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Development of ascorbyl palmitate nanocrystals applying the nanosuspension technology

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Abstract

Ascorbyl palmitate (AP) is an antioxidant used in both cosmetics and food industry. Owing to its poor solubility and instability caused by oxidation having been observed in several colloidal systems, the aim of this study was to investigate the feasibility of applying the nanosuspension technology by high-pressure homogenization (HPH) (DissoCubes® technology) to enhance the chemical stability of AP, followed by lyophilization. Sodium dodecyl sulfate (SDS) and Tween 80 were chosen as emulsifying agents to stabilize the developed AP nanosuspensions. After 3 months of storage at three different temperatures (4 °C, 25 °C and 40 °C), the photon correlation spectroscopy (PCS) analysis of AP nanosuspensions revealed that the mean particle size of those stabilized with SDS significantly increased compared to those stabilized with Tween 80. The results observed from both atomic force microscopy (AFM) and scanning electron microscopy (SEM) revealed AP nanocrystals of cubic-like shape. The percentage of AP remaining in nanosuspensions stabilized with Tween 80 was higher than 90% after 3 months storage at 4 °C, 25 °C and 40 °C. To increase the chemical stability of AP nanosuspensions, a drug powder was prepared by lyophilization. The effect of the presence of cryoprotectant trehalose on the physical stability was evaluated at different concentrations. After redispersing the lyophilized product, the mean size of AP nanosuspensions without trehalose was significantly higher compared with the system with trehalose. After 3 months of storage at 25 °C the mean size of lyophilized AP nanosuspensions remained constant. X-ray diffraction revealed the crystalline character of AP nanocrystals after HPH and lyophilization.

Keywords: Nanosuspensions; Ascorbyl palmitate; Lyophilization; Atomic force microscopy; Scanning electron microscopy; Tween 80; Sodium dodecyl sulfate (SDS)

1. Introduction

According to the Biopharmaceutics Classification System (BCS), a drug with poor solubility but having high intestinal permeability is classified as a drug of BCS Class II. This class of drugs generally possesses low bioavailability due to its low dissolution rate. However, this problem can be overcome by increasing the solubility of the drug (Müller and Akkar, 2004). Several techniques have been used to achieve high drug solubility, for instance, organic solvents (e.g. water–ethanol mixtures), solubilization techniques (e.g. surfactant molecules) and formation of an aqueous soluble complex (e.g. cyclodextrin) (Müller et al., 2001). However, these approaches have limitations regarding their applicability. An alternative procedure to improve the solubility of drugs is the reduction of the particle size, which leads to an increased surface area and therefore the dissolution rate increases. This procedure is called micronization, i.e. the drug is reduced to a micrometer size between 0.1 μm and 25 μm; however, only a negligible amount below 1 μm in the nanosize range is found. Nevertheless, micronization was found not sufficient to achieve the desired blood concentrations of drugs with very low solubility. Thus, nanonization has been introduced to

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achieve a particle size between 100 nm and 1000 nm (Müller and Akkar, 2004; Müller et al., 2001, 1998). Advantages of this technique are not only related to the increase of the drug dissolution rate but also to the increase of its saturation solubility (C_S) (Müller et al., 2000). There are several methods used to reduce the particle size of a drug powder to a nanometer size, for example, precipitation (Trotta et al., 2001; Sucker, 1998), jet milling, pearl milling (Liversidge and Conzentino, 1995) and high-pressure homogenization (HPH) (Müller et al., 2000). However, the method besides pearl milling frequently used for preparing the drug nanocrystals is the HPH technique. This method has several advantages over the others, for example, simplicity of the process, ease of large-scale production and a reduced product contamination (Müller and Akkar, 2004; Müller and Bohn, 1998).

Several advantages of nanosuspensions have been reported such as bioavailability improvement, reduction of variation in bioavailability and mucoadhesiveness. Besides, stability enhancement of chemically labile drugs has been reported by their formulation as nanosuspensions in comparison to aqueous solutions (Müller and Keck, 2004; Moschwitzer et al., 2004).

To transform aqueous dispersions into dry powder, one can use lyophilization and spray drying procedures. Spray drying usually requires high temperature in the process which is not suitable for thermolabile drugs. In such cases, lyophilization is the most suitable procedure. In the lyophilization process, a cryoprotective agent such as mannitol, trehalose or sucrose is added to the solution to avoid particle aggregation after reconstitution of the system (Müller and Bohn, 1998).

Ascorbly palmitate (AP) is soluble in organic solvents such as methanol but it shows very low solubility in water. It has been previously reported the chemical instability of AP after its incorporation into colloidal systems, i.e. microemulsions, liposomes and solid lipid nanoparticles (SLN) (Kristl et al., 2003; Üner et al., 2005; Špiclin et al., 2001). AP can suffer degradation by oxidation and/or hydrolysis (Kristl et al., 2003; Üner et al., 2005; Špiclin et al., 2001). Therefore, to overcome this problem these formulations should be transformed in a dry powder form. In this study, AP nanosuspensions were prepared by HPH, which were subsequently transformed to dry powder by lyophilization, to enhance the chemical stability of AP. The physicochemical properties of nanosuspensions in terms of particle size, polydispersity index (PI), zeta potential before and after lyophilization were investigated. Moreover, polymorphism of nanosuspensions was elucidated by wide angle X-ray scattering (WAXS) in comparison to the bulk active ingredient. The chemical stability of AP nanocrystals was assessed by determining the percentage of active present in the formulations stored at three different temperatures (4 °C, 25 °C, and 40 °C) during a period of 3 months.

2. Materials and methods

2.1. Materials

Ascorbyl palmitate (AP), sodium dodecyl sulfate (SDS), and trehalose were purchased from Sigma–Aldrich (Deisenhofen, Germany). Tween®80 (polyoxyethylene-20-sorbitan monooleate) was obtained from Uniqema (Everberg, Belgium). Methanol (HPLC grade) was obtained from Merck (Darmstadt, Germany). Ultra purified water was supplied from a MilliQ Plus system, Millipore (Schwalbach, Germany).

2.2. Preparation of AP nanosuspensions

Nanosuspensions containing 6% AP were produced by HPH (Micron LAB40, Homogenizer Systems, Germany). Briefly, the AP powder was dispersed in a surfactant solution (SDS or Tween 80) using an Ultra-Turrax T25 (Jahnke & Kunkel GmbH, Staufen, Germany) at 8000 rpm for 1 min. The dispersion was further processed by HPH applying two homogenization cycles at 150 bar, 500 bar and at 1000 bar, followed by 20 homogenization cycles at 1500 bar.

2.3. Lyophilization of AP nanosuspensions

AP nanosuspensions stabilized with Tween 80 were selected to observe the possibility of using lyophilization to enhance the chemical stability of AP nanocrystals. The freshly prepared nanosuspensions were lyophilized without and with cryoprotective agent (i.e. trehalose) at different concentrations (1, 2, 5, and 10%, w/w). Briefly, AP nanosuspensions were rapidly cooled down to −70 °C for 2 h followed by primary drying at 1.03 mbar and secondary drying at 0.001 mbar.

2.4. Particle size analysis

The mean particle size (z-ave) and the polydispersity index (PI) were determined by photon correlation spectroscopy (PCS) with a Malvern Nanosizer (Malvern Instruments, UK). Prior to the measurement, the samples were diluted with double distilled water to a suitable scattering intensity and redispersed by handshaking before the measurement.

2.5. Zeta potential analysis

The zeta potential (ZP) is a measure of the electric charge at the surface of the particles indicating the physical stability of colloidal systems. The ZP values higher than |30 mV| indicate electrostatic long-term stability of aqueous dispersions (Müller, 1996). In this study, the ZP values were assessed by determining the particle electrophoretic mobility using the Malvern Nanosizer (Malvern Instruments, UK). The measurements were performed in ultrapurified water adjusted to a standardized conductivity of 50 μS/cm with sodium chloride solution (0.9%, w/v) to avoid changes in ZP values due to day-to-day variations occurring in the conductivity of the water. The pH was in the range of 5.5–6.0. The ZP values were calculated using the Helmholtz–Smoluchowsky equation.

2.6. Atomic force microscopy (AFM)

The morphology of AP nanocrystals was investigated by AFM. Non-contact mode AFM was applied in this study. Imaging was performed on SPI 3800N (Seiko Instrument, Japan).

Typical resonance frequencies of these tip cantilever systems were about 134 kHz. Scan speed was set at 0.5 Hz and scan sizes were taken from 20 μm to 2 μm. Prior to analysis, the samples were diluted to the ratio of 1:100 with double-distilled water and then slightly dropped onto the glass slide and dried at ambient conditions to circumvent the movement of particles. The data were represented in topographic and phase imaging. In AFM, the topographic is created by monitoring the force between the tip and the surface, while the phase imaging is the mapping of the phase lag between the periodic signal driving the cantilever and the oscillations of the cantilever. Therefore, changes in the phase lag often signify changes in the properties of the sample surface.

2.7. Scanning electron microscopy (SEM)

The size and shape of AP nanocrystals were also evaluated by SEM. Prior to analysis, the samples were diluted with ultrapurified water to obtain a suitable concentration. Then, the samples were spread on a sample holder and dried using vacuum. They were subsequently coated with gold (JFC 1200 fine coater, JEOL, Japan) and examined by a scanning electron microscope (JSM 6301F, JEOL, Japan).

2.8. Differential scanning calorimetry (DSC)

Thermal analysis was performed using a Mettler DSC 821e apparatus (Mettler Toledo, Gieβen, Switzerland). The samples were weighed in 40 μl aluminum pans, approximately 1–2 mg based on the AP content in the formulation. Heating runs were performed from 25 °C to 150 °C at the heating rate of 5 K/min. An empty aluminum pan was used as a reference. The DSC parameters including the melting point and melting enthalpy were evaluated using the STAR^e Software (Mettler Toledo, Switzerland).

2.9. X-ray diffraction

X-ray diffraction was performed by wide-angle X-ray scattering (WAXS, 2θ 0.6°–40°) on a Philips PW1830X-ray generator (Philips, Amedo, the Netherlands) with a copper anode (Cu Kα radiation, 40 kV, 25 mA, $\lambda = 0.15418$ nm), using a Goniometer PW18120 as a detector. The samples were mounted onto a thin glass capillary prior to the analysis by WAXS. The obtained data were typically collected with a step width of 0.04° and a count time of 2 s.

2.10. Chemical stability

All samples were kept in siliconized glass vials at three different temperatures (4 °C, 25 °C and 40 °C) with nitrogen flushing over the top of the formulation to avoid the presence of oxygen. The chemical stability of AP nanosuspensions was followed up during 3 months. At predetermined time intervals, approximately 50 mg sample was weighed and dissolved in 10 ml of methanol, which were then sonicated in an ultrasonic bath for 5 min. The suspensions were then cooled down to room temperature, and further diluted with a mixture of methanol and water (pH adjusted to 2.5 with hydrochloric acid) at the ratio of 9:1. The percentage of AP remaining in the nanosuspensions was quantified by high performance liquid chromatography (HPLC). Briefly, HPLC analysis was performed using a Kroma System 2000 running in the isocratic modus. The system consisted of a HPLC pump 220, an Auto-sampler T360 and a UV detector 430. The stationary phase of HPLC system consisted of 250 mm × 4.6 mm i.d. column packed with 5 μm Luna-NH_2 (Phenomenex, Germany). The mobile phase comprised methanol and 0.02 M phosphate buffer pH 3.5 (70:30). The flow rate was 1 ml/min. The UV detector was set at the wavelength of 254 nm. The injection volume was 20 μl. All samples were analyzed in triplicate. The Kontron HPLC software was used for the analysis of the results.

2.11. Statistics

The reported data represent the mean value ± standard deviation (S.D.). Significance of difference was evaluated using one-way ANOVA at the probability level of 0.05.

3. Results and discussion

3.1. Particle size analysis of AP nanosuspensions

In this study, two different surfactants (Tween 80 and SDS) were chosen to stabilize the AP nanocrystals. Immediately after production, the mean particle size (z-ave) of AP nanosuspensions stabilized with Tween 80 and SDS were 365 nm (PI ∼ 0.3) and 348 nm (PI ∼ 0.2), respectively. The z-ave of AP nanosuspensions stabilized with SDS significantly increased reaching values higher than 1 μm after 3 months of storage at room temperature (25 °C) and at 40 °C (Fig. 1). The crystal growth could be explained by the Ostwald ripening. Generally, Ostwald ripening is a result of the difference in solubility between small and large particles due to a higher degree of curvature of the smaller particles leading to a higher solubility compared with the larger ones (Müller and Keck, 2004; Martin, 1993). As a result, the small particles dissolve and deposit at the surface of larger particles, which show lower solubility. Subsequently, the small particles disappear while the overall particle growth increases during storage. Concerning the polydispersity index (PI) values, it was found that AP nanosuspension stabilized with Tween 80 was higher than that with 0.3% SDS. Generally, the higher the PI values, the more pronounced Ostwald ripening occurs. However, Ostwald ripening can be avoided by selecting a suitable surfactant. As depicted in Fig. 2, the AP nanocrystals stabilized with Tween 80 remained in the nanometer size during 3 months of storage at three different temperatures. This could be explained by the steric hindrance of Tween 80 avoiding the Ostwald ripening. This effect was not observed when using SDS to stabilize AP nanosuspensions.

3.2. Zeta potential values

To predict the long-term physical stability of the colloidal systems, the zeta potential was evaluated during storage time. This

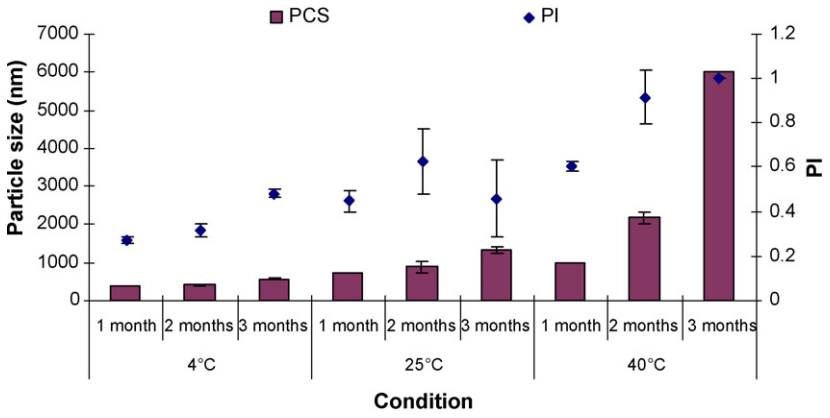

Fig. 1. Mean particle size (z-ave) and polydispersity index (PI) of AP nanosuspensions stabilized with 0.3% SDS after 3 months of storage at three different temperatures.

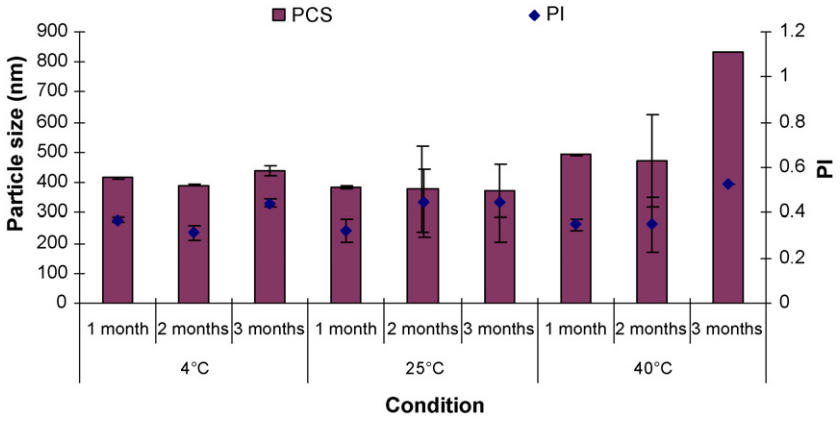

Fig. 2. Mean particle size (z-ave) and polydispersity index (PI) of AP nanosuspensions stabilized with 1% Tween 80 after 3 months of storage at three different temperatures.

analysis is based on the assessment of the movement of the particles in an electric field. After 3 months of storage, it was found that the ZP values of the AP nanosuspensions stabilized with SDS noticeably decreased from −64 mV to −50 mV and those stabilized with Tween 80 decreased from −56 mV to −48 mV. Nonetheless, both revealed ZP values higher than |30 mV| indicating a good physical stability of the nanocrystals. Generally, the choice of surfactant is based on the required physicochemical properties of the nanocrystals and on the intended administration route (Müller et al., 2006). For AP nanosuspensions stabilized with Tween 80, physical stability has been enhanced not only by electrostatic stabilization but also by steric hindrance of surfactant chains. From the results obtained, it could be therefore deduced that the physical stability of AP nanosuspensions is strongly dependent on the type of the surfactant. These results are in agreement with the data obtained from the particle size analysis.

3.3. AFM analysis

The morphology of AP nanocrystals, i.e. size and shape, has been evaluated by AFM in non-contact mode. Prior to the measurement, the surface of glass slide was checked to avoid analysis artifacts. The diluted samples were then dropped onto the glass slide and consequently dried at ambient temperature. Figs. 3 and 4 show the AFM images of AP nanosuspensions stabilized with 0.3% SDS and 1% Tween 80, respectively, after storage at room temperature (25 °C) for 3 months. AP nanocrystals were of cuboidal shape independently on the type of surfactant. Similar findings have been reported by Müller *et al.* (Müller et al., 2000; Müller and Keck, 2004). Nonetheless, AFM images revealed size differences between samples depending on the surfactant. Those stabilized with SDS revealed nanocrystal sizes of approximately 1 μm (Fig. 3), while those stabilized with Tween 80 showed sizes in the range between 300 nm and 500 nm (Fig. 4).

3.4. SEM analysis

The analysis by SEM has also been widely used to evaluate the morphology of drug nanocrystals (Müller et al., 2000). Prior to the measurement, the samples were diluted to have a suitable concentration, and then dropped on the carbon grid and dried at ambient temperature. Fig. 5(a and b) depicts the SEM images of AP nanocrystals after 3 months of storage at room temperature (25 °C). It was observed that the mean size of AP nanocrystals stabilized with SDS was higher than 1 μm while those stabilized with Tween 80 was lower than 1 μm. These data were in

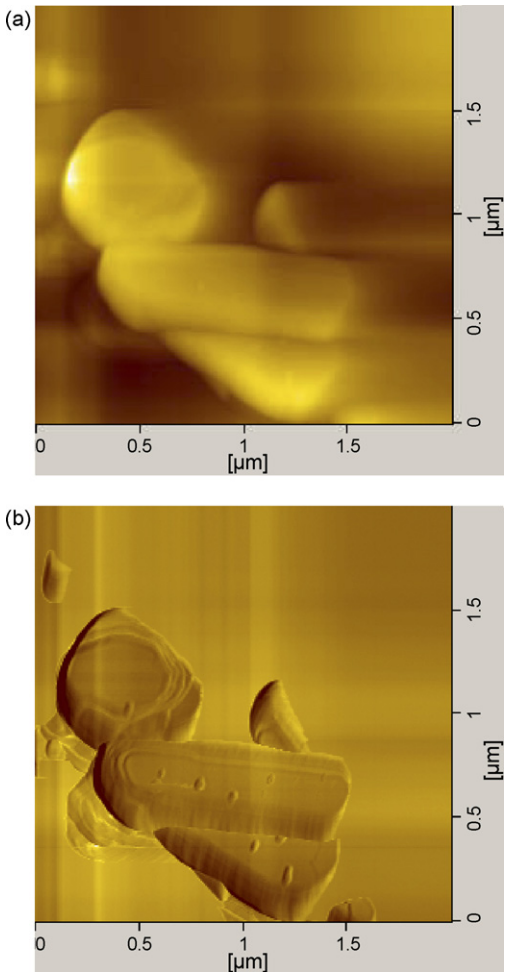

Fig. 3. AFM images in non-contact mode at 2 μm scan range of AP nanosuspensions stabilized with 0.3% SDS after 3 months of storage at room temperature; (a) topographic mode and (b) phase imaging.

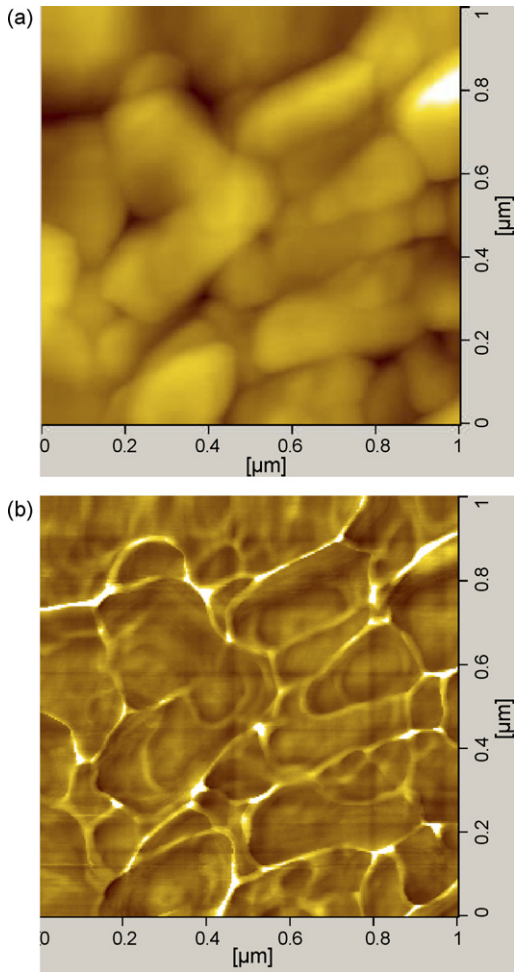

Fig. 4. AFM images in non-contact mode at 1 μm scan range of AP nanosuspensions stabilized with 1% Tween 80 after 3 months of storage at room temperature; (a) topographic mode and (b) phase imaging.

agreement with the obtained data from PCS and AFM analysis. In addition, the obtained SEM data support the AFM results showing cubic-like shape nanocrystals. Similar findings have also been reported for other drugs such as buparvaquone and RMKP22 (Müller and Jacobs, 2002; Müller and Peters, 1998).

3.5. DSC analysis

DSC was performed to investigate the effect of surfactants (Tween 80 vs. SDS) on the inner structure of AP nanosuspensions. The melting endotherm of bulk AP was recorded at ~117 °C. Although the melting curves of AP nanosuspensions stabilized with Tween 80 or with SDS were influenced by the presence of water, it was found that the melting endotherm of AP nanosuspensions stabilized with Tween 80 decreased to ~109 °C, whereas that of AP nanosuspensions stabilized with SDS was almost constant (~118 °C), in comparison to the bulk AP (i.e. 117 °C). Therefore, the decrease of the melting endotherm of AP nanosuspensions was not due to the effect of the particle size but rather due to the interaction of Tween 80 on the surface of the AP nanocrystals.

3.6. Chemical stability of AP nanosuspensions

Fig. 6 shows the percentage of AP remaining after 3 months of storage at three different temperatures. The amount of AP remaining in the formulations was mainly dependent on the type of surfactant and on the storage temperature. The highest percentage of active was measured in the nanosuspensions stabilized with Tween 80. Chemical instability of AP was significantly higher in the nanosuspensions stabilized with SDS stored at 40 °C (Fig. 6). Contrastingly, those stabilized with Tween 80 showed a good chemical stability when stored at three different temperatures during a period of 3 months. Moreover, the percentage of AP remaining was higher than 90%, being the mean nanocrystal size in the nanometer range (between 300 nm and 800 nm). Reasons for such differences in AP chemical stability might be the differences of AP solubility in the aqueous surfactant solutions. The degradation mechanism is generally more pronounced when the active is dissolved than when it is present in the solid state (Wells, 2002). Therefore, the higher amount of AP dissolving in the surfactant, the higher AP degradation is found. Comparing the solubility of AP in 1% Tween 80 and 0.3% SDS, it was found that the former one

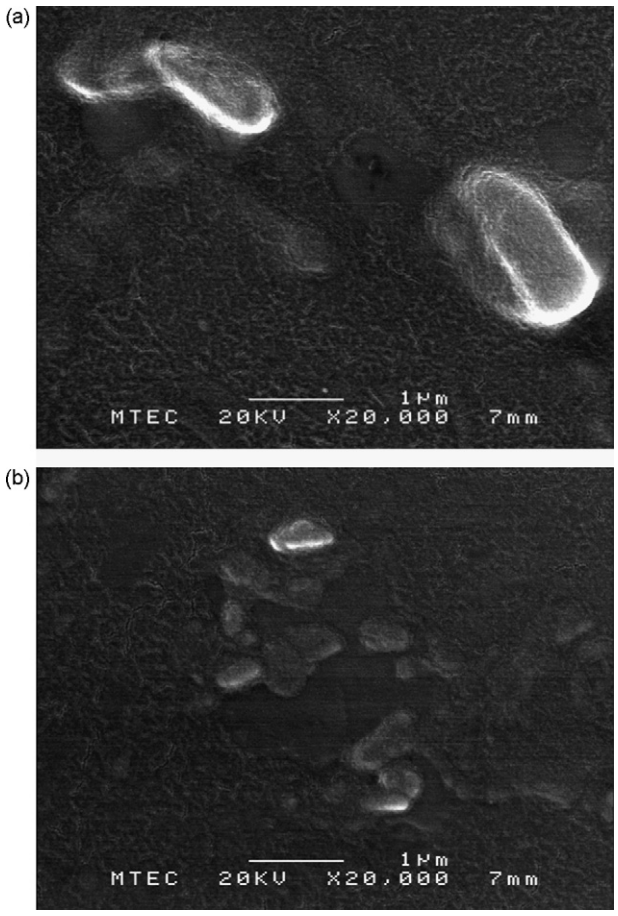

Fig. 5. SEM photographs of AP nanosuspensions after 3 months of storage at room temperature (25 °C); (a) AP nanosuspensions stabilized with 0.3% SDS and (b) AP nanosuspensions stabilized with 1% Tween 80.

showed higher solubility than the latter one meaning that the suitable type of surfactant could enhance the chemical stability of AP nanosuspensions. Therefore, the difference in AP solubility in the aqueous surfactant could not be used to explain in the experiment. Tween 80 is a non-ionic surfactant composed of polyethylene glycol chains. When nanocrystals are stabilized with this polymer, their surface will be surrounded by a thickened layer as confirmed by DSC. This might reduce the diffusion of oxygen molecules in the aqueous phase around the crystal and further exposure of the active nanocrystals to oxidative processes. However, a fully conclusive interpretation of the observed stability difference between the nanosuspensions stabilized by the Tween 80 and SDS cannot yet be given. As reported by other authors, the enhancement of chemical stability of AP entrapped in both w/o and o/w microemulsions, solid lipid nanoparticles (SLN), and nanostructured lipid carriers (NLC) stored under nitrogen-flushed conditions during 3 months of storage at room temperature could not be achieved, and the percentage of remaining AP was less than 80% (Kristl et al., 2003; Üner et al., 2005; Špiclin et al., 2001). Moreover, in pre-formulation studies performed in our study to develop a suitable AP nanosuspension, it has been observed that the chemical stability of AP nanosuspensions was higher than that of AP in methanol (data not shown). Therefore, the obtained results confirm that the chemical stability of AP could be improved when formulated as nanosuspension, in comparison to its methanolic solution and other colloidal carrier systems. Similar results were also reported for the enhancement of omeprazole and paclitaxel when formulated as nanosuspensions, in comparison to their aqueous solutions (Müller and Keck, 2004; Moschwitzer et al., 2004). This could be explained by the fact that only the surface of nanocrystals was exposed to oxygen and water (Müller and Keck, 2004; Moschwitzer et al., 2004). As a consequence, degradation took place only at the outermost surface of the particle protecting inner core of drug nanocrystals. For the AP nanosuspensions stored at 4 °C, almost 100% of active was present in all formulations after 3 months. Thus, the chemical stability of AP could be achieved by formulating the active as nanosuspension, selecting the suitable surfactant composition and storage temperature.

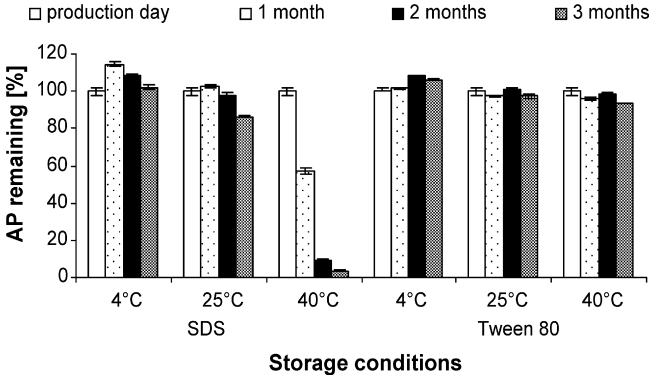

Fig. 6. Percentage of AP remaining in AP nanosuspensions stabilized with 0.3% SDS and 1% Tween 80, stored at three different temperatures during 3 months.

3.7. Lyophilization of AP nanosuspensions

Instability of AP has been already demonstrated after its incorporation into colloidal systems, such as microemulsions, nanoemulsions and liposomes (Kristl et al., 2003; Špiclin et al., 2001). The main mechanism of AP degradation is oxidation, which is usually more effective when the active is in solution rather than in solid state (Wells, 2002). Therefore, converting aqueous AP nanosuspensions into a dry powder might be an alternative procedure to enhance the chemical stability of this active. In preliminary studies, the chemical stability of AP as a dried powder has been evaluated under storage at 40 °C for 4 months with and without nitrogen flushing over the top of vial. The percentage of remaining AP was found to be higher than 98% for both storage conditions. Therefore, enhancement of chemical stability of AP nanosuspensions could be achieved by converting the system from aqueous into a dried powder form.

Generally, there are two methodologies to convert aqueous dispersions to dry powders, i.e. lyophilization and spray drying. Due to AP instability at high temperatures as previously reported, lyophilization was chosen in the present study to avoid the higher temperatures applied during spray drying. Trehalose was selected as a cryoprotective agent, being added to the AP nanosuspensions at the concentrations of 1%, 2%, 5% or 10% (w/w).

Table 1
Mean particle size (z-ave), polydispersity index (PI) and zeta potential (ZP) values of AP nanosuspensions stabilized with 1% Tween 80, immediately after reconstituting the lyophilized nanosuspensions and after 3 months of storage at room temperature (25 °C)

AP nanosuspensions	After lyophilization			After 3 months of storage at 25 °C		
Formulations	z-ave (nm)	PI	ZP (mV)	z-ave (nm)	PI	ZP (mV)
0% trehalose	537 ± 23	0.414 ± 0.057	−41.1 ± 2.1	689 ± 19	0.301 ± 0.092	−41.4 ± 1.5
1% trehalose	380 ± 6	0.299 ± 0.012	−40.4 ± 0.3	399 ± 3	0.205 ± 0.017	−41.5 ± 2.5
2% trehalose	368 ± 7	0.233 ± 0.045	−41.4 ± 1.2	377 ± 2	0.203 ± 0.005	−41.0 ± 1.8
5% trehalose	360 ± 5	0.220 ± 0.016	−41.2 ± 0.3	360 ± 11	0.219 ± 0.015	−40.3 ± 1.5
10% trehalose	346 ± 0	0.250 ± 0.040	−45.0 ± 1.1	362 ± 8	0.213 ± 0.055	−41.8 ± 2.1

To investigate the feasibility of producing lyophilized AP nanosuspensions, those stabilized with Tween 80 have been selected due to stability reasons. Table 1 depicts the effect of trehalose concentration on the mean size of AP nanocrystals assessed by PCS immediately after reconstituting the lyophilized powder and after 3 months of storage at 25 °C. After reconstituting the lyophilized AP nanosuspensions, the mean nanocrystal size was significantly higher, in comparison to the systems with added cryoprotectant (∼540 nm vs. ∼360 nm). The absence of trehalose was also responsible for the increased size during storage time due to particle aggregation. On the contrary, adding trehalose to the formulation led to a reduction of particle aggregation. Using one-way ANOVA to compare the mean particle size of lyophilized AP nanosupensions with and without trehalose addition, it was shown that the optimum amount of cryoprotectant was between 2% and 10% ($p > 0.05$). During 3 months of storage, the mean nanocrystal size remained below 380 nm, the ZP being always higher than −40 mV.

The ZP values slightly decreased after the lyophilization of samples. Nonetheless, the values obtained for both before and after lyophilization were higher than −40 mV. In addition, trehalose did not reveal a particular effect on the recorded ZP values. After 3 months of storage, the ZP values of all formulations remained unchanged emphasizing the optimal physical stability of the systems (Table 1). Therefore, both physical and chemical stabilities of the AP nanosuspensions could be achieved by transforming the aqueous dispersion into a dried powder by lyophilization.

3.8. X-ray diffraction analysis

X-ray diffraction has been used to analyze potential changes in the inner structure of AP nanocrystals due to the high-energy input during HPH. The extent of such changes depends on the chemical nature and on physical hardness of the active ingredient, as well as on the applied power density (Müller et al., 2001). Fig. 7 compares the diffractograms of bulk active (raw material) and of AP nanosuspensions stabilized with Tween 80 after lyophilization without the cryoprotective agent (trehalose). The obtained patterns reveal that after applying 20 cycles at 1500 bar by HPH, AP maintained a crystalline character.

From a literature, the Nanomorph™ technology of the company Soligs (Germany) leads to amorphous drug nanoparticles, being spherical in shape due to the amorphous character (not cuboid as nanocrystals). In general, drugs in the amorphous state show higher solubility than in the crystalline state. Therefore, from theoretical consideration, an amorphous drug nanoparticle of a poorly soluble compound should show best dissolution properties. However, the amorphous state is not long-term stable and it might change to a more crystalline state (Carstensen,

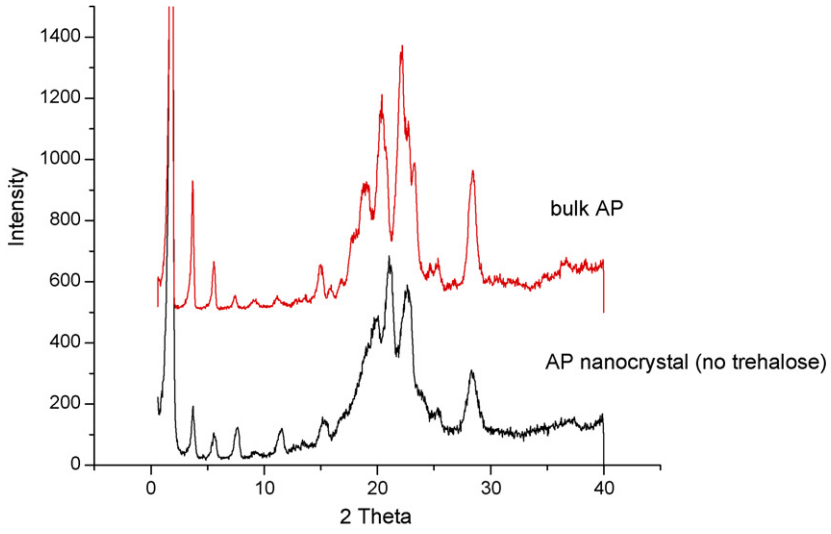

Fig. 7. X-ray diffraction patterns of bulk AP and lyophilized AP nanocrystals.

2001). This process cannot be predicted leading to unpredictable drug release and drug stability during its shelf life (Carstensen, 2001). Consequently, the presence of drug in a crystalline state and/or in a partial amorphous state might be suitable to increase its chemical stability and thus decrease aqueous solubility.

4. Conclusions

The physicochemical properties of AP nanosuspensions (mean nanocrystal size, zeta potential, and chemical stability) were found to be dependent on the type of stabilizer (surfactant). It was found that the mean size of AP nanosuspensions stabilized with Tween 80 remained in the nanometer range and the amount of active determined by HPLC was more than 90% when stored at three different temperatures during 3 months. The obtained results after lyophilization revealed that the nanocrystal agglomeration of formulations lyophilized without trehalose was more pronounced than those with trehalose. The suitable concentration of cryoprotectant was found to be between 2% and 10%. After 3 months of storage at room temperature, the z-ave of the lyophilized AP nanosuspensions stabilized with Tween 80 did not increase. From the X-ray diffractograms, it was shown that AP remained in a crystalline state which is physicochemically and thermodynamically more stable than AP in an amorphous state.

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