International Journal of Pharmaceutics 420 (2011) 93–100

Contents lists available at SciVerse ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Personalised Medicine

Nanosuspensions as advanced printing ink for accurate dosing of poorly soluble drugs in personalized medicines

Jana Pardeike[a,*], Daniela M. Strohmeier[a,b], Nina Schrödl[b], Christine Voura[b], Michael Gruber[b], Johannes G. Khinast[b], Andreas Zimmer[a]

[a] Institute of Pharmaceutical Sciences, Department of Pharmaceutical Technology, Karl-Franzens Universität Graz, Universitätsplatz 1, 8010 Graz, Austria
[b] Research Center Pharmaceutical Engineering GmbH, Inffeldgasse 21a/II, 8010 Graz, Austria

ARTICLE INFO

Article history:
Received 24 May 2011
Received in revised form 9 August 2011
Accepted 15 August 2011
Available online 22 August 2011

Keywords:
Nanosuspension
Personalized medicine
Folic acid
Dissolution velocity
Saturation solubility
Inkjet type printing technique

ABSTRACT

Folic acid was used as a model drug to demonstrate the advantages of formulating poorly soluble drugs as nanosuspensions and their use in an inkjet-type printing technique to produce personalized medicines. 10% folic acid nanosuspensions stabilized with Tween 20, a stabilizer showing the best wetting potential for folic acid, were prepared via high pressure homogenization. The particle size of the folic acid nanosuspension was well below 5 μm being a prerequisite for inkjet type printing technique. A good reproducibility of the particle size of folic acid nanosuspension prepared via high pressure homogenization was found. As indicated by the zeta potential the formulation showed a good storage stability. High pressure homogenization had no influence on the crystalline state of folic acid. An increase in the saturation solubility by 53.7% was found reducing the particle size from the micrometer range to the nanometer range. The dissolution velocity of the folic acid nanosuspension was significantly enhanced compared to a folic acid suspension, i.e. after 5 min 78.6% of the folic acid was dissolved from the nanosuspension and only 6.2% from the suspension. Moreover, the printing of 10% folic acid nanosuspension could be successfully demonstrated.

1. Introduction

About 40% of the new drugs in the development pipelines and about 70% of drugs produced via synthesis or high throughput screening are poorly soluble in aqueous media and many of them simultaneously in organic solvents (Merisko-Liversidge and Liversidge, 2008; Merisko, 2002). These drugs often present a challenging problem in formulation development as poor solubility creates delivery problems such as low oral bioavailability and fluctuation in absorption.

Examples of attempts that have been made to overcome the solubility problem include dissolution in aqueous mixtures (e.g. water–ethanol mixtures), solubilization using surfactants (e.g. Cremophor EL, Tween 20), formation of complexes (e.g. cyclodextrins), solid dispersions (e.g. mannitol) and salt formation (e.g. alkali metal salts, hydrochlorides). However, these approaches are partial of limited success as they require certain chemical properties of the drugs, e.g. molecular weight or functional groups. Drug nanocrystals/nanosuspensions have been suggested to be a universal delivery system for orally applied drugs for which the dissolution velocity is the rate-limiting step for absorption, i.e. class II and IV drugs in the biopharmaceutical classification system (BCS) (Shegokar and Müller, 2010).

Drug nanocrystals are pure solid drug particles with a mean particle size below 1 μm, generally between 200 nm and 500 nm (Keck and Müller, 2006). Although the term nanocrystals implies a crystalline structure, particles can be crystalline, partially crystalline or completely amorphous. A nanosuspension consists of drug nanocrystals, a stabilizing agent (typically surfactants or polymeric stabilizers) and a liquid dispersion medium (Müller and Akkar, 2004). The dispersion medium can be water, mixtures of water and other non-aqueous media (e.g. water–ethanol mixtures) or non-aqueous media (e.g. polyethylene glycol, oils).

Nanocrystals possess enhanced saturation solubility and consequently an increased dissolution velocity as well as enhanced mucoadhesion (Keck and Müller, 2006; Müller and Akkar, 2004; Shegokar and Müller, 2010). Furthermore, there are no regulatory issues as excipients, i.e. stabilizers and dispersion media, required for nanosuspension production are available with GRAS status for various applications (Müller and Jacobs, 2002). With regards to oral application, nanocrystals have the following advantages: an improved absorption and thus improved dose-bioavailability, improved dose proportionality, reduced fed/fasted state variability, reduced inter-subject variability and better patient compliance

* Corresponding author. Tel.: +43 316 380 8291; fax: +43 316 380 9100.
E-mail address: jana.pardeike@uni-graz.at (J. Pardeike).

0378-5173/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2011.08.033

as fewer oral units need to be taken (Dolenc et al., 2010; Li et al., 2009; Müller et al., 2001; Xia et al., 2010).

Formulations for oral application can easily be prepared from nanosuspensions. Aqueous nanosuspensions may be administered directly (e.g. Megace ES®). Furthermore, aqueous nanosuspensions can be used as granulation fluid in the tablet production or as a wetting agent for the extrusion mass in pelletization. Aqueous nanosuspensions can be transferred into drug nanocrystal powders, which then can be compressed into tablets or filled into capsules via spray-drying or lyophilization. Tablets (e.g. Rapamune®, Triglide®) and capsules (e.g. Emend®, Tricor®) are solid dosage forms containing drug nanocrystals produced via one of the above mentioned methods which can be found on the market.

Conventional pharmaceutical dosage forms, such as tablets or capsules, contain predefined amounts of active pharmaceutical ingredients. As a consequence, many patients, particularly women, children and elderly persons, may be confronted with under- or overdosage, which can lead to reduced effects or side effects. Personalized medicine aims at an individualized therapy specifically tailored to the requirements and needs of an individual patient. Factors such as age, weight, height, race, gender and disease state of the individual patient, are considered and translated in precisely tailored oral delivery forms to provide individualized therapy.

The present work investigated nanosuspensions with regards to the preparation of a personalized oral dosage form using a microdosing technology. The applied method for the production of individually dosed drugs was based on an inkjet-type printing technique under which all active pharmaceutical ingredients and related excipients required for a particular patient are directly printed on an edible substrate of a special paper carrier that can be rolled up and inserted into a hard gelatin capsule for oral administration (Voura et al., 2011). The advantages of this technique are (I) the possibility of on-demand manufacturing of a personalized oral dosage for individual patients, (II) precise dosing of low-dose drugs and/or drugs with a small therapeutic window, (III) multi-dosing by printing multiple drug layers on one paper carrier strip using barrier coatings and (IV) no need for the development of complex formulations (e.g. multilayer tablets).

The BCS class 4 substance folic acid was used as a model drug in the present study. Folic acid is practically insoluble in water and most organic solvents. The aim of this work was to show that the particle size of a poorly soluble drug, i.e. folic acid, can be reduced via high pressure homogenization in a reproducible way to a size below 5 μm, which is a precondition for printability using microdrop technology. Furthermore, the stability of the obtained folic acid nanosuspension was investigated. The influence of the size reduction on saturation solubility and dissolution behavior was studied and compared with a macrosuspension of folic acid. Moreover, the drop formation of 10% (w/w) folic acid nanosuspension was investigated.

2. Materials and methods

2.1. Materials

The following materials were used in the present study: folic acid (DSM, Heerlen, The Netherlands), Tween 20 (Cognis, Monheim, Germany), Tween 40 (Sigma–Aldrich, Steinheim, Germany), Tween 80 (Croda, Nettal, Germany), sodium lauryl sulfate (Merck, Hohenbrunn, Germany), Tagat S (Kwizda, Vienna, Austria), TPGS (Cognis, Monheim, Germany), Solutrol HS15 (BASF, Ludwigshafen, Germany), Cremophor EL (BASF, Ludwigshafen, Germany), Cremophor RH 40 (BASF, Ludwigshafen, Germany) and Lutrol F68 (BASF, Ludwigshafen, Germany).

2.2. Contact angle measurement

Contact angles were determined by goniometry on cover slides coated with folic acid. Briefly, folic acid was dissolved in dimethylformamide (Carl Roth, Karlsruhe, Germany), the solution was evenly spread over a cover slide and dimethylformamide evaporated forming of a dense folic acid film. 10 μl of purified water or 0.1% (w/v) surfactant/stabilizer solution in purified water was applied to the folic acid film. The contact angle was measured using an Easy Drop G1 (Krüss, Hamburg, Germany). The experiment was carried out in triplicate.

2.3. Preparation of folic acid suspension and nanosuspension

Folic acid suspension was obtained suspending 10% (w/w) folic acid in an aqueous 3% (w/w) Tween 20 solution using a mortar and a pestle. For nanosuspension production a Panda 2 K, NS1001L Spezial (GEA Niro Soavi, Lübeck, Germany) equipped with a water jacket for temperature control was used. Briefly, 10% (w/w) folic acid was suspended in an aqueous 3% (w/w) Tween 20 solution. The dispersion was homogenized running two cycles at 500 bar followed by 30 cycles at 1000 bar. The temperature was set at 5 °C. Each batch had a volume of 50 ml.

2.4. Photon correlation spectroscopy

The particle size of folic acid nanosuspension was investigated by photon correlation spectroscopy (PCS) using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) ($n = 3$). PCS yields the mean particle size and the polydispersity index (PI) as a measure of the width of the particle size distribution (Müller and Schuhmann, 1996).

2.5. Laser diffractometry

The particle size of folic acid suspension and nanosuspension was determined via laser diffractometry (LD) using a Mastersizer 2000 (Malvern Instruments, Malvern, UK). The Mie theory was used for data evaluation. Water with a refractive index (RI) of 1.33 was used as measuring medium. The real RI of folic acid is 1.755. The imaginary RI of this compound was 0. The LD data were evaluated using the diameters 50% (LD 50), 90% (LD 90), 95% (LD 95) and 99% (LD 99), which means that either 50%, 90%, 95% or 99% (volume distribution) of the measured particles are below the given size ($n = 3$).

2.6. Zeta potential

The zeta potential was measured using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). Measurements were performed in distilled water adjusted with 0.9% (w/v) sodium chloride solution to a conductivity of 50 μS cm^{-1} and a pH of 5.5–6.0 to obtain information on the surface charge. Moreover, measurements were performed in the original dispersion media to estimate the thickness of the diffuse layer. The zeta potential was calculated by applying the Helmholtz–Smoluchowski equation ($n = 3$).

2.7. Stability study

To investigate the physical and chemical long-term stability of the nanosuspension, samples were stored at 5 ± 3 °C, 25 ± 2 °C and 25 ± 2 °C with constant agitation of 150 rpm. The particle size of the formulation was measured over a period of 30 days by PCS and LD. Chemical long-term stability was investigated by measuring the

folic acid content on the day of production and on day 30 using HPLC analysis.

2.8. HPLC method

A reverse phase HPLC-UV/MS method modified after Heudi et al. (2005) was used to quantify folic acid. Standards were dissolved in ultra purified water (TKA MicroPure, Niederelbert, Germany). An ACQUITY UPLC H-Class Bio (Waters, Eischborn, Germany) system with an autosampler model Sample Manager-FTN, a pump system model Quaternary Solvent Manager and a PDA Detector linked to an Empower 2 data acquisition and process system was used. 10 μl was injected onto an ACQUITY UPLC BEH C18 1.0 mm × 50 mm (1.7 μm) column (Waters, Eischborn, Germany). The mobile phase was run with a flow rate of 0.4 ml/min utilizing a gradient of an aqueous 0.15% acetic acid solution (Sigma–Aldrich, Vienna, Austria) and acetonitrile (Bartelt, Graz, Austria). The UV-spectrum was recorded at 275 nm.

2.9. X-ray diffraction

X-ray diffraction was used to study the crystallinity of folic acid suspension and folic acid nanosuspension. Locust bean gum was added as thickening agent to the formulations in order to make the liquids accessible to the measurement without a drying step. Diffraction patterns were measured using a Philips PW1830 X-ray generator (Philips, Amedo, The Netherlands) with a copper anode ($\ddot{e}$ = 1.5418 Å, 40 kV, 20 mA) and a Goniometer PW18120 as detector. The samples were scanned from 0.6° to 40°, at 2θ with a step size of 0.04° and a step time of 0.5 s. Bragg's equation was used for data evaluation.

2.10. Saturation solubility

The solubility of folic acid from 10% (w/w) suspension and 10% (w/w) nanosuspension was determined in distilled water. For this purpose, 20 μl of each formulation was added to 5.0 ml of distilled water (n = 5). The samples were agitated at 150 rpm at 25 °C for 3 days. The samples were then filtered through 0.02 μm filters (Anotop 25 Plus, Whatman, Maidstine, UK) to remove the excess of the un-dissolved folic acid. The dissolved amount of folic acid was determined via HPLC. For statistical evaluation the two sample t-test for unpaired data was performed after testing for normal distribution using the Shapiro–Wilk test (α = 5%).

2.11. Dissolution study

Dissolution testing of folic acid suspension and nanosuspension was performed under sink conditions using a Dissolution Pharma Test Type PTW S3C (Bartelt, Graz, Austria). 500 μl of the test formulations was placed in 1000 ml of an aqueous 5% (w/v) sodium dodecyl sulfate solution. The test was carried out using the paddle method with a rotation speed of 100 rpm at 37 ± 0.5 °C. At predetermined time intervals 1.00 ml samples were withdrawn, filtered through 0.02 μm filters (Anotop 25 Plus, Whatman, Maidstine, UK) and 1.00 ml of fresh dissolution medium was added to replenish the dissolution medium. The amount of dissolved folic acid was determined by HPLC. The experiment was carried out in triplicate. The Wilcoxon test was used (α = 5%) for statistical evaluation.

2.12. Printing of folic acid nanosuspension

Printing of the folic acid nanosuspension was performed using an inkjet-based microdosing dispenser head (MD-K-140, Microdrop Technologies GmbH, Norderstedt, Germany) with an aperture size of 100 μm. The dispenser head was cleaned with water and then filled with the prefiltered (5 μm syringe filter, ReZist 30 PTFE, Whatman, Maidstine, UK) nanosuspension. The dosing device was continuously operated at a frequency of 200 Hz, an electric voltage of 100 V and an impulse width of 25 μs. All printing experiments were carried out at 20 °C and under ambient conditions.

Table 1
Contact angles obtained with purified water and 0.1% (w/v) surfactant/stabilizer solutions on folic acid films (n = 3, $\bar{x} \pm$ SD).

Surfactant/stabilizer	Contact angle [°]
Purified water	40.60 ± 0.98
Tween 20	11.90 ± 0.00
Tween 40	13.23 ± 5.91
Tween 80	19.43 ± 5.28
TPGS	17.60 ± 4.98
Sodium dodecyl sulfate	16.93 ± 2.79
Lutrol F68	18.20 ± 7.01
Solutrol HS15	23.67 ± 1.35
Cremophor RH 40	19.97 ± 2.85
Cremophor EL	24.77 ± 4.80
Tagat S	14.70 ± 5.23

3. Results and discussion

3.1. Contact angle measurement

In order to produce well stabilized drug nanosuspensions, the stabilizer used should (I) have a sufficient affinity to the particle surface, (II) possess a high diffusion rate to cover newly created surface areas of the drug generated during high pressure homogenization and (III) be present in a sufficient amount to fully cover the particle surface to prevent agglomeration and ensure long-term stability (Mishra et al., 2009; Müller and Jacobs, 2002). The affinity/wettability of a drug by a stabilizer solution can be evaluated by contact angle measurements. A small contact angle between the drug substance and the stabilizer was found to be a good predictor of the stabilizer's suitability for the production of nanosuspensions via high pressure homogenization (Cerdeira et al., 2010; Pardeike and Müller, 2010). Hence, the contact angles between folic acid films and solutions of surfactants/stabilizers with GRAS status for oral application were measured (Table 1). The highest contact angle was measured with purified water on folic acid films, i.e. 40.6°. All surfactants/stabilizers reduced the contact angle due to the lower surface tension of the surfactant/stabilizer solution compared to purified water. The best wetting of folic acid was obtained with Tween 20 solution. With 0.1% (w/v) Tween 20 solution the contact angle was reduced to 11.9°. Thus, Tween 20 was chosen as stabilizing agent for the folic acid nanosuspension.

3.2. Preparation of folic acid suspension and nanosuspension

In the present study high pressure homogenization was used for folic acid nanosuspension preparation. The achievable particle size of nanosuspensions prepared via high pressure homogenization using a piston gap homogenizer depends on (I) the applied homogenization pressure and hereby the width of the homogenization gap, (II) the number of applied homogenization cycles, (III) the production temperature, (IV) the hardness of the material subjected to homogenization and (V) the fineness of the starting material (Keck and Müller, 2006; Möschwitzer et al., 2004; Müller and Jacobs, 2002; Müller et al., 2001; Müller and Peters, 1997).

10% (w/w) folic acid was suspended in an aqueous 3% (w/w) Tween 20 solution. The particle size of the suspension was investigated by LD and light microscopy (Figs. 1 and 2 (left)). By LD measurements an LD 50 of 2.83 μm, an LD 90 of 6.66 μm, an LD 95 of 8.48 μm and an LD 99 of 13.64 μm were obtained. The particle size measured by LD was in agreement with the particle size

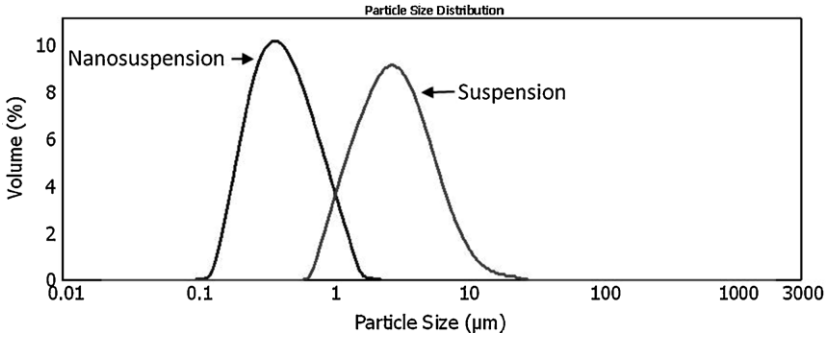

Fig. 1. LD volume distribution curves of folic acid bulk material suspension and nanosuspension.

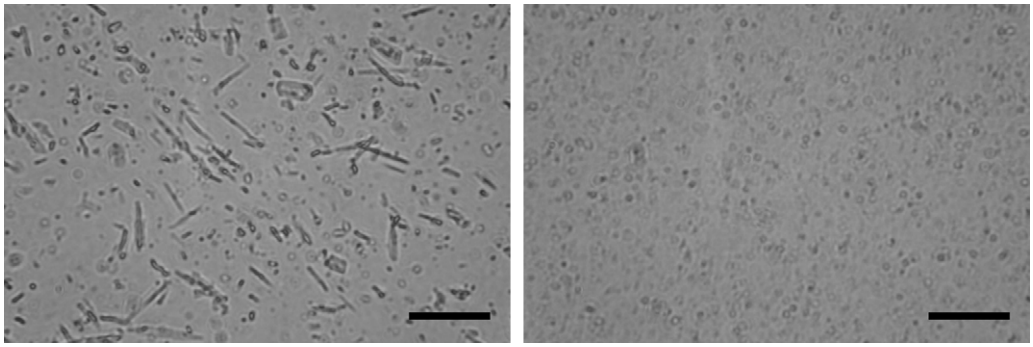

Fig. 2. Light microscope pictures with 1000-fold magnification of folic acid suspension (left) and folic acid nanosuspension (right). The bar refers to 20 μm.

observed by light microscopy. Furthermore, a broad particle size distribution of the folic acid suspension can be seen in the light microscope image.

Subjecting the folic acid suspension to high pressure homogenization (30 cycles at 1000 bar) led to a particle size in the nanometer-range and a narrow particle size distribution. The 10% (w/w) folic acid nanosuspension had an average particle size of 407 nm and a PI of 0.288. The LD volume distribution curve and a light microscope image of the folic acid nanosuspension are shown in Figs. 1 and 2, respectively. For folic acid nanosuspension an LD 50 of 0.42 μm, an LD 90 of 0.91 μm, an LD 95 of 1.09 μm and an LD 99 of 1.40 μm were measured. The light microscope image (Fig. 2 (right)) shows a uniform particle size of the folic acid nanosuspension. Particle size analysis techniques demonstrated that the folic acid particles could be reduced to a size well below 5 μm which is a prerequisite for printing the formulation using an inkjet-based microdosing dispenser head.

The reproducibility of the achieved particles size of the 10% (w/w) folic acid nanosuspension was investigated in three different batches by PCS and LD measurements. To determine the reproducibility the relative standard deviation (RSD) was calculated. The results are shown in Table 2. The RSD was below 10% for all measuring parameters, indicating a good reproducibility of the particle size of the folic acid nanosuspension. These findings are in agreement with the previously published data and the reports of a good reproducibility of the particle size for nanosuspensions produced via high pressure homogenization (Grau et al., 2000; Peters, 1999).

Table 2
Inter batch reproducibility of the particle size of folic acid nanosuspension.

	Batch 1	Batch 2	Batch 3	RSD [%]
PCS [nm]	407	373	411	5.3
PI	0.288	0.275	0.279	2.4
LD 50 [μm]	0.418	0.439	0.407	3.9
LD 90 [μm]	0.906	1.021	0.926	6.5
LD 95 [μm]	1.087	1.272	1.162	7.9
LD 99 [μm]	1.401	1.511	1.647	8.1

3.3. Crystalline state evaluation

Due to the high energy input during high pressure homogenization changes in the crystalline state of the material subjected to high pressure homogenization, i.e. change of polymorphic modification, increase of the amorphous fraction or creation of completely amorphous particles, might occur (Möschwitzer and Müller, 2006; Teeranachaideekul et al., 2008). Changes in the crystalline state can affect (I) the solubility, (II) the dissolution velocity, (III) the oral bioavailability as well as (IV) the stability of a pharmaceutical formulation (Möschwitzer and Müller, 2006; Wang et al., 2011).

Therefore, the influence of high pressure homogenization on the crystalline structure of folic acid was investigated via X-ray diffraction. Fig. 3 shows the X-ray diffractograms of folic acid suspension and folic acid nanosuspension. Both, the diffraction patterns of the suspension and nanosuspension, show a high peak intensity caused

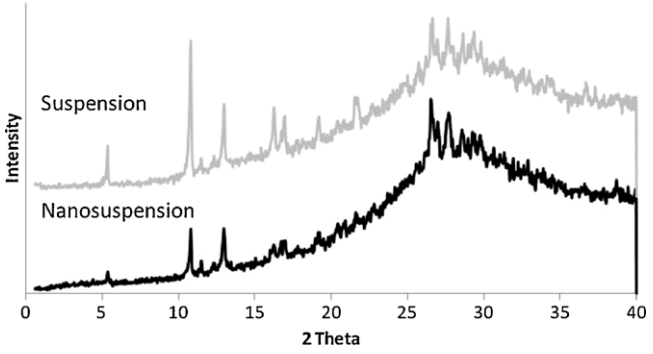

Fig. 3. X-ray diffraction patterns of folic acid suspension and folic acid nanosuspension.

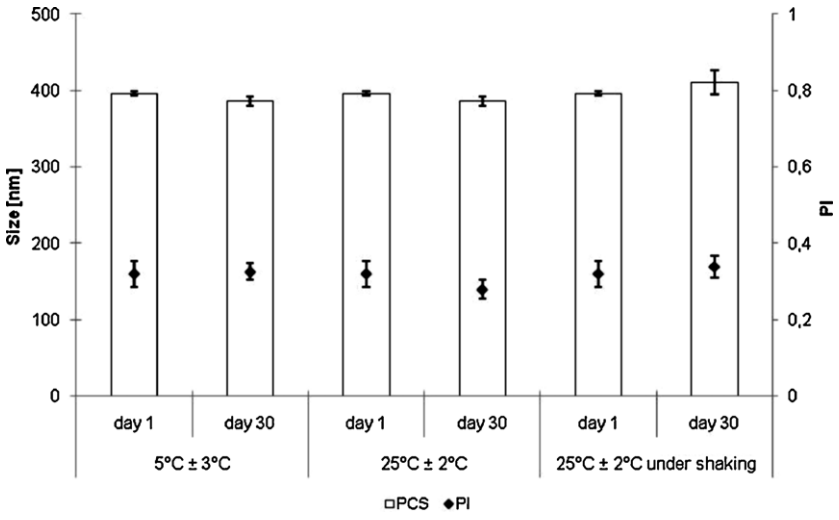

Fig. 4. Particle size and PI of folic acid nanosuspension measured by PCS at day 1 and day 30 storing the samples refrigerated, at room temperature and at room temperature under constant shaking.

by the crystalline state of folic acid. There is no difference in the peak location for folic acid suspension and nanosuspension indicating the presence of the same crystalline structure of folic acid in both formulations. Furthermore, the peak location is in agreement with the reported peak location for folic acid (Vora et al., 2002).

3.4. Zeta potential

The zeta potential can be used to predict storage stability of dispersed systems. If the zeta potential is measured in a media with low electrolyte concentration, e.g. with a conductivity of 50 $\mu S\,cm^{-1}$, predictions on the surface charge density, i.e. the Nernst potential, can be made. Measuring the zeta potential of dispersed particles in the original dispersion medium provides information regarding the thickness of the diffuse layer. In both cases it may be assumed that the higher the measured zeta potential is, the better is the physical long-term stability of the dispersion (Jacobs and Müller, 2002; Mishra et al., 2009; Müller, 1996; Müller and Jacobs, 2002).

In distilled water adjusted to a conductivity of 50 $\mu S\,cm^{-1}$, a zeta potential of -50.8 ± 0.7 mV was measured for folic acid nanosuspension. This value indicates a well charged surface promoting long-term stability of the nanosuspension (Riddick, 1968). In the original medium, which was the storage medium for the folic acid nanosuspension, a zeta potential of -38.9 ± 1.0 mV was obtained. It is well known that absolute zeta potential values above 30 mV measured in the original dispersion medium indicate a good physical stability since the diffuse layer has a sufficient thickness to prevent particle aggregation via electrostatic repulsion (Ney, 1973). Thus, the zeta potentials measured in both dispersion media indicate good long-term stability of the folic acid nanosuspension.

3.5. Stability study

A stability study was performed by storing the folic acid nanosuspension over 30 days under 3 conditions, i.e. refrigerated, at room temperature and at room temperature under constant shaking. These conditions were chosen to determine instabilities as low temperatures can promote particle growth due to solubility reduction and therefore recrystallization. Agitating the samples during storage can mimic shipping and handling conditions of the formulation, which induces kinetic energy into the system and in some cases, can reduce the physical stability of the formulation due to particle agglomeration/aggregation.

The particle size of folic acid nanosuspension measured by PCS and LD during the observation period under the chosen storage conditions is shown in Figs. 4 and 5, respectively. By PCS measurements small changes in the mean particles size of the bulk population can

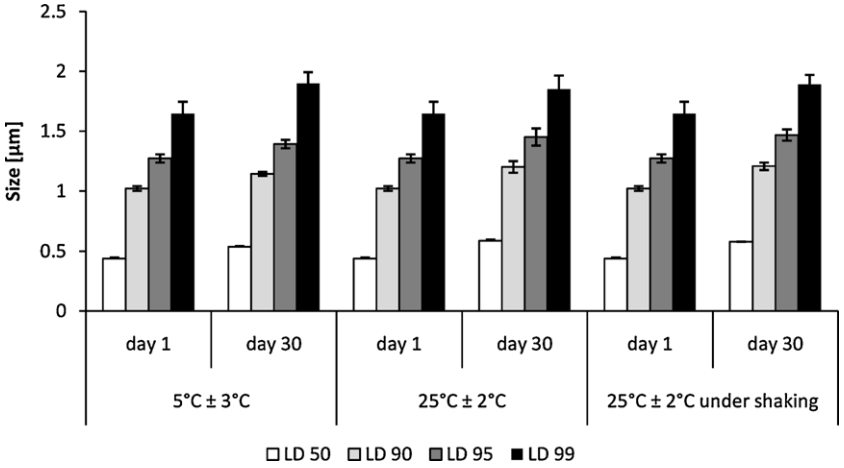

Fig. 5. Particle size of folic acid nanosuspension measured by LD at day 1 and day 30 storing the samples refrigerated, at room temperature and at room temperature under constant shaking.

be detected. LD measurement is a useful tool to detect changes of the size of larger particles as well as agglomeration and aggregation taking place in the formulation. Under all storage conditions no changes in particle size were detected by PCS measurements. Moreover, only minor changes were detected by LD measurements that would not affect the printing process via inkjet-type printing technique. The nanosuspension was physically stable. These findings are supported by a high zeta potential of folic acid nanosuspension both in distilled water adjusted to a conductivity of 50 μS cm^{-1} and in the original dispersion medium. Physical long-term stability of up to 3 years of drug nanosuspensions was reported in the literature (Peters, 1999).

In addition to physical stability, chemical stability of folic acid was evaluated. The folic acid content was constant from the day of production until day 30 of storing the nanosuspension under the three storage conditions. The nanosuspension was chemically stable during the observation period.

3.6. Saturation solubility

Saturation solubility is defined as the maximum quantity of a compound (solute) that can be dissolved in a certain quantity of a specific solvent at a specified temperature (Latscha and Klein, 1995). However, it is well known that the saturation solubility also depends on the modification of polymorphic compounds (Giron, 1995; Grunenberg, 1997) and the particle size in case particles <1 μm are present (Grant and Brittain, 1995). According to the crystalline state investigation, an increase in saturation solubility of folic acid due to the presence of a less energetic polymorphic or an amorphous form can be excluded. In order to obtain information regarding the effect of the particle sizes of folic acid nanosuspension and suspension on the saturation solubility, solubility studies were performed.

The folic acid suspension had a saturation solubility of 8.96 ± 1.94 μg/ml. Reducing the particle size of folic acid to the nanometer range led to an increase of the saturation solubility to 13.77 ± 3.50 μg/ml. There was a significant difference between the saturation solubility of folic acid in suspension and nanosuspension (α = 5%).

The Kelvin equation and the Ostwald–Freundlich equation describe the principle of the increase in saturation solubility if the particle size is reduced below 1 μm. Briefly the Kelvin equation describes the vapour pressure over a curved surface of a liquid droplet in a gas phase. The vapour pressure increases with an increasing curvature caused by a decreasing particle size. This equation that describing the transition of molecules from a liquid phase to a gas phase can also be applied to the transition of molecules from a solid phase to a liquid phase. The vapour pressure is then replaced by the dissolution pressure. Saturation solubility is the equilibrium between dissolving and recrystallizing molecules. Increasing the dissolution pressure shifts the equilibrium resulting in an increase in saturation solubility.

The Ostwald–Freundlich equation not only describes the dependency of saturation solubility on the particle size but offers another possible explanation for the increase in saturation solubility via the creation of high energy surfaces that occur when microparticles are disrupted into nanoparticles. Lyophobic inner surfaces of the particle will be exposed to the aqueous dispersion medium. According to this equation the saturation solubility is a function of the interfacial energy. Differences in the interfacial energy cause differences in the saturation solubility of polymorph forms (Kipp, 2004; Müller and Akkar, 2004). The same may be valid for differences in saturation solubility for nanosuspensions and microparticulate suspensions (Müller and Peters, 1997).

In the literature various examples are given for an increase in saturation solubility decreasing the particle size from the micrometer range to the nanometer range (Mauludin et al., 2009; Pardeike and Müller, 2010; Wang et al., 2011; Xia et al., 2010). The findings for folic acid are well in agreement with the theory and earlier findings for other compounds.

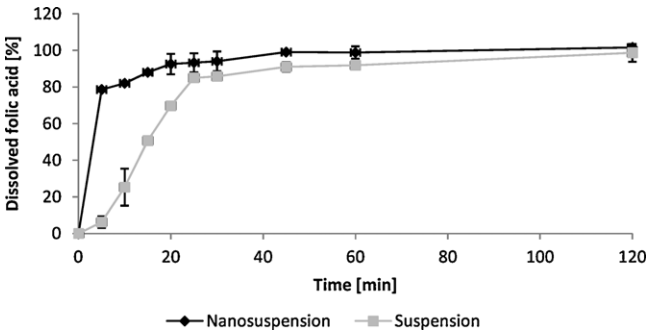

Fig. 6. Dissolution profile of folic acid nanosuspension and folic acid suspension in 5% sodium dodecyl sulfate solution (n = 3).

3.7. Dissolution velocity of folic acid nanosuspension and suspension

The dissolution study was carried out under sink conditions. To obtain sink conditions for the poorly soluble folic acid 5% sodium dodecyl sulfate was added to the dissolution medium. The dissolution profiles of folic acid nanospuspension and folic acid suspension are shown in Fig. 6. The dissolution of folic acid from the nanosuspension was significantly faster than from folic acid suspension (α = 5%). Within the first 5 min 78.6% and 6.2% of folic acid dissolved from the nanosuspension and suspension, respectively. More than 90% of folic acid was dissolved from the nanosuspension within 20 min. The dissolution of a similar amount of folic acid from the suspension took 45 min.

The dissolution velocity depends on (I) the surface area of solid particles, (II) the diffusional transport of dissolved material and (III) the saturation solubility of the solute. An increase in dissolution velocity is proportional to an increase in surface area which occurs when the particle size is reduced (Mosharraf and Nyström, 1995). This is described by the Noyes-Whitney equation. The Prandtl equation describes the reduction of the diffusional distance with an increasing curvature of ultrafine particles, which is an additional factor accelerating the dissolution velocity (Anderberg et al., 1988; Nyström and Bisrat, 1988).

Dissolution studies on folic acid nanosuspension and suspension clearly showed an increase in the dissolution velocity due to the reduction of the particle size from the micrometer-range to the nanometer-range, which had previously been reported for other poorly soluble compounds (Ambrus et al., 2009; Dolenc et al., 2010; Mauludin et al., 2009; Wang et al., 2011; Xia et al., 2010).

3.8. Printing of folic acid nanosuspension

For the reliable and reproducible production of monodisperse microdroplets, the properties of the used ink must be within certain limits. The requirements for a stable droplet ejection are (I) the elimination of particles that are large enough to clog the ejection hole and (II) a viscosity that is sufficiently low to produce a liquid jet (Lee, 2003). In addition, density and surface tension of the dispensed liquid have considerable influence on the microdrop formation (Jang et al., 2009).

The dispensed droplets were observed using a stroboscope diode that was synchronized with the actuation signal of the droplet generator. Its delay relative to the electric pulse of the

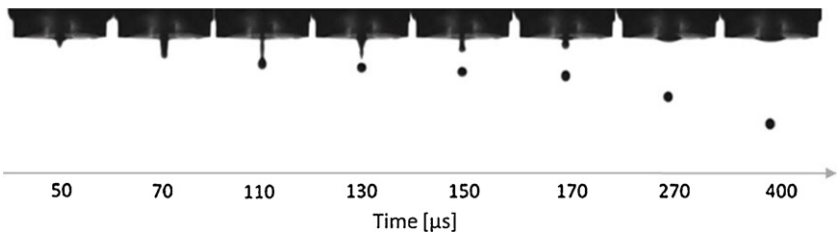

Fig. 7. Visualization of the drop formation. The pulse width is 25 μs, the pulse amplitude 100 V and the frequency 200 Hz. The final droplet size is 68.4 μm.

droplet generator can be varied between 0 and 2 ms, allowing the observation of the drop formation stages.

Fig. 7 shows various stages in the development of a microdroplet. At the early stages a liquid jet is ejected from the aperture, which destabilizes and forms a spherical shaped droplet. The rest of the liquid column is drawn back into the nozzle. The droplet break off occurs approximately 120 μs after the initial driving pulse.

The droplet size and velocity were calculated based on the droplet images using an in-house Matlab routine. Depending on the variation of the operating parameters of the dosing device droplet diameters between 56 and 84 μm and droplet velocities between 0.5 and 1.5 m/s were obtained. By keeping frequency, electric voltage and impulse width constant, precise droplets with a highly reproducible diameter and velocity are achieved, which guarantees a high accuracy of the dosing for the production of personalized medicines.

The experiments showed that the particle size of the folic acid nanosuspension was sufficiently small, enabling undisturbed microdrop formation. Even when the process had been halted for about an hour no clogging of the drop ejector occurred. Furthermore, no overly wetted nozzle orifice and no buildup of solid deposits in or around the ejection hole were observed, which indicates that the developed nanosuspension is suitable for inkjet-type printing technique used in the production of personalized medicines.

The potential of using nanosuspension for dispensing APIs using microdrop technology compared to the application of drug solutions in inkjet type printing is miscellaneous. The possibility of processing poorly soluble drugs, the increase of stability of the formulation, the wider range of applications for different APIs and the increase of the saturation solubility as well as the dissolution velocity are only some advantages. Especially for printing of formulations with high API concentrations the effect of recrystallization of the dissolved API is excluded which often occurs when dosing highly concentrated solutions with an API content close to the saturation solubility. This recrystallization leads to a clogging of the ejector nozzle or an inhomogeneous application of the drug respectively which prevents an accurate and reliable production of personalized dosage forms.

4. Conclusion

A nanosuspension that met the prerequisites for an inkjet-type printing technique with a particle size well below 5 μm and an active content of 10% (w/w) was prepared via high pressure homogenization in a reproducible manner from the poorly soluble folic acid. Furthermore the nanosuspension had a good storage stability. Reducing the particle size to the nanometer range led to a significant increase in saturation solubility and dissolution velocity of folic acid compared to suspension, which is advantageous for the oral absorption of poorly soluble drugs. It was also demonstrated that 10% (w/w) folic acid nanosuspension can be printed on edible paper carriers via inkjet-type printing technique for the production of personalized medicines. Combining the advantages of formulating poorly soluble drugs as nanosuspensions to reduces oral absorption problems with the inkjet-type printing technology for the preparation of personalized medicines can contribute to an effective and safe therapy for individual patients.

Acknowledgement

We acknowledge the financial support of the Austrian Federal Government in the framework of the COMET competence center funding program as well as from G.L. Pharma. We thank Maxi Pardeike for illustrating the graphical abstract.

References

Anderberg, E.K., Nyström, C., Bisrat, M., 1988. Physicochemical aspects of drug release VII. The effect of surfactant concentration and drug particle size on solubility and dissolution rate of felodipine, a sparingly soluble drug. Int. J. Pharm. 47, 67–77.

Ambrus, R., Kocbek, P., Kristl, J., Sibanc, R., Rajko, R., Szabo-Revesz, P., 2009. Investigation of preparation parameters to improve the dissolution of poorly water-soluble meloxicam. Int. J. Pharm. 381, 153–159.

Cerdeira, A.M., Mazzotti, M., Gander, B., 2010. Miconazole nanosuspensions: influence of formulation variables on particle size reduction and physical stability. Int. J. Pharm. 396, 210–218.

Dolenc, A., Govedarica, B., Dreu, R., Kocbek, P., Srcic, S., Kristl, J., 2010. Nanosized particles of orlistat with enhanced in vitro dissolution rate and lipase inhibition. Int. J. Pharm. 396, 149–155.

Giron, D., 1995. Thermal analysis and calorimetric methods in the characterisation of polymorphs and solvates. Thermochim. Acta 248, 1–59.

Grant, D.J.W., Brittain, H.G., 1995. Solubility of pharmaceutical solids. In: Brittain, H.G. (Ed.), Physical Characterization of Pharmaceutical Solids. Marcel Dekker, New York, pp. 321–386.

Grau, M.J., Kayser, O., Müller, R.H., 2000. Nanosuspensions of poorly soluble drugs—reproducibility of small scale production. Int. J. Pharm. 196, 155–159.

Grunenberg, A., 1997. Polymorphism and thermal analysis of pharmaceutical agents. Pharm. Unserer Z. 26, 224–231.

Heudi, O., Kilinc, T., Fontannaz, P., 2005. Separation of water-soluble vitamins by reversed-phase high performance liquid chromatography with ultra-violet detection: application to polyvitaminated premixes. J. Chromatogr. A 1070, 49–56.

Jacobs, C., Müller, R.H., 2002. Production and characterization of a budesonide nanosuspension for pulmonary administration. Pharm. Res. 19, 189–194.

Jang, D., Kim, D., Moon, J., 2009. Influence of fluid physical properties on ink-jet printability. Langmuir 25, 2629–2635.

Keck, C.M., Müller, R.H., 2006. Drug nanocrystals of poorly soluble drugs produced by high pressure homogenisation. Eur. J. Pharm. Biopharm. 62, 3–16.

Kipp, J.E., 2004. The role of solid nanoparticle technology in the parenteral delivery of poorly water-soluble drugs. Int. J. Pharm. 284, 109–122.

Latscha, H.P., Klein, H.A., 1995. Analytische Chemie. Springer Verlag, Berlin/Heidelberg.

Lee, E.R., 2003. Fluid engineering of microdrop ejectors. In: Lyshevski, S.E. (Ed.), Microdrop Generation. CRC Press LCC, pp. 177–188.

Li, X., Gu, L., Xu, Y., Wang, Y., 2009. Preparation of fenofibrate nanosuspension and study of its pharmacokinetic behavior in rats. Drug Dev. Ind. Pharm. 35, 827–833.

Mauludin, R., Müller, R.H., Keck, C.M., 2009. Kinetic solubility and dissolution velocity of rutin nanocrystals. Eur. J. Pharm. Sci. 36, 502–510.

Merisko-Liversidge, E.M., Liversidge, G.G., 2008. Drug nanoparticles: formulating poorly water-soluble compounds. Toxicol. Pathol. 36, 43–48.

Merisko, E., 2002. Liversidge nanocrystals: resolving pharmaceutical formulation issues associated with poorly water-soluble compounds. In: Marty, J.J. (Ed.), Particles. Marcel Dekker, Orlando.

Mishra, P.R., Al Shaal, L., Müller, R.H., Keck, C.M., 2009. Production and characterization of Hesperetin nanosuspensions for dermal delivery. Int. J. Pharm. 371, 182–189.

Möschwitzer, J., Achleitner, G., Pomper, H., Müller, R.H., 2004. Development of an intravenously injectable chemically stable aqueous omeprazole formulation using nanosuspension technology. Eur. J. Pharm. Biopharm. 58, 615–619.

Möschwitzer, J., Müller, R.H., 2006. Spray coated pellets as carrier system for mucoadhesive drug nanocrystals. Eur. J. Pharm. Biopharm. 62, 282–287.
Mosharraf, M., Nyström, C., 1995. The effect of particle size and shape on the surface dissolution rate of micronized practically insoluble drugs. Int. J. Pharm. 122, 35–47.
Müller, R.H., 1996. Zetapotential und Partikelladung in der Laborpraxis. Wissenschaftliche Verlagsgesellschaft, Stuttgart.
Müller, R.H., Akkar, A., 2004. Drug nanocrystals of poorly soluble drugs. Encyclopedia Nanosci. Nanotechnol. 2, 627–638.
Müller, R.H., Jacobs, C., 2002. Buparvaquone mucoadhesive nanosuspensions: preparation, optimisation and long-term stability. Int. J. Pharm. 237, 151–161.
Müller, R.H., Jacobs, C., Kayser, O., 2001. Nanosuspensions as particulate drug formulations in therapy. Rationale for development and what we can expect for the future. Adv. Drug Deliv. Rev. 47, 3–19.
Müller, R.H., Peters, K., 1997. Nanosuspension for the formulation of poorly soluble drugs 1. Preparation by a size-reduction technique. Int. J. Pharm. 160, 229–237.
Müller, R.H., Schuhmann, R., 1996. Teilchengrößenmessung in der Laborpraxis. Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart.
Ney, P., 1973. Zetapotentiale und Flotierbarkeit von Mineralien. Springer Verlag, Wien/New York.
Nyström, C., Bisrat, M., 1988. Physicochemical aspects of drug release VIII. The relation between particle size and surface specific dissolution rate in agitated suspensions. Int. J. Pharm. 47, 223–231.
Pardeike, J., Müller, R.H., 2010. Nanosuspensions: a promising formulation for the new phospholipase A2 inhibitor PX-18. Int. J. Pharm. 391, 322–329.
Peters, K., 1999. Nanosuspensionen-Ein neues Formulierungsprinzip für schwerlösliche Arzneistoffe. PhD, Freie Universität Berlin, Berlin.
Riddick, T.M., 1968. Zeta-Meter Manual. Zeta-Meter Inc., New York.
Shegokar, R., Müller, R.H., 2010. Nanocrystals: industrially feasible multifunctional formulation technology for poorly soluble actives. Int. J. Pharm. 399, 129–139.
Teeranachaideekul, V., Junyaprasert, V.B., Souto, E.B., Müller, R.H., 2008. Development of ascorbyl palmitate nanocrystals applying the nanosuspension technology. Int. J. Pharm. 354, 227–234.
Vora, A., Riga, A., Dollimore, D., Kenneth, S.A., 2002. Thermal stability of folic acid. Thermochim. Acta 392–393, 209–220.
Voura, C., Schroedl, N., Gruber, M.M., Strohmeier, D., Eitzinger, B., Bauer, W., Brenn, G., Khinast, J.G., Zimmer, A., 2011. Printable medicines: a microdosing device for producing personalised medicines. Pharma Technol. Eur. 23, 32–36.
Wang, Y., Liu, Z., Zhang, D., Gao, X., Zhang, X., Duan, C., Jia, L., Feng, F., Huang, Y., Shen, Y., Zhang, Q., 2011. Development and in vitro evaluation of deacety mycoepoxydiene nanosuspension. Colloids Surf. B Biointerfaces 83, 189–197.
Xia, D., Quan, P., Piao, H., Sun, S., Yin, Y., Cui, F., 2010. Preparation of stable nitrendipine nanosuspensions using the precipitation-ultrasonication method for enhancement of dissolution and oral bioavailability. Eur. J. Pharm. Sci. 40, 325–334.