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Development of an oral rutin nanocrystal formulation

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ABSTRACT

Dried rutin nanocrystals have been prepared by lyophilization and investigated regarding their physicochemical properties with respect to re-dispersability, particle size, morphology and dissolution behavior. Photon correlation spectroscopy (PCS) and laser diffractometry (LD) were employed to determine the particle size. Morphology of the particles was analyzed by light microscopy. Lyophilized rutin nanocrystals were incorporated into tablets and the dissolution behavior of the tablets was evaluated. Very fine particles of lyophilized rutin could be completely re-dispersed in the water. The PCS size average and polydispersity index (PI) of lyophilized rutin were of 721 nm and of 0.288 after re-dispersion. The rutin nanocrystal-loaded tablets were produced using direct compression. The dissolution velocity of the rutin nanocrystal-loaded tablet was superior compared to rutin microcrystal-loaded and a marketed tablet. After 30 min rutin was released and dissolved completely from the nanocrystal tablets in water. In contrast, only 71% and 55% of the total amount of rutin were dissolved from the microcrystal tablets and the marketed tablet, respectively. The improving dissolution behavior of the rutin nanocrystal-loaded tablet should lead to a better bioavailability of the poorly soluble rutin in the body.

1. Introduction

Rutin (2-(3,4-dihydroxyphenyl)-4,5-dihydroxy-3-[3,4,5-trihydroxy-6-[(3,4,5-trihydroxy-6methyl-oxan-2-yl)oxymethyl]oxan-2-yl]oxy-chromen-7-one) also known as quercetin-3-rutinoside or sophorin is a flavonol glycoside comprised of the flavonol quercetin and the disaccharide rutinose (Fig. 1). It is a polyphenolic compound widely distributed in higher plants. High concentrations for example are found in buckwheat seed, fruits and fruit rinds, especially in citrus fruits (e.g. orange, grapefruit, lemon, lime). Rutin has significant scavenging properties on oxidizing species such as OH radical, superoxide radical, and peroxyl radical (Calabro et al., 2005). Furthermore it has several pharmacological activities including antiallergic (Chen et al., 2000), anti-inflammatory and vasoactive (Ihme et al., 1996), antitumor (Deschner et al., 1991), antibacterial, antiviral and anti-protozoal properties (Panasiak et al., 1989). As an outcome of these biological effects, it has been widely used in treating these diseases. Moreover, it has also been reported that rutin has other therapeutic effects such as hypolipidaemic (Park et al., 2002), cytoprotective (Janbaz et al., 2002), antispasmodic (Mata et al., 1997) and anticarcinogenic (Webster et al., 1996). Rutin offers an advantage over myricetin, quercetagenin and other flavonoids, which on some occasions behave as prooxidant agents and catalyze oxygen radical production (Hodnick et al., 1986). Therefore, it is worth considering it being a potential but non-toxic and non-oxidizable molecule. The disadvantage of the molecule is its poor solubility in aqueous media, being the reason for its poor bioavailability. Therefore it imposes yet some restraints to further pharmaceutical use, especially for oral administration (Miyake et al., 2000). Oral administration is also desired for the application of rutin as nutritional supplement to be taken daily (Rutin, 2008).

Besides other approaches (e.g. solid dispersions, the use of cosolvents or cyclodextrines) particle diminution to the sub micron range is a powerful formulation approach to increase the dissolution rate, the saturation solubility and in turn to enhance the oral bioavailability of poorly soluble drugs (Hecq et al., 2005; Keck and Müller, 2006; Merisko-Liversidge et al., 2003; Müller et al., 2000a, 2003; Müller and Akkar, 2004; Müller and Keck, 2007). The concept of oral nanosuspensions has been specifically used to increase the rate and extent of the absorption of drugs, which have poor and erratic dissolution. In the current decade, the concept of nanosuspensions could be commercially exploited by pharmaceutical companies as micronization did in the last few decades. Therefore drug nanocrystals represent an alternative to existing drug delivery technologies for poorly soluble compounds (Liversidge and Cundy, 1995; Müller et al., 2000b).

Drug nanonization can be achieved through different techniques, where one distinguishes between the bottom-up and the top-down technologies. Bottom-up technologies are precipitation methods (Auweter et al., 2002; List and Sucker, 1988; Sucker and Gassmann, 1994; Wu et al., 2008), whereas top-down technologies

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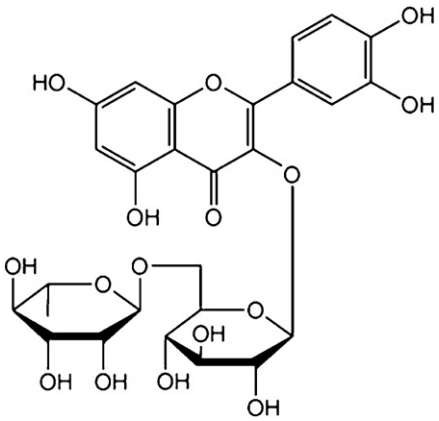


Fig. 1. Chemical structure of rutin.

start from coarse drug macrosuspensions. The diminution is achieved either by pearl/ball milling (Liversidge and Cundy, 1995; Liversidge et al., 1992), high pressure homogenization, either in water (Müller et al., 1999a) or in water free or water reduced media (Müller et al., 2000c), by combination technologies, e.g. precipitation and subsequent high pressure homogenization (Kipp et al., 2003) or ball milling and subsequent high pressure homogenization (Petersen, 2006).

In the literature many data are available covering the formulation and optimization of nanosuspensions (Müller, 1998; Müller et al., 2006; Weder and Van Hoogevest, 1998). Liversidge and Cundy have reported that if bioavailability is truly dissolution rate limited, particle size reduction can significantly improve the oral bioavailability of the drug (Liversidge and Cundy, 1995). It has been also investigated that producing nanosuspensions for oral administration leads to effective therapeutic concentrations in the blood because solubility and absorption problems in the gastrointestinal tract have been overcome by extensive size reduction (Hecq et al., 2005; Jinno et al., 2006; Liversidge and Conzentino, 1995; Liversidge and Cundy, 1995; Müller et al., 1999b; Rao et al., 2008). Therefore, the present work of formulating rutin as a nanosuspension has significant importance in enhancing its bioavailability. Dried rutin nanocrystals were prepared by lyophilization to enable the formulation of a solid oral dosage from. Re-dispersion of the lyophilized rutin in water was intensively investigated and the particle sizes were analyzed by photon correlation spectroscopy (PCS) and laser diffractometry (LD). Finally rutin nanocrystal-loaded tablets were developed using direct compression and its dissolution behavior was evaluated in various dispersion media.

2. Materials and methods

2.1. Materials

Rutin was purchased from Sigma Aldrich GmbH (Deisenhofen, Germany). Rutin nanosuspensions were stabilized by sodium dodecyl sulfate (Fluka Chemie GmbH, Deisenhofen, Germany). Avicel PH 101, AcDiSol (Lehmann & Voss & Co., Hamburg, Germany), EXPLOTAB (JRS PHARMA GmbH + Co.KG, Rosenberg, Germany), talc and magnesium stearate (BASF AG, Ludwigshafen, Germany) were used as tablet excipients. Milli-Q Plus double-distilled water (Millipore GmbH, Schwalbach, Germany) was used as dispersion medium. The other chemicals were of analytical reagent grade.

2.2. Methods

2.2.1. Preparation of rutin nanosuspension

The rutin nanosuspension (R-NS) was produced via high pressure homogenization (HPH) using a Micron Lab40 (APV Deutschland GmbH, Unna, Germany) applying 20 cycles at 1.5×10^8 Pa. The nanosuspension obtained contained 10% (w/w) rutin and 0.2% (w/w) sodium dodecyl sulfate (SDS) as stabilizer.

2.2.2. Lyophilization

The R-NS was dried using lyophilization. In a 20 ml vial 2 ml of the R-NS were frozen at −70 °C. The frozen rutin nanocrystals were freeze dried for 24 h (Christ® Alpha I-5, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany).

2.2.3. Re-dispersability and phase separation

The re-dispersability of rutin nanosuspensions stored in glass bottles was determined by tilting the bottle up and down by hand until the sediment was dispersed in the aqueous phase uniformly. The number of times tilted was noted and rated as fast, medium and low. Phase separation was determined visually in all formulations during long-term storage.

2.2.4. Particle size analyses and physical stability of rutin nanosuspensions

Photon correlation spectroscopy (PCS, Zetasizer Nano ZS, Malvern Instruments, UK) and laser diffractometry (LD, Coulter LS 230, Beckman-Coulter, Germany) were employed to determine the particle size of the re-dispersed rutin nanocrystals. PCS yields the mean diameter of the bulk population (*z*-average) and the polydispersity index (PI) as measure for the width of the size distribution. Laser diffractometry yields a volume distribution. LD results were analyzed using Mie theory with the optical parameters 1.456 for the real refractive index and 0.01 for the imaginary refractive index.

2.2.5. Light microscopy

Light microscopy was performed using an Orthophlan microscope (Leitz, Wetzlar, Germany). The employed magnification was 1000 fold (oil immersion) and each sample was investigated 3 times.

2.2.6. HPLC analysis of rutin

Drug concentrations were determined by high performance liquid chromatography (HPLC). A modified method after Day and Williamson (2001) has been developed. The chromatographic system consisted of a KromaSystem 2000 (Kontron Instruments GmbH, Neufahrn, Germany), a solvent delivery pump equipped with a 20 μl loop and a rheodyne sample injector. The analytical column was an Eurospere C18 RS (25 cm × 4.6 mm lC). As solvent system acetic buffer (pH 4.8) and acetonitrile in a ratio of 80:20 were used. A diode array detector (DAD 540) was used as UV detector and operated at 255 nm.

2.2.7. Preparation of the tablets and drug release

The formulations of the rutin tablets are shown in Table 1. The mass of each tablet prepared was calculated to have the same content of rutin and a total mass equal to the marketed tablet (100 mg). The tablet mass was prepared for 200 tablets by adding the lyophilized rutin nanocrystals to the tablet excipients

Table 1
Composition of rutin tablet formulations A to C and the market formulation; AV = Avicel PH 101, AC = AcDiSol, Ex = Explotab, Mg = magnesium stearate, T = Talc, * not known.

Formulation	Rutin nanocrystals	Rutin microcrystals	Excipients (mg)				
			AV	*AC*	*Ex*	*Mg*	*T*
A	50 mg	–	42	5	–	2	1
B	50 mg	–	42	–	5	2	1
C	–	50 mg	42	5	–	2	1
Market tablet	–	50 mg	*	*	*	*	*

(Table 1) in a tumbler (Turbula, Willy A. Bachofen AG, Muttenz, Switzerland). The tablets were prepared using direct compression by a single punch tablet machine (Korsch Pressen GmbH, Berlin, Germany).

The dissolution tests were performed using a USP XXIII rotating paddle apparatus with a Pharmatest PTW SIII (Pharma Test Apparatebau GmbH, Hainburg, Germany) at 37 °C and a rotating speed of 100 rpm. Tablets were placed in the dissolution chamber containing the dissolution media (900 ml). At certain times, samples were drawn from each dissolution chamber. The samples were filtered through Sartorius® 0.1 μm filters (Sartorius AG, Goettingen Germany). From each vial an aliquot was withdrawn with a 1 ml glass syringe (Poulten & Graf GmbH, Wertheim Germany) and assayed by HPLC to evaluate the amount of rutin dissolved. Any dilution of the samples was intentionally avoided, to prevent any possible interference with the chemical equilibrium, particularly by considering the presence of colloidal particles.

3. Results and discussion

3.1. Preparation of the rutin nanosuspension

The aqueous nanosuspension produced by HPH had a mean particle diameter of 727 nm (PCS). The absence of larger particles or aggregates was proved by LD and light microscopy. No particles > 2.5 μm (D (v) 99% = 2.34 μm) were observed.

3.2. Lyophilization and re-dispersability

In case of oral administration solid dosage forms are preferred, because of several advantages (e.g. better handling, ease of administration, etc.). For the incorporation of nanocrystals into solid dosage forms (e.g. tablets, capsules, pellets or effervescent tablets), the transformation of the liquid aqueous nanocrystals into a dry nanocrystal crystal powder is a pre-requisite. The choice of the drying technology is a critical point, because it needs to be ensured that the nanocrystals can be re-dispersed as separated particles and do not aggregate, which would lead to a loss of their special properties. Lyophilization was performed to dry the aqueous rutin nanosuspension. The lyophilized rutin nanocrystals were further investigated with respect to re-dispersability.

Dried powders of nanocrystals are not only designed to re-disperse into nanometer-sized particles when placing the dosage form in water but also in alternate water-based environments (e.g. gastric fluid or simulated gastric fluid, etc.). Therefore the aim of this study was also to investigate if the lyophilized powder can be re-dispersed completely prior formulating it as tablet, capsule, pellet and effervescent tablet (solid dosage form).

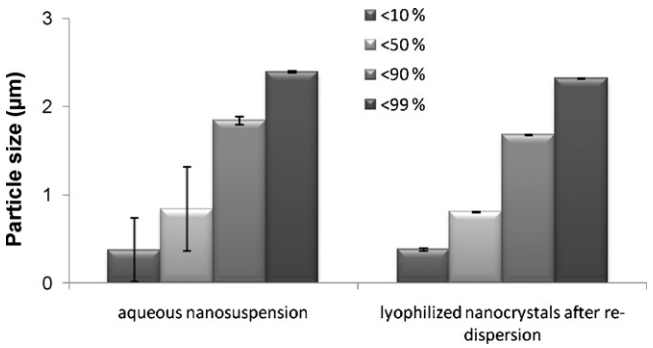


Fig. 2. Particle size distributions (LD data) of the aqueous rutin nanosuspension (left) and of the re-dispersed lyophilized nanocrystals (right). The error bars represented the standard deviation of three repeated measurements.

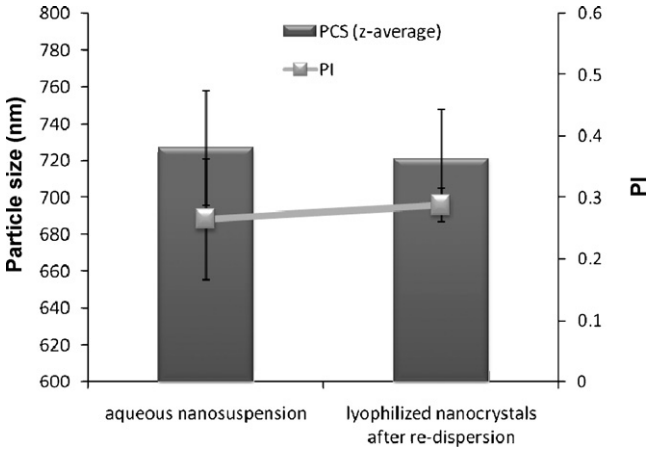


Fig. 3. Particle size (*z*-average, PCS data) and polydispersity index (PI) of the aqueous rutin nanosuspension (left) and the re-dispersed lyophilized nanocrystals (right).

Upon the addition of water the lyophilized rutin nanocrystals could be easily re-dispersed without aggregates or agglomerates. Fig. 2 shows that the particle size distribution of the re-dispersed rutin nanocrystals is not so much different to the original aqueous rutin nanosuspension. The d50% of lyophilized rutin nanocrystals was similar to the d50% of the rutin nanosuspension after production.

The particle size (PCS) of the original rutin nanosuspension was 727 nm with a polydispersity index of 0.265. The PCS size average and polydispersity index of re-dispersed rutin were of 721 nm and of 0.288 after re-dispersion (Fig. 3). The data are practically identical to the original rutin nanosuspension. These results confirm

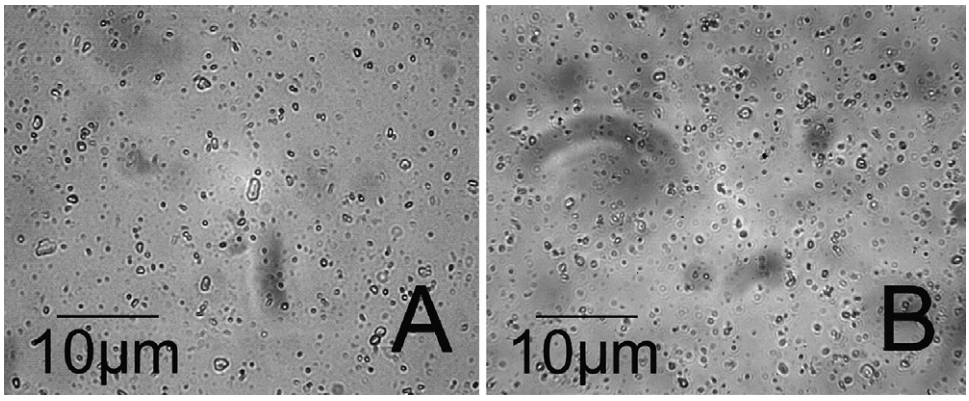


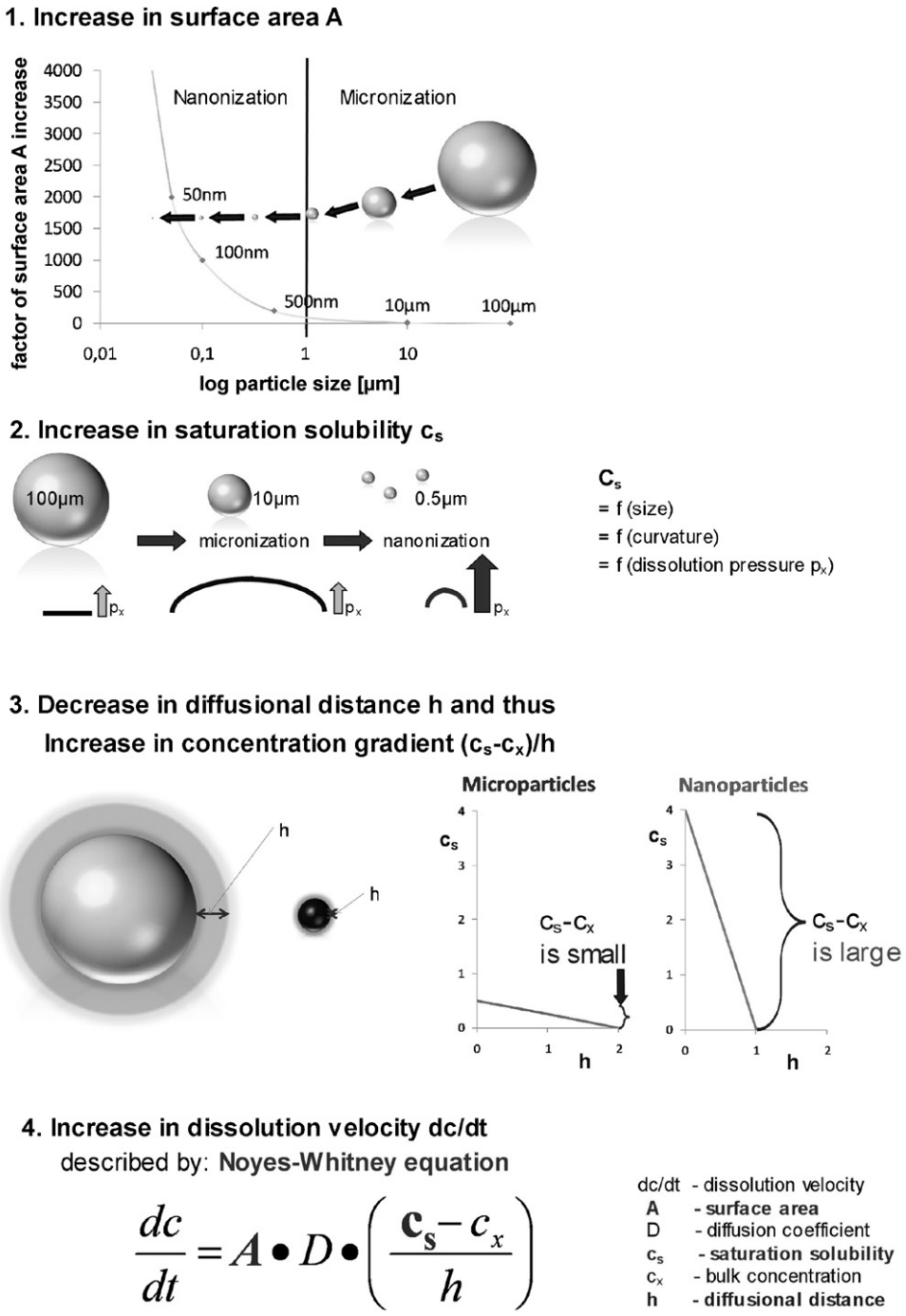
Fig. 4. Images of light microscopy of the aqueous nanosuspension after production (A) and of re-dispersed lyophilized rutin nanocrystals (B).

that the transform process of the rutin aqueous nanosuspensions to lyophilized rutin nanocrystals (dry powder) using a freeze dryer definitely did not or just little influenced the particle size (e.g. growth by agglomeration).

Usually, in many processes of the lyophilization, cryoprotectants are added to the nanosuspension to protect nanoparticles or nanosuspensions from freezing damage (damage due to ice formation) and to minimize the particle size growth during lyophilization (Liversidge et al., 1994). In this study it was found that added cryoprotectant did not minimize nanocrystal growth efficiently. Surprisingly, it was found that lyophilized rutin nanocrystals without any cryoprotectant can be re-dispersed in water properly.

3.3. Polarized light microscopy

Light microscopy images of rutin nanosuspension and re-dispersed rutin nanocrystals were visibly similar. No differences in particle size could be observed between the rutin nanosuspension and re-dispersed lyophilized rutin nanocrystals (Fig. 4). These pictures reveal that the particles of the lyophilized rutin nanocrystals were distributed homogenously as single particles.



$$\frac{dc}{dt} = A \bullet D \bullet \left(\frac{c_s - c_x}{h} \right)$$

Fig. 5. Transfer of microcrystals to nanocrystals leads to an increase in surface area (upper). Increase in saturation solubility c_s, decrease in diffusional distance h and increase in the concentration gradient $c_s - c_x/h$. All effects increase the dissolution velocity dc/dt, modified after (Keck and Müller, in press).

This is well in agreement with the low polydispersity index value.

3.4. Dissolution velocity

An outstanding feature of nanocrystals is the increase in saturation solubility and consequently an increase in the dissolution velocity of the compound. Based on the Noyes–Whitney equation (Noyes and Whitney, 1897), this increase in dissolution velocity takes place in addition to the increase caused by the enlargement of the surface area, e.g. exploited in micronized products (Müller et al., 2000b). By decreasing the particle size (e.g. to the nanometer range), consequently the surface area of the particulate is further increased. In addition, the Noyes–Whitney equation also describes that the dissolution velocity dc/dt depends on the concentration gradient $(c_s - c_x)/h$ (c_s is the saturation solubility; c_x the equilibrium concentration in the bulk phase and h the diffusional distance) and the Prandtl equation describes that the diffusional distance h is reduced for small particles. Thus, the simultaneous increase in the saturation solubility c_s and the decrease in h lead to an increased concentration gradient $(c_s - c_x)/h$, enhancing the dissolution velocity in addition to the surface effect (Müller et al., 2000b, 1998, 2003; Müller and Akkar, 2004). Fig. 5 summarizes these effects.

An increase in dissolution velocity and also an increase in saturation solubility can also be achieved by changing the crystalline state of the material (e.g. from crystalline to amorphous or partially amorphous). Due to thermodynamic reasons the preservation of the amorphous state is critical; therefore the production of nanocrystals should lead to crystalline particles.

The crystalline state of the rutin nanocrystals investigated in this study remained unchanged (100% crystalline) upon both, high pressure homogenization and lyophilization (Mauludin, 2008).

3.5. Preparation and dissolution behavior of tablet dosage forms

In general oral administration is the first choice for the administration of drug nanocrystals. When a drug is given orally to the patients, the bioavailability finally depends on the solubility of the drug (class II drugs) and the absorption (class III and IV drugs) in the gastrointestinal tract. In the past many active compounds have been failed because their poor solubility has limited the in vivo absorption and did not lead to effective therapeutic concentrations. The problem of poor solubility combined with low absorption are for example true for the compounds atovaquone (marketed product: Wellvone) and buparvaquone. Atovaquone is the approved drug to be used for the treatment of opportunistic *Pneumocystis carinii* infections in HIV patients (Public Health Service, 2000). Poor bioavailability could be overcome by particle size reduction of the both drugs using nanonization techniques (Jacobs et al., 2001; Müller et al., 2001; Schöler et al., 2001). The size-dependency comes only into effect for particles having a size below approximately 1 μm (submicron particulate)—a phenomenon observed in tabletting which leads to an increase of the dissolution rate of such fine drug particles (Buckton and Beezer, 1992; Hu et al., 2004). Thus

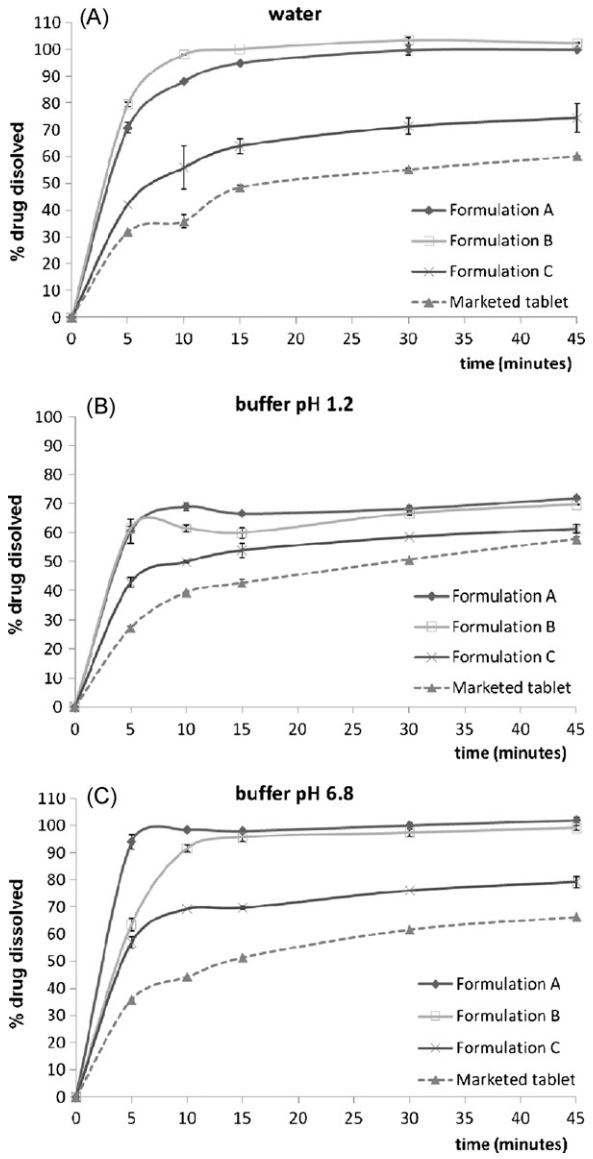


Fig. 7. Percentage of dissolved rutin from nanocrystal-loaded tablets (formulation A, B) and from microcrystal-loaded tablets (formulation C and marketed tablet) in water (A, upper), buffer of pH 1.2 (B, middle) and buffer of pH 6.8 (C, lower).

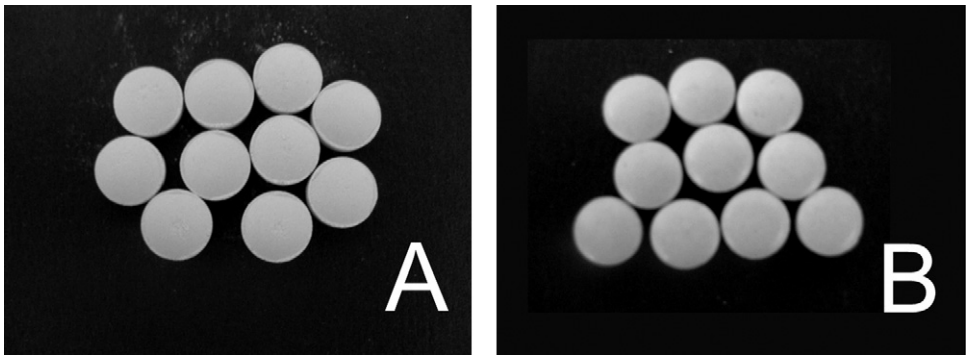


Fig. 6. Final product of nanocrystal-loaded tablet (A) and marketed tablet (B).

the production of rutin nanocrystals could increase the distinctly poor solubility and slow dissolution of rutin and consequently lead to an improved oral bioavailability. The transform of the aqueous nanosuspension into a solid oral dosage form would ease the application of the drug. Therefore the lyophilized nanocrystals were formulated as tablets. The formulations of rutin nanocrystal-loaded tablets can be seen in Table 1. Avicel PH 101, AcDiSol, Explotab are excipients usually used for direct compression. These excipients have good characteristics for direct compression such as good compressibility and an excellent flow ability. Therefore those excipients were chosen as filler, binder and super disintegrating agents (Busignies et al., 2006; Munoz et al., 1998; Takagi et al., 2007). As lubricant, antiadherent and glidant the combination of magnesium stearate and talc was used. Lyophilized rutin nanocrystals were admixed to the tablet excipients by a tumbler and afterwards compressed by a single punch tablet machine using direct compression. Direct compression was chosen to minimize agglomeration of the rutin nanocrystal during longer processing by granulation. The final product of the rutin nanocrystal-loaded tablets can be seen in Fig. 6. These tablets were further evaluated with respect to their dissolution behavior.

The dissolution test of the rutin tablets was performed using a USP XXIII rotating paddle apparatus. Three formulations of rutin have been prepared for this study. Formulation A and B contained rutin nanocrystals and formulation C contained rutin microcrystals (Table 1).

Dissolved rutin from all formulations was evaluated in three different dissolution media. The dissolution velocity of rutin from the rutin nanocrystal-loaded tablet (nanocrystal tablet) was faster compared to the rutin microcrystal-loaded tablet (microcrystal tablet). Within 5 min almost 80% of rutin was dissolved from the nanocrystal tablet (formulation B) in water. It was definitely better compared to the microcrystal tablet from which only about 40% of rutin was dissolved in the same conditions. Moreover within 30 min rutin was dissolved completely from the nanocrystal tablets (formulation A and B) in water (Fig. 7A). In contrast, after 30 min only 71% was dissolved from the microcrystal tablets in water.

Not only in water, but also in buffer of pH 1.2 the dissolution velocities of the nanocrystal tablets were superior when compared to the microcrystal tablet (formulation C). In formulation A and B 68% and 67% of the rutin was dissolved within 30 min, respectively, whereas only 59% of the rutin was dissolved from the microcrystal tablet (Fig. 7B). The pH dependent solubility can be explained by the chemical structure. Rutin is a weak acid, thus the solubility is decreased in acidic condition. In buffer at pH 6.8 with buffer strength of 93.051 mM, formulations A and B are distinctly faster in releasing rutin compared to formulation C. The difference was similar to water. Within 5 min 95% of the rutin could be dissolved from the nanocrystal tablet (formulation A) and only within 10 min all of the rutin was dissolved completely. Compared to formulation C, the dissolved rutin from formulation B was definitively increased. Only 50% of rutin could be dissolved from formulation C within 5 min. In a period of 10 min only 68% of the drug content was dissolved (Fig. 7C).

3.6. Dissolution of drug nanocrystals-loaded tablet and marketed tablet

In all of three dissolution media, the nanocrystal-loaded tablets (formulation A and B) were superior compared to the marketed tablet. Within 5 min almost 80% of rutin was dissolved from formulation B in water. It was definitely better compared to the marketed tablet from which only about 30% of rutin was dissolved in the same conditions. In addition, within 30 min 100% rutin was dissolved from the nanocrystal-loaded tablets (formulation A and B) in water and buffer of pH 6.8. In the same time, 68% and 67% of rutin were dissolved also from nanocrystals tablets (formulation A and B) in buffers having a pH of 1.2. In contrast, in the marketed after 30 min only 55%, 51% and 62% of rutin were dissolved in water, and buffers having a pH of 1.2 and 6.8, respectively. The dissolution velocity profiles of rutin from the different tablet formulations investigated are also shown in Fig. 7.

Recently, the particle size reduction effectiveness of drug substances-loaded tablets on oral bioavailability has been intensively investigated. It has been proven that particle size reduction leads to improved oral bioavailability in the body. Takano et al. have specified that particle size reduction leads to improved dissolution rate and bioavailability. In addition, the rate-limiting steps of oral absorption were simulated. An increase in the dissolution rate and administered dose showed a shift from dissolution rate-limited to solubility-limited absorption. In the study in dogs, the particle size reduction improved the oral absorption (Takano et al., 2008). Such studies provide a powerful tool to predict dose nonlinearity and will aid in the development of formulating poorly soluble drugs (Biopharmaceutical Classification System (BCS) class II drugs).

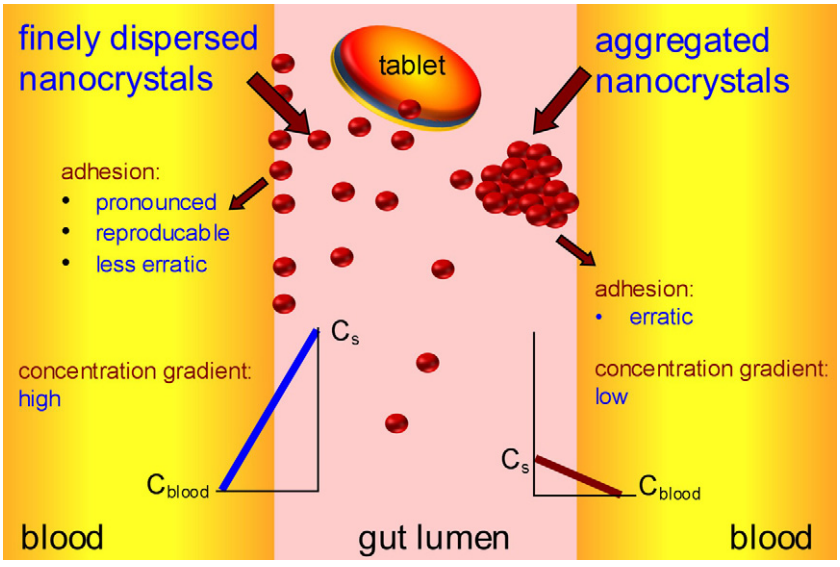


Fig. 8. Mechanism of action: finely dispersed nanocrystals versus aggregated nanocrystals (similar to micrometer crystals).

According to Hintz et al., a computer method has been devised to describe the theoretical dissolution rate of a polydisperse powder under non-sink conditions based on its weight percent particle size distribution. It is shown that finer particles in the size distribution showed an improved dissolution behavior. Moreover the particle size distributions were used to simulate their effect on the amount of drug absorbed orally (Hintz and Johnson, 1989). Based on those results, it has been suggested a promising rutin tablet dosage form for oral administration. It leads to superior physicochemical properties and should overcome the in vivo absorption problem of the poorly soluble rutin as class II BCS drug. Fig. 8 summarizes the effects on bioavailability enhancement in the gut. Important is that the nanocrystals are released as fine nanocrystals. It could be shown that a slight aggregation does not yet impair the dissolution velocity (Keck et al., 2004), but pronounced aggregation will decrease the dissolution velocity strongly (Fichtinger, 2004).

4. Conclusion

Lyophilized rutin nanocrystals could provide superior physicochemical properties. Very fine particles of lyophilized rutin could be re-dispersed completely in the water. This characteristic is a critical point for improving the dissolution behavior of drugs especially from a tablet dosage form. Rutin nanocrystal-loaded tablets could be produced using direct compression. The dissolution velocity of rutin from the rutin nanocrystal-loaded tablets was superior compared to the rutin microcrystal-loaded tablet or the marketed tablet. Improving the dissolution behavior of rutin nanocrystals-loaded tablet leads to better bioavailability of the poorly soluble rutin in body, in case dissolution in gastrointestinal tract is the rate-limiting step.

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