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Nanosuspensions: A promising formulation for the new phospholipase A_2 inhibitor PX-18

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ABSTRACT

PX-18 is a new highly potent phospholipase A_2 inhibitor with a poor aqueous solubility. Therefore, it was formulated as nanosuspensions with an active content of 1% (w/w), 5% (w/w) and 10% (w/w) using high-pressure homogenization. By contact angle measurements on compressed discs of PX-18, Tween 80 was identified as best wetting agent of PX-18 and therefore used to stabilize the nanosuspensions. The achieved particle size of all nanosuspensions was well in the nanometer range. For the 1% (w/w) PX-18 nanosuspension an average particle size below 50 nm was measured by photon correlation spectroscopy (PCS). A good reproducibility of the mean particle size was found in between different batches. As postulated by the zeta potential of the nanosuspensions, a good physical stability over an observation period of 180 days was obtained when stored at 5 ± 3 °C. PX-18 formulated as nanosuspensions was chemically stabile. An increase in saturation solubility was found after formulating PX-18 as nanosuspension compared to bulk material, favourable for increasing bioavailability.

1. Introduction

Phospholipase A_2 (PLA_2) hydrolyzes fatty acids from the sn-2 position of glycerophospholipids resulting in free fatty acids such as arachidonic acid and lysophospholipids (Diaz and Arm, 2003; Arni and Ward, 1996; Dennis, 1997). PLA_2 reaction is the rate-limiting step for the metabolism of arachidonic acid by one of several enzymatic pathways for the production of lipid mediators (eicosanoids) (Pruzanski et al., 1997). These lipid mediators are implicated in several physiological and pathological processes including inflammation, immune responses, asthma, sleep regulation, hemostasis, parturition, maintenance of renal function, pain and fever (Diaz and Arm, 2003; Schaloske and Dennis, 2006).

PLA_2 has attracted considerable interest as a pharmacological target. The control of the arachidonic acid production by inhibiting PLA_2 appears to be advantageous for the treatment of phatological conditions induced by phospholipase derived mediators, because the inhibition of selective pathways of eicosanoid production, e.g. cycloooxygenases or lipoxygenases, results in an upregulation of an alternative way (Yedgar et al., 2000). In contrast to that, the inhibition of PLA_2 results in the suppression of several important classes of pro-inflammatory lipids, e.g. prostaglandins and leukotrienes. Hence, the use of PLA_2 inhibitors has been considered to be an attractive therapeutic strategy in the treatment of inflammation-related diseases and tissue injuries (Balsinde et al., 1999).

PX-18 (2-*N*,*N*-Bis(oleoyloxyethyl)amino-1-ethanesulfonic acid) is a new highly potent PLA_2 inhibitor (Fig. 1) (Franson and Ottenbrite, 1997; Franson and Ottenbrite, 2002). Like approximately 40% of the drugs in the development pipelines and about 60% coming directly from the synthesis PX-18 is poorly soluble in aqueous media (Merisko, 2002). As proven by the market introduction of nine pharmaceutical products containing drug nanocrystals of poorly soluble drugs, i.e. Rapamune®, Emend®, TriCor®, Megace® ES, Triglide®, Avinza®, Facalin® XR, Ritalin® LA and Zanaflex Capsules™, formulating poorly soluble drugs as nanocrystals is a promising approach to overcome this problem. Drug nanocrystals are pure solid drug particles with a mean particle size below 1 μm. Dispersions of drug nanocrystals in liquid dispersion media stabilized with stabilizing agents, typically surfactants or polymeric stabilizers, are called nanosuspensions (Müller and Akkar, 2004). Nanosuspensions have several advantages, e.g. increased saturation solubility and consequently an increased dissolution velocity. Due to the nanosize, nanosuspensions show an increased adhesion to surfaces. This results in an enhanced bioavailability of active compounds. Due to these advantages nanosuspensions have been under investigation for various application routes including parenteral (Jacobs et al., 2000), peroral (Bushrab and Müller, 2003; Hecq et al., 2005; Müller and Jacobs, 2002), dermal (Müller et al., 1999), ocular (Müller et al., 1999) and pulmonary (Hernandez-Kirstein, 2006; Jacobs and Müller, 2002).

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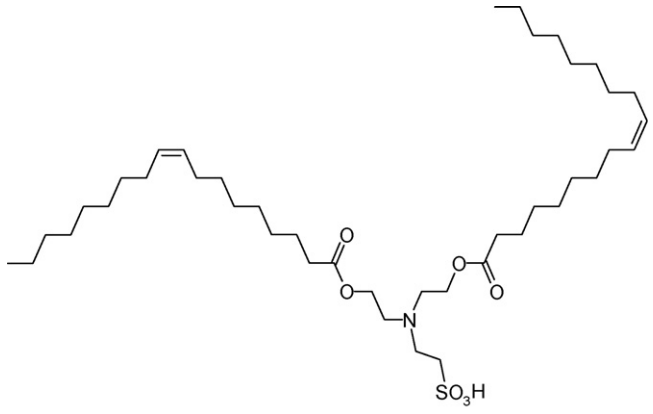

$C_{42}H_{78}O_7NS$

M = 741.56 g/mol

M.P.: 100-110°C

Fig. 1. Chemical structure, molecular formula, molecular weight (M) and melting point (M.P.) of PX-18.

This paper describes the development of PX-18 nanosuspensions applying high-pressure homogenization. Investigations on the reproducibility of this method and the physical and chemical stability of the nanosuspensions were performed. Furthermore, the saturation solubility of PX-18 bulk material and the obtained nanosuspensions were compared.

2. Materials and methods

2.1. Materials

PX-18

used in this work was synthesized by the Department of Inorganic and Organic Chemistry of the Charles University (Hradec Kralove, Czech Republic). The surfactants/stabilizers Brij 56 (ICI Surfactants, Cleveland, Great Britain), Inutec SP1 (Nordmann, Rassmann, Hamburg, Germany), L.A.S. (Gattefossé, Saint-Priest, France), Lipoid E80 (Lipoid, Ludwigshafen, Germany), Montanov 202 (Kreglinger Europe, Antwerp, Belgium), Phospholipon 80 (Nattermann, Köln, Germany), Pluronic F68 Prill (BASF, New Jersey, USA), Tagat S (Goldschmidt, Essen, Germany), Tegin M Pellets (Goldschmidt, Essen, Germany), Tween 80 (Sigma Aldrich, Deisenhofen, Germany) and Plantacare® 2000 (Cognis, Monheim, Germany) were under investigation as potential stabilizers for the nanosuspensions.

2.2. Contact angle measurement

Contact angles were determined performing goniometry on compressed discs of PX-18 bulk material using a Contact Angle Meter G1 (Krüss, Hamburg, Germany). 0.1% (w/v) solutions of the surfactants/stabilizers in purified water were prepared. 500 mg of PX-18 bulk material were compressed to a disc with a diameter of 1 cm using a single punch tablet machine (Emil Korsch Maschinenfabrik, Berlin, Germany).

2.3. Preparation of PX-18 nanosuspensions

A Micron LAB 40 (APV Deutschland GmbH, Unna, Germany) equipped with a water jacket for temperature control was used for the production of 1% (w/w), 5% (w/w) and 10% (w/w) PX-18 nanosuspensions. Each batch had a volume of 40 ml. All nanosuspensions were stabilized using 1% (w/w) Tween 80 as wetting agent. The according amount of PX-18 bulk material was dispersed in aqueous surfactant solution using a mortar and pestle. The dispersions were pre-homogenized running two cycles at 150 bar followed by two cycles at 500 bar and two cycles at 1000 bar. The pre-homogenization process was conducted at 5 °C. The obtained pre-dispersions were subjected to high-pressure homogenization applying 20 cycles at 1500 bar and 5 °C.

2.4. Laser diffractometry (LD)

The particle size of PX-18 bulk material and of the 5% (w/w) and 10% (w/w) PX-18 nanosuspensions was determined by laser diffractometry (LD) using a Coulter LS 230 (Beckman-Coulter, Krefeld, Germany). 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 PX-18 is 1.496. The imaginary RI of this compound is 0. The LD data were evaluated using the diameters 50% (LD 50), 90% (LD 90) and 95% (LD 95), which means that either 50, 90 or 95% (volume distribution) of the measured particles are below the given size. Each measurement was performed in triplicate. The volume distribution curves of three succeeded measurements were compared to obtain information if dissolution took place. No differences could be seen between the curves indicating no dissolution of the particles.

2.5. Photon correlation spectroscopy (PCS)

The particle size of all nanosuspensions was investigated by photon correlation spectroscopy (PCS) using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK) performing 30 runs per measurement. 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). 1% (w/w) PX-18 nanosuspensions were measured without further dilution to avoid dissolution of the particles. 5% (w/w) and 10% (w/w) PX-18 nanosuspensions were diluted with Milli-Q water succinct before the measurement. No decrease in particle size was observed during PCS measurements for the 5% (w/w) and 10% (w/w) nanosuspensions indicating that no dissolution took place.

2.6. Light microscopy

A light microscope (Leitz, Wetzlar, Germany) equipped with a CMEX-1 digital camera (Euromex microscopes, Arnheim, Netherlands) connected to Image Focus software version 1.3.1.4. (Euromex microscopes, Arnheim, Netherlands) was used.

2.7. Scanning electron microscopy (SEM)

SEM studies were performed at Zentraleinrichtung Elektronenmikroskopie (Technische Universität Berlin, Berlin, Germany) using a Hitachi S-4000 (Hitachi High-Technologies, Europe, Krefeld, Germany). PX-18 nanosuspensions were applied to carbon object holders and dried before analyses.

2.8. Zeta potential

The zeta potential was measured using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). PX-18 nanosuspensions were diluted with distilled water adjusted with 0.9% (w/v) sodium chloride solution to a conductivity of 50 $\mu S\,cm^{-1}$. The pH was in the range of 5.5–6.0. Furthermore, the zeta potential was measured in the original medium, which was obtained by filtration of the nanosuspensions through Centrisart filters with a molecular weight

cut-off of 300,000 (Sartorius, Göttingen, Germany). A conductivity of 58.2 μS cm^{-1} and a pH of 3.4 were measured in the original dispersion medium. The zeta potential was calculated using the Helmholtz–Smoluchowski equation.

2.9. Stability study

To investigate the physical and chemical long-term stability of the nanosuspensions, they were stored at 5 ± 3 °C and 25 ± 2 °C. The particle size of the formulations was measured over 180 days by PCS and LD. No LD measurements were performed for the 1% (w/w) PX-18 nanosuspension due to the limitation of this measurement technique at the lower size range. Due to the small particle size of the 1% (w/w) PX-18 nanosuspension and therefore the high transparency of the samples no sufficient obscuration could be obtained for LD measurements. Chemical long term stability was investigated of the physically most stable formulation at the day of production (day 0) and day 180 by HPLC measurement.

2.10. HPLC method

A reverse phase HPLC-UV method was used to quantify PX-18. To make PX-18 detectable by UV-light a chromatophoric system was attached by derivatisation reaction (Pardeike and Müller, 2008). As derivatisation agent p-nitrobenzyl-*N*,*N*′-diisopropylisourea (PNBDI) (Sigma–Aldrich, Deisenhofen, Germany) was chosen. 5 mM PNBDI were dissolved in acetonitrile (Mallinckrodt Baker, Deventer, Netherlands). PX-18 was dissolved in a mixture of acetonitrile and *N*,*N*-dimethylformamide (Merck, Darmstadt, Germany) (1:1, v/v) without exceeding 2.5 mM. PNBDI and PX-18 solution were mixed in a ratio 1:1. The derivatization reaction was carried out over 24 h at 75 °C. An autosampler model 560, a pump system model 525 and a diode array detector model 540 (Kontron Instruments, Groß-Zimmern, Germany) linked to a KromaSystem 2000 v. 1.70 data acquisition and process system were used. 20 μl were injected onto a Superspher 100 RP-18 (4 μm) endcapped 250 mm × 4 mm column with a matching pre-column (Knauer, Berlin, Germany). The mobile phase was run with a flow rate of 1 ml/min using a gradient of acetonitrile and distilled water. The UV-spectrum was recorder at 273 nm.

2.11. Saturation solubility

To determine the saturation solubility of PX-18 bulk material, the compound was suspended in 1% (w/w) Tween 80 to a final active concentration of 5% (w/w) using mortar and pestle. The saturation solubility of nanosuspensions with an active content of 1% (w/w), 5% (w/w) and 10% (w/w) was determined. 2.0 ml of each formulation were added to 20.0 ml of distilled water. The samples were stored at 25 °C shaking with 100 rpm in an Innova 4230 refrigerated incubator shaker (New Brunswick Scientific, New Jersey, USA). After 1 week the samples were filtered through 0.02 μm filters (Anotop 25 Plus, Whatman, Maidstine, Great Britain) to remove the excess of PX-18 or PX-18 nanosuspensions, respectively. 10.0 ml of the filtrate were collected and freeze dried. The amount PX-18 present was analyzed by HPLC.

3. Results and discussions

3.1. Contact angle measurement

A three-phase system composed of a gas phase, a liquid and a solid is creating a contact angle at the point where the three phases meet. The contact angle is a quantitative measurement of the wetting of a solid by a liquid. Table 1 provides an overview of the contact angles obtained with purified water and different surfactant/stabilizer solutions. Due to the fact that surfactants exhibit a lower surface tension compared to purified water, for all surfactant solutions smaller contact angles were measured on the compressed disc than for purified water. For PX-18 the best wetting was obtained with 0.1% (w/v) Tween 80 solution. Purified water showed a contact angle of 51.6° on the compressed PX-18 disc. With 0.1% (w/v) Tween 80 solution the contact angle was reduced to 23.2°. Therefore, Tween 80 was chosen as stabilising agent for PX-18 nanosuspensions.

Table 1
Contact angles obtained for purified water and 0.1% (w/v) surfactant/stabilizer solutions on compressed discs of PX-18 ($n = 3$, $\bar{x} \pm$ SD).

Liquid	Contact angle (°)
Purified water	51.6 ± 0.6
Brij 56	30.5 ± 1.3
Inutec SP1	32.8 ± 0.6
Lipoid E80	38 ± 0
L.A.S.	26 ± 1
Nontanov 202	35 ± 0.6
Phospholipon 80	37.8 ± 0.8
Plantacare® 2000	25.6 ± 0.6
Pluronic F68	28 ± 0
Tagat S	29 ± 0.6
TegoAcid S40P	42.3 ± 0.6
Tween 80	23.2 ± 0.3

3.2. Preparation and particle size of PX-18 nanosuspensions

Fig. 2 shows the particle size of the PX-18 bulk material suspended in 1% (w/w) Tween 80 solution investigated by LD measurement and light microscopy. The bulk material has a particle size in the micrometer range and an inhomogeneous particle size distribution.

The homogenization process reduces the mean particle size and simultaneously narrows the width of the size distribution (Müller and Peters, 1997). A decrease in particle size with an increasing number of applied homogenization cycles was observed for PX-18 nanosuspensions by PCS and LD measurements (Figs. 3 and 4). Moreover a continuous narrowing of the width of the particle size distribution could be observed by a decrease in PI (Fig. 3).

While the PCS mean diameter of the 5% (w/w) and 10% (w/w) PX-18 nanosuspensions changes only slightly with increasing cycle number above 5–10 cycles (Fig. 3 (middle and lower)), the reduction of the LD 90 and LD 95 values is still pronounced up to cycle 20 (Fig. 4). This is due to the fact that with an increasing cycle number a

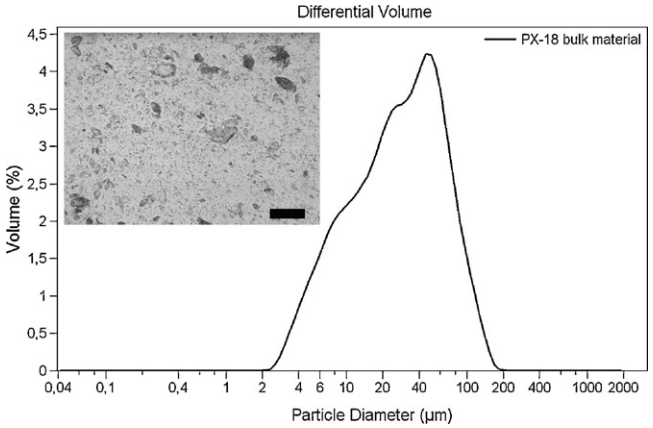

Fig. 2. Particle size of PX-18 bulk material investigated by LD and light microscopy (bar refers to 100 μm).

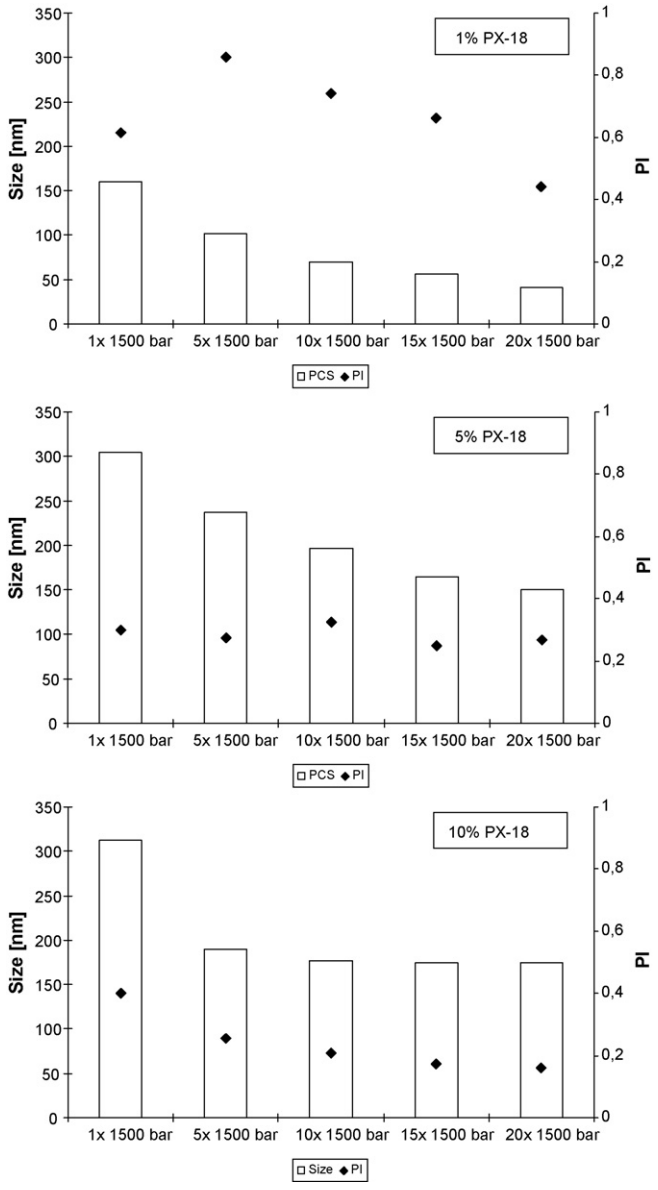

Fig. 3. Decrease in particle size and PI measured by PCS during the production of a 1% (w/w) (upper), 5% (w/w) (middle) and 10% (w/w) PX-18 nanosuspension (lower).

more uniform product is reached by the reduction of particles in the micrometer range. During the homogenization process, particles break at imperfections of their crystal structure. With a decreasing particle size the number of imperfections is reduced. Therefore, the force required to break the particles increases with a decreasing particle size. This can be observed in Figs. 3 and 4 by a rather exponential decrease in particle size than a linear decrease. If the forces in the homogenizer are equal to the interaction forces in the crystal, no further decrease in particle size will be observed, even when additional homogenization cycles are applied. Therefore, a decrease of the LD 90 and LD 95, being more sensitive to larger particles in the formulation, can be seen up to cycle number 20 while the average particle size measured by PCS stays nearly constant.

Fig. 5 shows an SEM picture of a 1% (w/w), 5% (w/w) and 10% (w/w) PX-18 nanosuspension. The picture of the 1% (w/w) PX-18 nanosuspension (Fig. 5 (left)) shows an agglomerate of the ultrafine (about 40 nm) PX-18 nanocrystals. Agglomeration of PX-18 nanocrystals might be caused by sample preparation for SEM. Investigating the size of the single particles of the agglomerate, particles smaller 50 nm can be seen. The picture therefore confirms the particle size of 41 nm measured by PCS (Fig. 3 (upper)). It was reported previously that nanocrystals with different shapes, e.g. cube-shape, rode-shape and needle-shape were obtained by high-pressure homogenization (Müller and Peters, 1997). PX-18 nanocrystals with a spherical shape were obtained at a concentration of 1% (w/w) whereas rod-shaped particles were obtained with this procedure at a concentration of 5% (w/w) and 10% (w/w). The SEM picture in Fig. 5 (middle) shows a 5% (w/w) PX-18 nanosuspension. The rod-shape nanocrystals were homogeneous in size. The particles had a length of approximately 140–180 nm and a width of approximately 60–100 nm. Therefore, the particle sizes of the 5% (w/w) PX-18 nanosuspensions detected by SEM is well in agreement with the findings of the particle size measurements based on light scattering. One single large rode-shaped PX-18 particle with a length of approximately 980 nm and a width of approximately 520 nm can be seen in the SEM picture of the 10% (w/w) nanosuspension (Fig. 5 (right)). In Fig. 6 a light microscope picture of the 10% (w/w) PX-18 nanosuspension taken with 1000× magnification can be seen. These pictures confirm the small particle size measured by LD, a homogeneous particle size distribution as well as the small number of larger particles detectable by LD in the 10% (w/w) PX-18 nanosuspension.

It could be shown, that 1% (w/w), 5% (w/w) and 10% (w/w) PX-18 nanosuspensions with a mean particles size well below 1 μm (PCS data) could be prepared applying 20 homogenization cycles at 1500 bar at 5 °C. In addition, the LD diameters 95% were also distinctly below 1 μm, i.e. 450–650 nm. It is well known, that the achievable particle size reduction depends on the hardness of the material subjected to the homogenization process (Keck and Müller, 2006; Müller and Akkar, 2004). In the literature average particles sizes measured by PCS of approximately 460 nm for a 1% (w/w) buparvaquone nanosuspension (Hernandez-Kirstein, 2006), 600 nm for a 1% (w/w) budesonide nanosuspension (Jacobs and Müller, 2002) and 920 nm for a 1% (w/w) omeprazol nanosuspension (Möschwitzer, 2005) were reported after applying 20 homogenization cycles at 1500 bar. For the 1% (w/w) PX-18

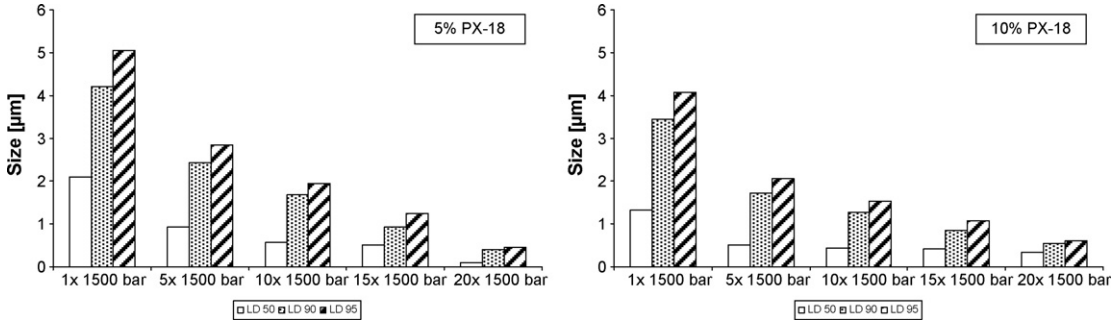

Fig. 4. Decrease in particle size measured by LD during the production of a 5% (w/w) (left) and 10% (w/w) nanosuspension (right).

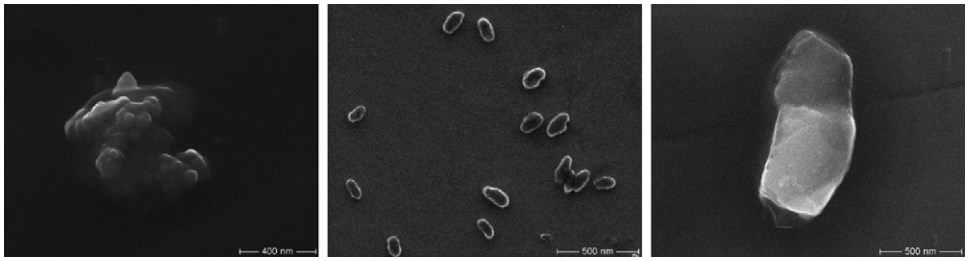

Fig. 5. SEM picture of 1% (w/w) (left), 5% (w/w) (middle) and 10% (w/w) PX-18 nanosuspension (right).

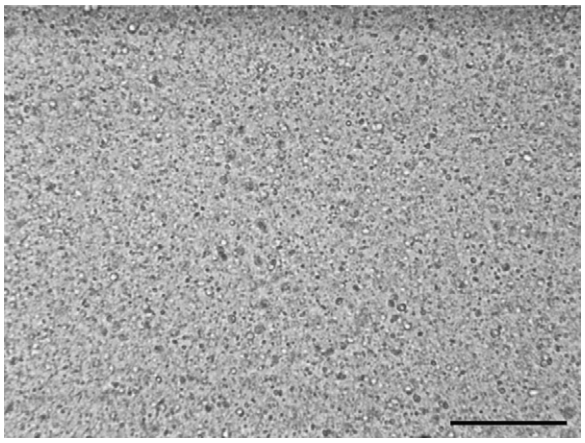

Fig. 6. Light microscope picture of 10% (w/w) PX-18 nanosuspension (bar refers to 25 μm).

nanosuspension an average particle size measured by PCS <50 nm was obtained using the same production conditions. Particle sizes as small as this have previously only been reported for nanosuspensions produced by a combination technology, where the material is lyophilized and afterwards subjected to high-pressure homogenization (H96 technology) (Möschwitzer and Lemke, 2005). Also at higher concentrations, i.e. 5% (w/w) and 10% (w/w) PX-18, relatively small particles were obtained under the applied conditions (approximately 150 nm), which provide evidence that PX-18 is a soft material. In the literature an average particle size measured by PCS of approximately 760 nm for a 5% (w/w) omeprazol nanosuspension (Möschwitzer, 2005), 940 nm for a 10% (w/w) omeprazol nanosuspension (Möschwitzer, 2005) and 435 nm for a 10% (w/w) budesonide nanosuspension (Jacobs and Müller, 2002) were reported. For some materials it was reported, that the particle size decreases with an increasing drug concentration (Jacobs and Müller, 2002). This was explained by the higher disintegration forces by increased collision of crystals in the homogenization gap, when higher amounts of drug are present. Increasing the amount of PX-18 from 5% (w/w) to 10% (w/w) a smaller particle size was measured by LD after the first homogenization cycle at 1500 bar for the 10% (w/w) PX-18 nanosuspension. With increasing cycle number the particle size of the 5% (w/w) PX-18 nanosuspension decreased more than the particle size of the 10% (w/w) PX-18 nanosuspension. This is in contrast to the above cited reports. However, it should be kept in mind that these reports deal with hard crystalline materials. In case of PX-18 the material is soft, relatively easy to disperse. This material is therefore rather to compare with easily to disperse oil-in-water nanoemulsions, e.g. lipofundin and intralipid. For these emulsions it is well documented in the literature, that 20% emulsions are larger in PCS mean diameter droplet size than 10% emulsions, e.g. 250–300 nm versus 200–250 nm (Müller and Heinemann, 1992; Müller and Heinemann, 1993). The increased diameters with higher concentration of the homogenized material can be explained via the power density (Krause and Müller, 2001). The production conditions were kept constant for the formulations with different concentrations, which means the energy to disintegrate the particles was identical in all three productions despite the fact that the amount of material to be disintegrated was increased. However, it could be shown that high-pressure homogenization applying 20 cycles at 1500 bar at 5 °C can be used to formulate PX-18 suspensions with particles in the nanometer range and a narrow particle size distribution.

Table 3
Results of the reproducibility study obtained by LD measurements.

Formulation	Value	Average particle size (μm)				
		Batch 1	Batch 2	Batch 3	Mean	RSD (%)
5% PX-18	LD 50	0.095	0.093	0.110	0.099	9.7
	LD 90	0.400	0.364	0.418	0.388	9.7
	LD 95	0.450	0.404	0.487	0.447	9.3
10% PX-18	LD 50	0.347	0.353	0.375	0.358	4.1
	LD 90	0.541	0.570	0.649	0.587	9.5
	LD 95	0.604	0.643	0.704	0.650	7.8

3.3. Reproducibility of the particle size

The reproducibility of the particle size of PX-18 nanosuspensions was evaluated on three different batches of each concentration. The particle size was evaluated by PCS and LD. For the evaluation of the reproducibility the relative standard deviation was used. The results of this study are shown in Tables 2 and 3. A good reproducibility of the mean particle size in between the batches was obtained for all PX-18 nanosuspensions. The relative standard deviation was ≤10% at all measurement points. This is well in agreement with earlier findings, where a good reproducibility of the particles size was reported for nanosuspensions produced by high-pressure homogenization (Grau et al., 2000; Peters, 1999).

Table 2
Results of the reproducibility study obtained by PCS measurements.

Formulation	Average particle size (nm)					PI				
	Batch 1	Batch 2	Batch 3	Mean	RSD (%)	Batch 1	Batch 2	Batch 3	Mean	RSD (%)
1% PX-18	41	48	44	44.3	7.9	0.443	0.424	0.441	0.436	2.4
5% PX-18	146	151	134	143.7	6.1	0.274	0.268	0.253	0.265	4.1
10% PX-18	175	202	185	187.3	7.3	0.169	0.185	0.200	0.185	8.4

Table 4
Zeta potential values of the PX-18 nanosuspensions ($n = 30$, mean ± SD).

Formulation	Distilled water adjusted to 50 μS cm^{-1} (mV)	Original medium (mV)
1% PX-18	−50.6 ± 2.5	−19.8 ± 6.1
5% PX-18	−50.8 ± 5.7	−19.3 ± 4.9
10% PX-18	−56.2 ± 6.8	−19.0 ± 5.1

3.4. Zeta potential

To investigate the surface properties of the nanosuspensions the zeta potential was measured in distilled water adjusted to a conductivity of 50 μS cm^{-1}. Furthermore, the zeta potential was measured in the original dispersion medium. The results are shown in Table 4. The PX-18 nanosuspensions had a zeta potential higher than |−50 mV| measured in distilled water adjusted to a conductivity of 50 μS cm^{-1}, whereas the zeta potential measured in the original medium was below |−20 mV|.

The zeta potential allows only predicting the electrostatic stabilization. Further stabilizing factors or destabilizing factors cannot be predicted with the zeta potential. Tween 80 performs an additional sterical stabilization of the nanocrystals. Uncharged steric stabilizers can adsorb to the surface of the nanocrystals. Due to the adsorption of the steric stabilizer the diffuse layer is protected against removal during the movement in the electrical field during zeta potential measurements. According to the dimensions of the steric stabilizer the shear plane is shifted further away from the particle surface (Fig. 7). This results in zeta potential measurements in a bigger distance from the Stern layer. Due to the exponential decay of the potential in the defuse layer, the measured zeta potential is smaller than in dispersions without steric stabilizers (Müller, 1996). It has been reported, that in a combined electrostatic and sterical stabilization zeta potential values of about |−20 mV| can be sufficient for physical stability (Jacobs and Müller, 2002). The high zeta potential values obtained in distilled water adjusted to a conductivity of 50 μS cm^{-1} indicate a high surface potential of the nanocrystals. Due to the dilution of the original medium with distilled water the concentration of Tween 80 at the particle surface might be lowered. As a result, the exposure of negatively charged particle surface increased. However, the zeta potential values obtained indicate a good physical stability of the PX-18 nanosuspensions.

3.5. Physical stability

The particle size of the PX-18 nanosuspensions stored at 5 ± 3 °C and 25 ± 2 °C was observed over a period of 180 days. The results of this study are shown in Figs. 8 and 9.

The nanosuspensions were physical stable when stored at 5 ± 3 °C. Under this condition no changes in the particle size distribution occurred. Physical long-term stability of nanosuspensions stored refrigerated for up to 3 years has previously been reported (Peters, 1999). Increasing the storage temperature leads to physical instability of the PX-18 nanosuspensions after 1 month of storage. The electrostatic repulsion between the nanocrystals in combination with steric stabilization was therefore not sufficient to prevent particle aggregation at higher storage temperatures. Increasing the temperature leads to a decrease of the dynamic viscosity. According

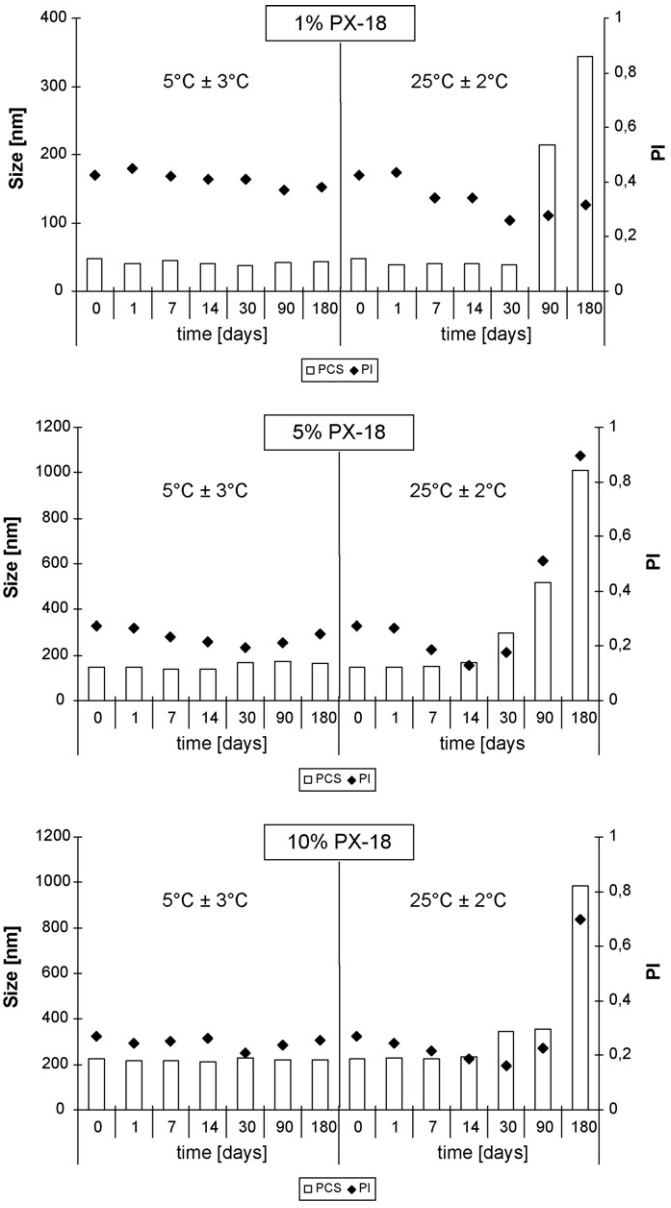

Fig. 8. Particle size and PI of 1% (w/w) (upper), 5% (w/w) (middle) and 10% (w/w) PX-18 nanosuspension (lower) measured by PCS over a period of 180 days.

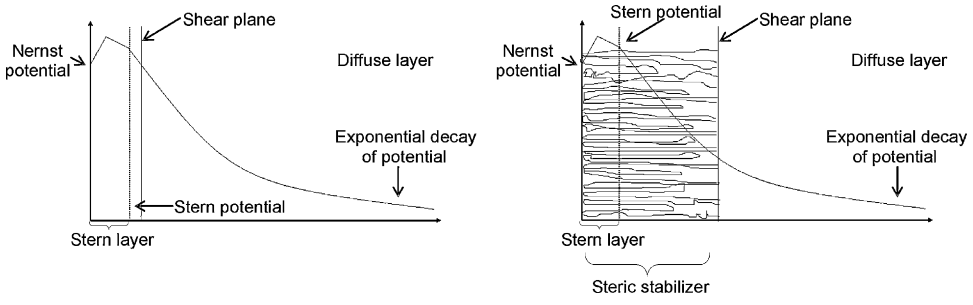

Fig. 7. Schematic overview of the location of shear plane in an electrostatic stabilized system (left) and in a sterical stabilized system (right).

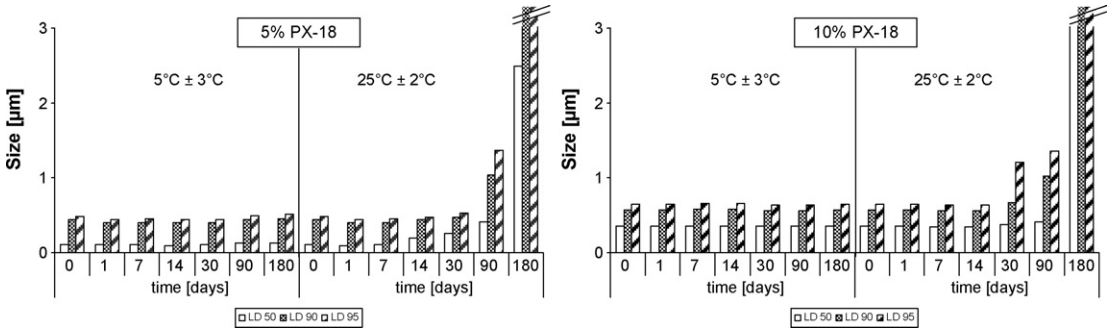

Fig. 9. LD values of a 5% (w/w) (left) and 10% (w/w) PX-18 nanosuspension (right) measured over a period of 180 days.

to the Stokes–Einstein equation an increase in temperature and a decrease in dynamic viscosity results in an increase of the diffusion constant. A higher diffusion constant leads to a faster diffusion of the particles. Having a higher kinetic energy, the repulsion between the particles can be overcome more easily, which results in particle aggregation.

In order to detect a possible chemical instability of PX-18 in the nanosuspensions, it was quantified by HPLC. Table 5 provides an overview of the results obtained in this study. All formulations with a very good physical stability showed also an excellent chemical stability. The drug content of all formulations was equal at the day of production and after 180 days of storage.

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 solvent at a specified temperature (Latscha and Klein, 1995). However, it should be taken into account that the saturation solubility also depends on the modification of polymorphic compounds (Beckman and Ames, 1998; Giron, 1995; Grunenberg, 1997) and the particle size in case particles approximately <2 μm are present. If the particle size is reduced below about 2 μm, the ratio between the surface area and the volume is increased that much, that the saturation solubility increases by an increased interaction surface between solute and solvent (Nyström and Bisrat, 1988; Torrado et al., 1998; Beckman and Ames, 1998). The principle of the increase in saturation solubility, if the particle size is reduced below about 2 μm, is described in the Kelvin equation and the Ostwald–Freundlich equation. The dissolution pressure increases with increasing curvature of the particles (Müller and Akkar, 2004; Keck and Müller, 2006). This leads to a higher solubility, which is actually a "kinetic" solubility. This kinetic solubility is higher than the "thermodynamic" equilibrium solubility of "normally" sized particles (»2 μm) of daily life.

The saturation solubility of PX-18 bulk material and the kinetic solubility of the developed nanosuspensions as well as their particle size measured by PCS are shown in Fig. 10. To compensate the influence of the surfactant used as stabilizer in the nanosuspensions, e.g. increased wetting of the material and solubilization, PX-18 bulk material was also suspended in 1% (w/w) Tween 80 before testing. According to the European Pharmacopoeia the PX-18 bulk material

Table 5
Drug content of PX-18 nanosuspensions at the day of production and after 180 days of storage at 5 ± 3 °C (n = 3, mean ± SD).

Formulation	Day 0 Concentration (%)	Day 180 Concentration (%)
1% PX-18	1.05 ± 0.08	1.14 ± 0.13
5% PX-18	5.33 ± 0.56	5.07 ± 0.09
10% PX-18	10.23 ± 0.67	10.68 ± 0.03

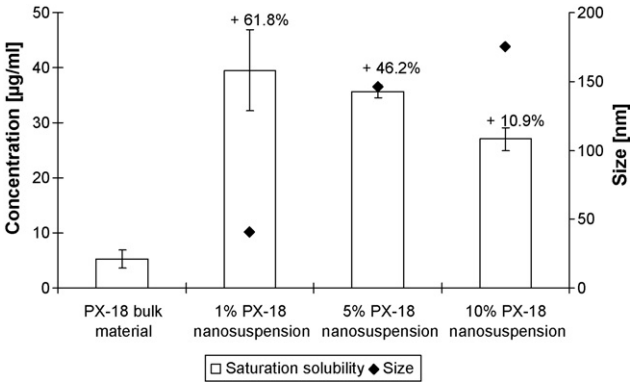

Fig. 10. The saturation solubility of PX-18 bulk material can be increased decreasing the particle size of PX-18 nanosuspensions.

with a saturation solubility of 24.4 μg/ml can be classified as practically insoluble. Producing nanosuspensions out of the bulk material leads to an increase in saturation solubility with decreasing particle size.

With the Kelvin equation and the Ostwald–Freundlich equation it was shown theoretically how the saturation solubility increases when decreasing the particle size from the micrometer range to the nanometer range. 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 (Peters, 1999). The results obtained with PX-18 are well in agreement with the theory and previously reported findings. The increase in kinetic solubility is increasing with decreasing particle size. The obtained results are in agreement with this because highest kinetic solubility was found for the 40 nm 1% PX-18 (w/w) nanocrystal suspension.

4. Conclusions

Producing nanosuspensions of the new poorly water-soluble PLA_2 inhibitor PX-18 by high-pressure homogenization makes the drug available for various administration routs. Physical and chemical stable nanosuspensions with a good reproducibility could be formulated with a drug contend of 1% (w/w), 5% (w/w) and 10% (w/w). Due to the small particle size (<1 μm) PX-18 nanosuspensions can even be injected intravenously. A further advantage of formulating PX-18 as nanosuspensions is the increase in saturation solubility, which is expected to contribute to an increased bioavailability of the drug.

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