular enzyme-inhibitor with anti-inflammatory properties for oral administration, was provided by Elbion AG (Radebeul, Germany). ECU-01, a heterocyclic derivate of a 3,5-dichloro-pyridine, is a poorly water-soluble drug substance. Acetone (Merck KG, Darmstadt, Germany) was of analytical grade. Water was used in double-distilled quality. Employed stabilizing agents were agar (Merck KG), calcium caseinate (Lactonat®EC, Lactoprot, Kaltenkirchen, Germany), chitosan (degree of deacetylation: 93.2%; 12.5 mPa s; Chitopure xp; Fish Contract, Bremerhaven, Germany), dextran 200 (Sigma, Deisenhofen, Germany), gelatin A (Merck KG), hydoxyethylcellulose (HEC; Natrosol®Pharm G, Natrosol®HHX, Hercules, Wilmington, USA), hydroxyethylstarch (HES 130, HES 450, Fresenius Kabi, Bad Homburg, Germany), hydroxypropylcellulose (HPC; Klucel® GF, Klucel® LF, Hercules, Wilmington, USA), methylcellulose (MC; Tylose® M 4000, Clariant, Frankfurt, Germany), methylhydroxyethylcellulose (MHEC; Tylopur® MH 50, Tylopur® MH 4000, Clariant, Frankfurt, Germany), hydroxypropylmethylcellulose (HPMC; Metolose® 60 SH 15, 4000, 10000, Shin Etsu, Tokyo, Japan), sodium carboxymethylcellulose (NaCMC; Tylopur® C 30, Clariant, Frankfurt, Germany), sodium alginate (Sigma, Deisenhofen, Germany), pectin (pectin Classic AU 204, low viscosity, Herstreith & Fox KG, Neuenburg, Germany), polyvinylalcohol (PVA; Sigma, Deisenhofen, Germany), and polyvinylpyrrolidone (PVP; Kollidon®30, BASF AG, Ludwigshafen, Germany). In the case of chitosan and gelatin, the pH was adjusted to 3.8 (acetic acid, Merck KG) and 2.4 (hydrochloric acid, Merck KG).

2.2. Crystallization procedure

Crystallization was carried out using the solvent change method by instantaneously mixing two liquids in the presence of a stabilizing agent. In the first step

ECU-01 was dissolved in acetone (1%). The stabilizing agents were dissolved in water (0.025%). Afterwards, 200 ml of the aqueous solution were poured rapidly under stirring conditions into 50 ml of the drug solution. For comparison of the stabilizing effects, also pure water was used. The particle size in the resulting dispersions was determined after 0, 60, and 120 min. A dispersion prepared using hydroxypropylmethylcellulose (HPMC 15) was spray-dried (Büchi 190 Mini Spray-Dryer, Büchi Labortechnik AG, Flawil, Switzerland). A micron-sized drug powder with a drug load of 90.08% is obtained. In this study, the spray-drying process was not employed to form particles as if solutions are spray-dried, but only to dry previously formed particles. In terms of reaching a tight particle size distribution, performing by the direct current spray-drying principle is possible as a suspension in spray-dried. Therefore, the product is exposed to less thermal stress. Because of the small particles, a high stream of volume (40 m^3/h) was used to obtain a higher yield.

2.3. Product characterization

2.3.1. Particle size

The volume particle size distribution was measured using a laser diffractometer (Helos, Sympatec GmbH, Clausthal Zellerfeld, Germany). The dispersions were diluted with water and measured in a cuvette. Spray-dried microcrystals were previously suspended in water. As a second determination method, microcrystals were measured in dry powder form after dispersing in air using compressed air (Helos Rodos). Particle size distribution is characterized by the X10 (10% below this size), the X50, X90 and the X99-value.

2.3.2. Powder dissolution

Dissolution studies were carried out in simulated gastric fluid according to the paddle method (USP) using an Erweka DT6 dissolution apparatus (Erweka, Heusenstamm, Germany). The stirring speed employed was 100 rpm, and the temperature was maintained at 37 ± 0.5 °C. Quantification of the dissolved amount of drug was carried out photometrically at 284 nm (Lambda40 UV-Vis Spectrometer, Perkin-Elmer, Connecticut, USA). All samples were analyzed in triplicate. The dissolution medium consists of 0.25% sodium dodecyl sulfate and 0.2% sodium chloride and is adjusted with hydrochloric acid to pH 1.2 ± 0.1 (all Merck KG). Surface tension is lowered by SDS in an attempt to mimic in vivo conditions as described by Pedersen et al. (2000). Because of their physiological relevance for dissolution testing of poorly water-soluble drugs the addition of a surfactant is generally to be preferred to e.g. hydroalcoholic mediums (Shah et al., 1989).

2.3.3. Scanning electron microscopy (SEM)

Scanning electron micrographs were taken using a Philips XL 20 (Philips, Eindhoven, The Netherlands). Samples were fixed on an aluminum stub with conductive double sided adhesive tape (Leit-Tabs, Plano GmbH, Wetzlar, Germany) and coated with gold in an argon atmosphere (50 Pa) at 50 mA for 50 s (Sputter Coater, Bal-Tec AG, Liechtenstein).

2.3.4. Specific surface area

The specific surface area was determined using the gas adsorption method. Calculation is based on the BET equation. A Surface Area Analyzer Gemini-2360 (Micromeritics Instrument Corporation, Norcross, USA) was employed.

2.3.5. Contact angle

The contact angle was measured by the sessile drop technique using a goniometer (G1, Krüss GmbH, Hamburg, Germany). A compressed disc of the powder (100 mg) was made at 30 kN for 90 s under vacuum. The contact angle between the disc and a single drop of water (25 μl) was determined 10 and 180 s after the droplet was put onto the disc. Determination was repeated 20 times.

2.3.6. Differential scanning calorimetry (DSC)

A differential scanning calorimeter (DSC7, Perkin-Elmer) was used. The equipment was calibrated using indium and zinc. Samples were heated at 10 °C/min in aluminum pans under nitrogen atmosphere. The onsets of the melting points and enthalpies of fusion were calculated by the software (Pyris, Perkin-Elmer).

2.3.7. X-ray diffractometry

Powder X-ray diffraction (PXRD) patterns were collected in transmission using an X-ray diffractometer with a rotating anode (Stoe and Cie GmbH,

Darmstadt, Germany) with Cu $K\alpha_1$ radiation (monochromator: graphite) generated at 200 mA and 40 kV. Powder was packed into the rotating sample holder between two films (PETP).

3. Results and discussion

During the crystal precipitation, a hydrophobic surface is formed. Due to the surface energy, the energy of the system increases. Thus, a stabilizing agent—provided that it has any affinity to the surface—covers the newly formed surface spontaneously. Thereby, the surface energy and consequently the enthalpy of the system are lowered. The small particles, which normally would aggregate in order to lower the surface energy, are stabilized sterically against crystal growth by a layer of protective polymer (Schott, 1985).

The first objective was to find stabilizing agents which can stop the molecular association and the crystal growth by forming a protective layer around the nucleation germs in order to obtain micron-sized crystals. In Fig. 2a, the particle size distribution is shown determined 60 min after precipitation. Differences can be clearly discerned. Some stabilizing agents (e.g. some cellulose ethers or PVA) stabilize the newly formed crystals and prevent a crystal growth. The X50 value is approximately 1 μm and a tight particle size distribution is obtained. If precipitation is carried out with pure water, a crystal growth is observed. Very similar particle sizes are observed in relation to pure water if agar and sodium carboxymethylcellulose are applied. Dextran, hydroxyethylstarch and PVP, which are classic crystallization delaying agents, take an intermediate position. In the presence of the positively charged macromolecules gelatin and chitosan particles with a mean size of 9 and 14 μm, respectively are formed after 60 min. The possibility of electrostatic repulsion did not lead to such a decrease of particle size as, e.g. HPMC. Only for HPMC, MHEC, and PVA a

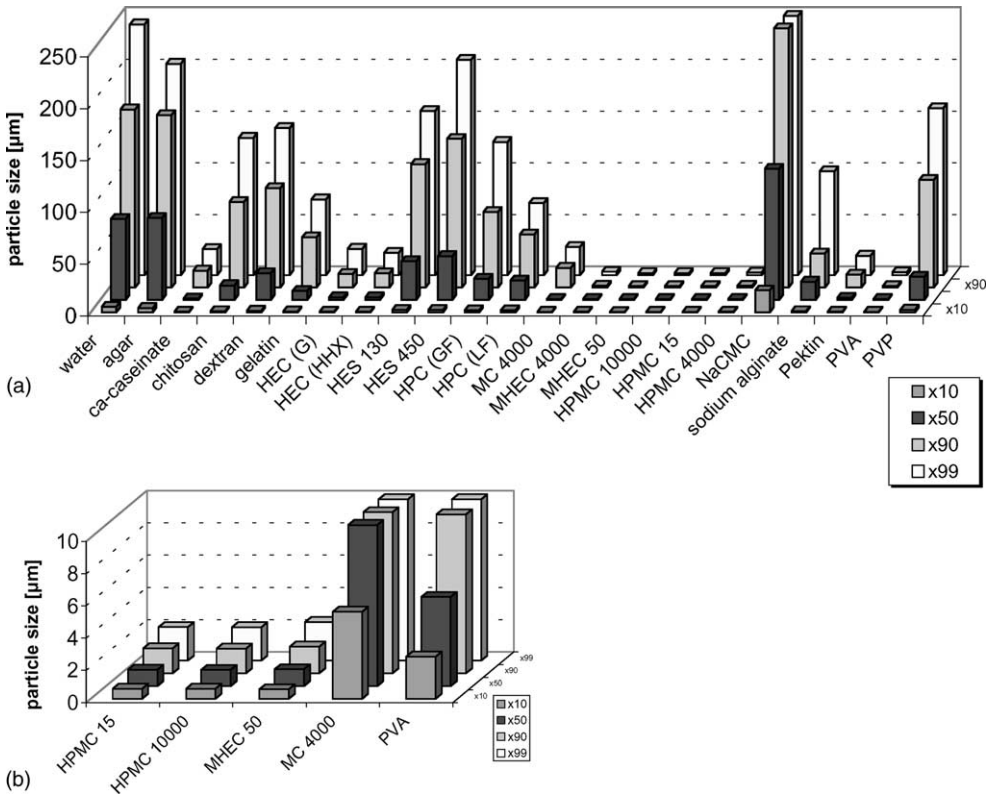

Fig. 2. Particle size distribution (dispersion): (a) 60 min after precipitation and (b) 120 min after precipitation.

mean particle size lower than 5 μm was achieved after 120 min (Fig. 2b). In the case of PVA a slight increase in particle size occurred during this time. The use of HPMC or MHEC resulted in effective stabilized small particles which did not show a significant change in particle size when comparing minute 60 and 120. From these results it can be concluded that the affinity of the stabilizing agent to the newly formed crystal surface is decisive. Crystal growth is protected by the adsorbed stabilizers. A dependency of the resulting particle size on the viscosity of the precipitating liquid (determined using an Ubbelohde-viscometer) cannot be found. Plotting the mean particle size against the dynamic viscosity the correlation coefficient $R^2 = 0.005$ is calculated by linear regression. Additives with a mainly hydrophilic molecule structure (e.g. agar, dextran, chitosan, hydroxyethylstarch and sodium carboxymethylcellulose) do not stabilize the formed crystals effectively. If a hydrophobic substituent is inserted to the stabilizer-molecule, the drug particle size decreases (HPMC, MHEC versus HPC). The difference between HPMC and MC can be explained with a different degree of substitution (DS). To obtain a water-soluble molecule, in MC the DS cannot be higher than 1; because of the hydroxypropyl groups, in HPMC the amount of methyl groups is higher (DS = 2). From the effectiveness of HPMC it can be inferred that molecules with a hydrophobic substituent and with hydratable functional groups are useful stabilizers of hydrophobic crystals. HPMC is a cellulose ether which is water-soluble and also soluble in acetone. Thus, it is not precipitated during the solvent change process. As HPMC shows surface activity (Chang and Gray, 1978), it can be adsorbed onto the newly created surface of the precipitated drug in order to lower the interfacial tension. Especially, cellulose ethers containing methoxyl or hydroxypropyl groups are adsorbed onto hydrophobic solid surfaces while more hydrophilic derivates (as NaCMC) show lower tendency for adsorbing onto the solid–liquid interface as described for hydrophobic silicon dioxide (Daniels and Barta, 1994). The effect of water-soluble cellulose polymers on suspension stability has been described by Law and Kayes (1983). The polymers are adsorbed onto polystyrene lattices and ibuprofen, which are put into a solution of the polymer. The redispersability of the suspensions is influenced due to steric stabilizing effects. In vitamin production nanoparticles are stabilized with gelatin. Here high amounts of gelatin (drug:gelatin = 1: 2.5) and further additives are necessary (Horn, 1989). These preparations are amorphous and can be called embeddings. In Fig. 3, the particle size distribution of ECU-01, 60 min after precipitation with pure water, is illustrated. In contrast, a dispersion prepared with HPMC shows a tight particle size distribution (Fig. 3). Two hours after the drug was precipitated, a homogeneous suspension with a mean particle size of 1.1 μm is still stable.

After spray-drying the formed dispersion, microcrystals were obtained as illustrated in Fig. 4. Uniformly formed crystals can be detected. In spite of carrying out the spray-drying according to the direct

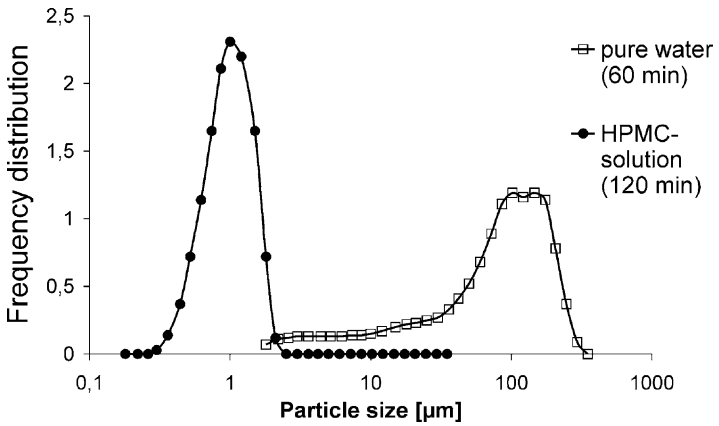

Fig. 3. Volume particle size distributions of the dispersion (60 min after precipitation with pure water and 120 min after precipitation with HPMC solution).

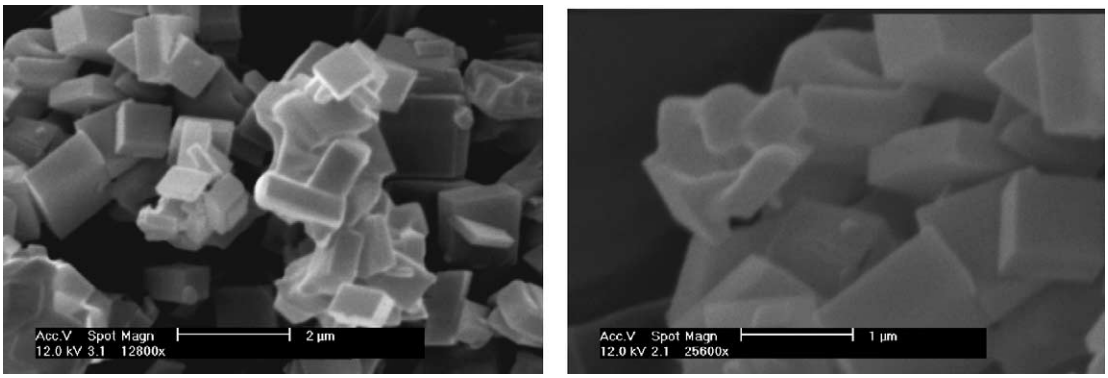

Fig. 4. SEM photographs of spray-dried microcrystals.

current principle, the size distribution is tight due to the fact, that the particle size is determined by the previously prepared dispersion. The crystals are covered with HPMC, no pure existing HPMC can be detected. Also the microcrystals do not have not such a smooth surface as the nonmicronized drug (Fig. 1). The crystals are isomorphic with the nonmicronized drug, the thermodynamically stable form polymorph I is formed as X-ray and DSC analysis showed. The whole drug crystallized, and based on the calculation of the heat of fusion no amorphous content was detected. The particle size distribution of the microcrystals is uniform as shown in Fig. 5. Because of the high surface area, some crystals are agglomerated.

After dispersing the crystals in water (without further excipients normally used in suspensions), a stable dispersion with a symmetric and tight particle size distribution is obtained. No crystal growth occurs. Four weeks after dispersing the spray-dried microcrystals in water, a partial sedimentation was observed, as was to be expected. But due to the homogeneous particle size, no caking occurred. After shaking, the dispersion (Fig. 6) was obtained. The particle size had not changed during the 4 weeks. The crystals are effectively protected against crystal growth. The protective layer on the crystal surface, which is further fixed on the crystals by the drying process, forms a diffusion barrier against Oswald ripening. In addition, a tight particle size distribution prevents Oswald ripening.

The microcrystals show a dramatic enhancement in dissolution rate (Fig. 7). Even when comparing with the unstable polymorph II, which has a lower lattice

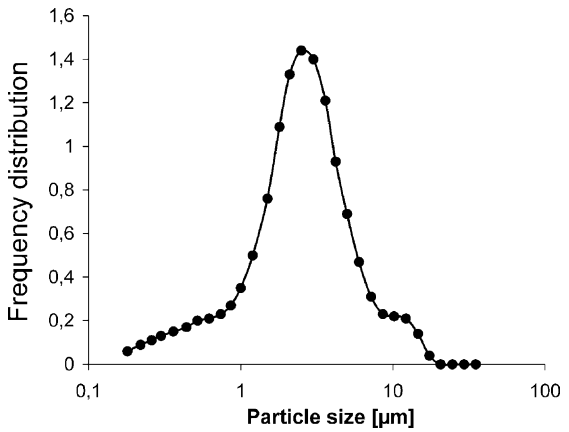

Fig. 5. Particle size distribution of the microcrystals (dispersed in air).

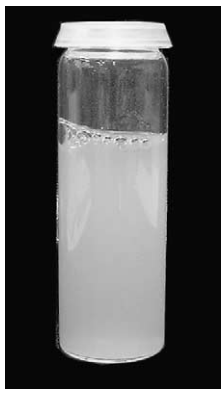

Fig. 6. Spray-dried microcrystals, dispersed in water (after 28 days).

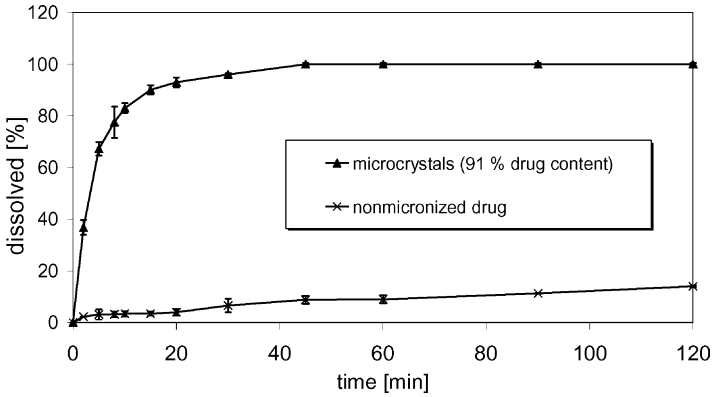

Fig. 7. Powder dissolution.

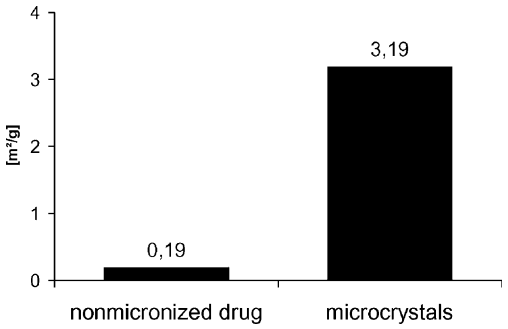

Fig. 8. Specific surface area.

energy, a further improvement in drug dissolution was reached (data unpublished). This effect can be explained by an increased specific surface (Fig. 8). This high surface is hydrophilized, the contact angle is decreased due to adsorbed hydrophilic HPMC (Fig. 9). Thus, by precipitating microcrystals in the presence of stabilizing protective polymers, a high and hydrophilized surface can be formed in a one process step without using any milling techniques. The microcrystals are suitable drug formulations for drug dissolution enhancement. They can be used in solid dosage forms or in suspensions.

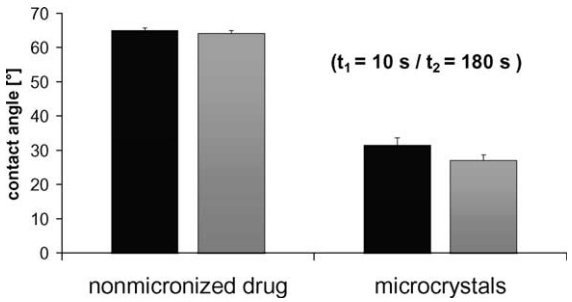

Fig. 9. Contact angle.

4. Conclusions

The preparation of microcrystals of poorly water-soluble drugs by a precipitation technique in the presence of protective hydrophilic polymers followed by spray-drying is suitable for the enhancement of drug dissolution. By this way, a large hydrophilized surface of the drug substance yields. In order to form a protective layer on the crystal surface, an applied polymer has to be affine to the hydrophobic crystal surface. Especially HPMC is able to stabilize the new drug substance ECU-01. Compared with, e.g. solid dispersions or cyclodextrin encapsulations, the amount of drug in the preparation is high (90.8%). The preparation of the microcrystals can be performed discontinuously as well as continuously using a static mixer. The manufacturing of the microcrystals requires a common equipment only and is able to perform in a single process step.

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