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Production and characterization of Hesperetin nanosuspensions for dermal delivery

Prabhat R. Mishra[a,b], Loaye Al Shaal[b], Rainer H. Müller[b], Cornelia M. Keck[b,*]

[a] Pharmaceutics Division, Central Drug Research Institute, Lucknow 226 001, India
[b] Department of Pharmaceutics, Biopharmaceutics & NutriCosmetics, Free University of Berlin, Kelchstr. 31, 12169 Berlin, Germany

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ABSTRACT

Nanosuspensions of Hesperetin were produced using four different stabilizers, Poloxamer 188, Inutec SP1, Tween 80 and Plantacare 2000, possessing different mechanisms of stabilisation. The nanosuspensions were characterized with regard to size (photon correlation spectroscopy (PCS), laser diffractometry (LD)) and charge (zeta potential measurements). A nanocrystal PCS size of about 300 nm was obtained after 30 homogenization cycles at 1500 bar with the stabilizers Poloxamer, Inutec and Plantacare. Tween was slightly less efficient to preserve the nanocrystal size directly after production (347 nm). The short-term stability was assessed by storage of the nanosuspensions at 4 °C, room temperature and 40 °C. As predicted from the zeta potential measurements, Inutec and Plantacare stabilized nanosuspensions were stable with no change in PCS diameter and LD diameter 99%. Slight increases in size were found for the Poloxamer and the Tween stabilized nanosuspensions, which is not considered to impair their use in dermal formulations.

1. Introduction

In many cases poorly soluble actives, pharmaceuticals and cosmetics, have bioavailability problems at their desired site of action, especially when the actives are simultaneously poorly soluble in aqueous and non-aqueous (organic) media (Müller et al., 1999b; Rabinow, 2004; Stegemann et al., 2007). In most cases, the poorly soluble actives are lipophilic; hence, in principle the molecules show a good permeability through lipophilic membranes. However, poor solubility is generally related to a slow dissolution velocity. Thus, when the few dissolved molecules have permeated a membrane in the body, dissolution from the crystals of the active is not fast enough to replace the permeated molecules (Fig. 1, left). The rate-limiting step for absorption of such drugs is the dissolution velocity. These drugs are the so-called class II drugs of the biopharmaceutical classification system (BCS) (Desai et al., 1996; Kasim et al., 2004; Martinez and Amidon, 2002). One approach to overcome this problem is the production of nanocrystals. Nanocrystals possess an increased dissolution velocity due to their large surface area A, but also because in addition they exhibit an increased saturation solubility c_s (Buckton and Beezer, 1992; Hecq et al., 2005; Jinno et al., 2006; Keck and Müller, 2008, in press; Müller et al., 2000; Müller et al., 1999a) (Fig. 1, right). Apart from fast dissolution, the increased saturation solubility c_s leads to an increased concentration gradient at membranes further promoting permeation (Fig. 1, right).

In pharmacy most of the attention is focussed on improved oral delivery of poorly soluble drugs because oral formulations are the largest market. Second area of interest for drug nanocrystals is their intravenous injection to reduce side effects (e.g. itraconazole (Rabinow et al., 2007) or for targeting to specific sites in the body (e.g. the brain (Kayser and Kiderlen, 2003; Keck, 2008; Schöler et al., 2001)). However, completely neglected was the dermal delivery of nanocrystals to enhance penetration and efficiency of actives, as well in the pharmaceutical but also the cosmetic field (Keck and Müller, 2008, in press; Rabinow, 2004). In principle, there is little difference between the penetration problems of dermal drugs and dermal cosmetic actives. The requirements regarding formulation characteristics (e.g. nanocrystal size), physical stability, tolerability/toxicity and desired controlled increased penetration are identical or at least similar (of course still having in mind the less strict regulatory hurdles for cosmetics).

To elucidate the principle production and formulation parameters affecting the size characteristics and physical stability of dermal nanocrystals, the active Hesperetin was used to produce nanocrystals. Hesperetin is the aglycone of hesperidin and its IUPAC name is ((S)-2,3-dihydro-5,7-dihydroxy-2-(3-hydroxy-4 methoxy-phenyl)-4H-1-benzopyran-4-one), see Fig. 2. It is a flavanone found in citrus fruits.

* Corresponding author. Tel.: +49 30 83850 631; fax: +49 30 83850 616.
E-mail address: ck@ckc-berlin.de (C.M. Keck).

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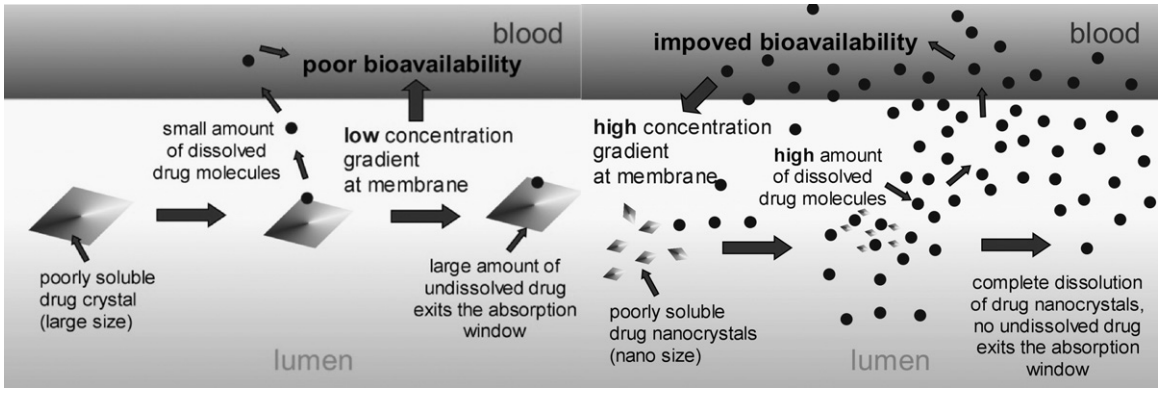

Fig. 1. Left: poor solubility of BCS class II drug due to low-dissolution velocity and low-concentration gradient at membrane; right: improved bioavailability by production of nanocrystals, processing an increased saturation solubility and a high dissolution velocity which leads to a high-concentration gradient at the membranes.

Hesperetin has antioxidative (Choi and Kim, 2008; Jeong et al., 2005; Vauzour et al., 2007) and also antiallergic properties (Lee et al., 2004). It is common knowledge, that oxidative stress, but also inflammation play a key role in the aging process (de Grey, 1999), thus Hesperetin, capable to prevent both, is thought to be a very effective anti-aging compound. However, oxidative stress and inflammation are also caused by sun exposure, leading to accelerated skin aging (Fischer, 2005). Thus, Hesperetin has not only a potential as oral, but also as dermal anti-aging compound, thought to be even more effective than its already very effective glycoside Hesperidin, which has strong antioxidative but no anti-inflammatory properties (Lee et al., 2004). In the study with hesperidin the oxidative stress to the skin was created by UV irradiation and the biological effect quantified in terms of the increase in the sun protection factor (SPF) (Müller et al., 2007; Petersen, 2006). A part of this effect is due to the good penetration characteristics of the lipophilic Hesperidin, compared to watersoluble derivatives such as Rutin-glucoside (Petersen, 2006). Hesperetin has an even lower solubility than Hesperidin (1.4 μg/ml versus 20 μg/ml of Hesperidin), the molecule has more lipophilic character, and should therefore penetrate even better when the solubility problem and the low-dissolution velocity can be overcome by nanocrystal production (Mitragotri, 2002; Scheuplein, 1968). Basically there is no difference in the procedure for producing nanocrystals for oral, intravenous or dermal administration/application. The only difference in the formulations is the choice of the stabilizers. For example sodium lauryl sulphate (SDS) is only suitable for oral but not for intravenous or dermal formulations. Therefore, in this study, nanosuspensions of Hesperetin were produced by high-pressure homogenization employing four different stabilizers suitable for dermal use. The stabilizers were of different class, i.e. low-molecular weight stabilizers (i.e. surfactants) and high-molecular weight polymers (i.e. steric stabilizers). The obtained nanocrystals were characterized in terms of size (laser diffractometry (LD), photon correlation spectroscopy (PCS), light microscopy) and particle charge (zeta potential). To assess the physical stability a short-term study was performed storing the differently stabilized nanosuspensions at three different temperatures, to elucidate which type of stabilization proved to be most effective.

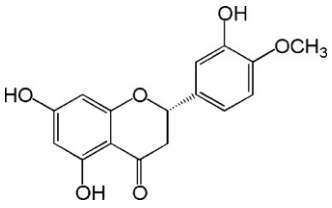

Fig. 2. Chemical structure of Hesperetin.

2. Materials and methods

2.1. Materials

Hesperetin was purchased from Exquim, S.A. (Spain). The stabilizers Tween 80 (polysorbate 80, Uniqema, Belgium); Lutrol F68 (poloxamer 188, BASF, Germany); Plantacare 2000 (alkyl polyglycoside, Cognis, Germany) and Inutec SP1 (inulin lauryl carbamate, Orafti Bio Based Chemicals, Belgium) were used for this study. As dispersion medium, freshly prepared double distilled and ultra purified water (milli-Q, Millipore GmbH, Germany) was used. 0.9% sodium chloride solution was purchased from B. Braun Melsungen AG (Germany).

2.2. Methods

2.2.1. Preparation of nanosuspensions

All nanosuspensions were produced by high-pressure homogenization (HPH) in pure water after (Müller et al., 1999a) using an LAB 40 (APV Deutschland GmbH, Germany) with a batch size of 40 ml. The formulations produced contained 5.0% (w/w) Hesperetin and 1.0% (w/w) of the respective stabilizer. Briefly, a suspension was obtained by adding 38.0 g of the freshly prepared surfactant solution (1.53% (w/w)) to 2.0 g of the coarse Hesperetin powder. Pre-milling was performed with an ultra turrax T25 (Janke and Kunkel GmbH, Germany) for 1 min at 10,000 rpm, followed by five pre-milling homogenization cycles at low pressures. High-pressure homogenization at 1500 bar was applied for 30 homogenization cycles to obtain the final products. Samples for characterization were collected after pre-milling, and after 1, 5, 10, 15, 20 and 30 homogenisation cycles at 1500 bar.

2.2.2. Physical stability

To investigate the physical stability of the different nanosuspensions and to identify the most suitable stabilizer, after the production each nanosuspension was divided into three vials and stored at three different temperatures (4 °C, 25 °C, and 40 °C) for 30 days. Characterization was carried out on day 0, day 3, day 7, day 14 and day 30. Day 0 is the day of production.

2.2.3. Characterization

Characterization of the samples was performed using dynamic light scattering, static light scattering techniques and light microscopy.

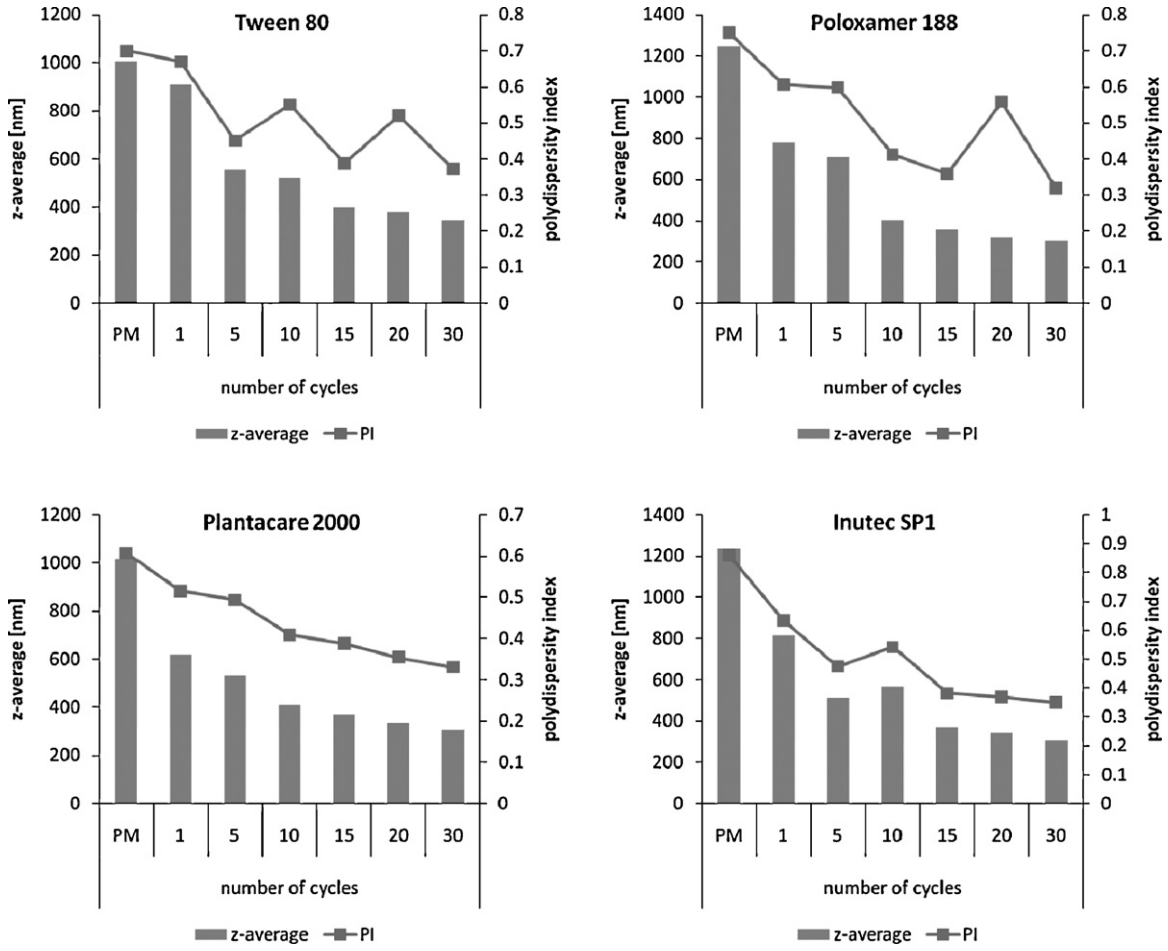

Fig. 3. Decrease in particle size (PCS data presented as *z*-average) and width of the size distribution (presented as polydispersity index (PI)) as a function of homogenization cycles for the four differently stabilized Hesperetin nanosuspensions (upper, left: Tween 80; upper, right: Poloxamer 188; lower, left: Plantacare 2000; lower, right: Inutec 2000, PM = values after pre-milling).

2.2.3.1. Dynamic light scattering. Dynamic light scattering, also known as PCS was performed using the zetasizer Nano ZS (Malvern Instruments, UK). The analysis yields the *z*-average of the sample, which is an intensity weighted mean diameter of the bulk population and the polydispersity index, which is a measure for the width of the size distribution. The measuring range of the zetasizer is from approximately 0.6 nm to 6 μm. Thus, to observe larger particles, static light scattering, also known as laser diffraction (LD), with a measuring range to up to 2000 μm was applied as additional characterization method.

2.2.3.2. Laser diffraction. Laser diffraction was performed using the Mastersizer 2000 (Malvern Instruments, UK). The instrument was operated with the Hydro S sample dispersion unit. Sonification prior and during the measurement was not performed to avoid the destruction of possible aggregates within the sample, being a sensitive marker for insufficient stabilization. LD yields volume-weighted diameters. The d(v)50%, d(v)90%, d(v)95% and d(v)99% were used as characterisation parameters. The d(v)50% represents the size where 50% of the particles are below the given size. D(v)95% and d(v)99% are sensitive parameters to quantify potential larger sized particles present, e.g. larger crystals that may remain in the suspension during homogenisation or aggregates formed due to insufficient stabilization. Thus, especially the d(v)99% is an important characterization parameter. All parameters have been analyzed using the Mie characterisation mode with the optical parameters 1.59 for the real refractive index and 0.01 for the imaginary refractive index.

2.2.3.3. Light microscopy. Light microscopy (Ortophlan, Leitz, Germany) was performed to analyse the morphology of the particles. Polarized light was used to observe the presence or absence of larger crystals or aggregates using the magnifications 160-fold and 1000-fold with and without polarized light.

2.2.4. Zeta potential measurements

The surface charge of the particles was assessed by zeta potential measurements using the Malvern zetasizer Nano ZS (Malvern Instruments, UK) and by applying a field strength of 20 V/cm. The zetasizer Nano measures the electrophoretic mobility of the particles, which was converted into the zeta potential using the Helmholtz–Smoluchowski equation built into the Malvern zetasizer software. The zeta potential of particles depends on the dispersion medium; therefore, the surface charge has been measured in double distilled water adjusted to 50 μS/cm using 0.9% NaCl solution and in the original dispersion medium.

3. Results and discussion

3.1. Size and size distribution as function of 20 homogenization cycles

Typical production parameters in nanocrystal production by high-pressure homogenization (HPH) are pre-milling of the prepared macrosuspension at increasing pressures followed by up to 20 cycles at 1500 bar. Premilling is a procedure used to diminute very large crystals of the coarse suspension prior to the high-

Table 1
PCS and LD particle sizes as a function of homogenization cycles for the four different Hesperetin nanosuspensions (PI = polydispersity index).

Stabilizer	Number of homogenization cycles	PCS		LD particle size (μm)			
		Size (nm)	PI	d(v) 50%	d(v) 90%	d(v) 95%	d(v) 99%
Tween 80	20	377	0.52	0.316	1.378	1.804	2.601
	30	346	0.371	0.295	1.093	1.467	2.358
Poloxamer 188	20	318	0.358	0.295	1.256	1.665	2.428
	30	301	0.318	0.277	0.96	1.257	1.838
Plantacare 2000	20	331	0.354	0.321	1.194	1.194	2.188
	30	304	0.330	0.303	0.961	0.961	1.786
Inutec SP1	20	342	0.368	0.297	1.252	1.649	2.380
	30	304	0.348	0.283	1.003	1.311	1.995

pressure homogenization. Premilling starts with the use of an Ultra turrax, followed by subsequent homogenization cycles at pressures below 1500 bar (e.g. 2 cycles at 200, 500 and 1 cycle at 1000 bar). The pre-milling avoids blocking of the homogenization gap of the piston gap homogenizer by large crystals present in the prepared macrosuspension. The width of the gap is a function of the applied pressure, being about 5 μm at 500 bar, but just 3 μm at 1500 bar (Müller et al., 2006). The large crystals are easy to break and thus they can be diminuted even at these relatively low pressures. In studies it could be shown, that increasing the pressure further to 2000 or even 4000 bar has little effect on reducing the particle size further (Fichera et al., 2004a,b). However, the wearing of the machine at such high pressures is very high, not considered as being acceptable in large-scale pharmaceutical production. Based on these experiences the production parameters (1500 bar) were chosen in this study, to avoid re-inventing the wheel. However, 30 cycles instead of 20 were employed to see if some unexpected effects would occur.

Fig. 3 shows the decrease in PCS diameter and polydispersity index (PI) as a function of homogenization cycles. Independent on the type of stabilizer, the most pronounced decrease in PCS diameter took place until cycle 15. Very little changes are observed within cycle 20 to cycle 30. The further decrease in the PCS diameter after was in the range of about 20–40 nm (Table 1), which does not justify 10 additional production cycles.

The profile of size reduction was similar for all four formulations. In parallel, the polydispersity index (PI) decreased with increasing cycle numbers. The decrease was most steadily for the Plantacare 2000 and Inutec stabilized nanosuspensions (Fig. 3, lower left and right). It was least for the Tween 80 stabilized systems (Fig. 3, upper left), the fluctuations in the PI indicating slight reversible aggregate formation during the homogenization, increasing the PI, which were de-aggregated in the next cycles. Input of energy in the homogenization process can also lead to aggregation in case of suspensions or coalescence in case of emulsions (Jahnke, 2001). The steady decrease in PI confirms that at higher cycles mainly remaining larger particles are removed, and only limited further size reduction of the bulk population takes place.

The laser diffractometry data (Fig. 4) are in line with the PCS results. There is little change in the diameter d(v)50% characterizing the bulk population when moving from cycle 15 to 30. However, there is still a further decrease in the diameter d(v)99%, being a sensitive measure of the presence of even a few larger particles, because LD yields a volume distribution, weighting a few large particles much more than the *z*-average from PCS.

However, for a more detailed interpretation of size effects by the different stabilizers, Figs. 3 and 4 are not sufficient. It is necessary to review the exact measured size data (Table 1). According to the theory, the resulting final nanocrystal size depends – apart from the hardness of the drug crystals – only on pressure and cycle number, not on the type of stabilizer (Müller et al., 1995; Peters, 1999). However, the stabilizers differ in their ability to preserve the generated small crystal size and to avoid aggregation of the generated fine crystals, i.e. immediately after the homogenization process and long term. In fact, if two stabilizers are similar efficient in stabilization, the obtained nanocrystal sizes of the suspensions will be identical after production.

After 20 cycles the PCS diameters are 377 nm (Tween 80), 318 nm (Poloxamer), 331 nm (Plantacare 2000) and 342 nm (Inutec) (Table 1). Assuming validity of the theory, it can be concluded that:

- Poloxamer was most efficient in stabilizing the generated nanocrystals.
- Plantacare 2000 and Inutec are slightly less sufficient in comparison to Poloxamer 188, but are similar efficient to stabilize.
- Tween 80 was least efficient.

The latter is in accordance with the observed fluctuation of the PI with increasing cycle number (Fig. 3, upper-left), indicating occurrence of aggregation during the homogenisation process of the Tween 80 stabilized suspension.

3.2. Size and size distribution as function of 30 homogenization cycles

Again, according to homogenization theory, dispersed particles can aggregate to a certain extent after leaving the homogenisation gap in case the stabilizer cannot sufficiently fast cover the newly generated surfaces. The ability to cover (diffusion velocity to the crystal surface) depends on the molecular weight (high-molecular weight surfactants diffuse more slowly) as well as on the de-aggregation velocity of the micelles providing monomeric surfactant for diffusion onto the generated new interfaces. Re-aggregation after leaving the homogenization gap is also more pronounced in case the stabilizing capacity of the stabilizer is not good. However, for such stabilizers, the formed aggregates can be removed by running additional homogenisation cycles. This finally should lead to full coverage of the surfaces and completely identical sizes (assumed the stabilizer has no lack in stabilizing capacity). After 30 homogenization cycles the PCS diameters are 301 nm, 304 nm and 304 nm for the nanosuspensions stabilized with Poloxamer, Plantacare and Inutec, being well in accordance with the theory for similarly effective stabilizing stabilizers. The Tween 80 stabilized nanosuspension exhibited a diameter of 346 nm (377 nm at 20 cycles). This indicates that in this nanosuspension a slight aggregation might have occurred. The aggregation can be considered as not being relevant for the use in a dermal products, because from the microscopic pictures, no differences were found between the different nanosuspensions (Fig. 5). The reason is that the aggregation is only very slight and therefore not accessible be light microscopy, but only by the sensitive PCS.

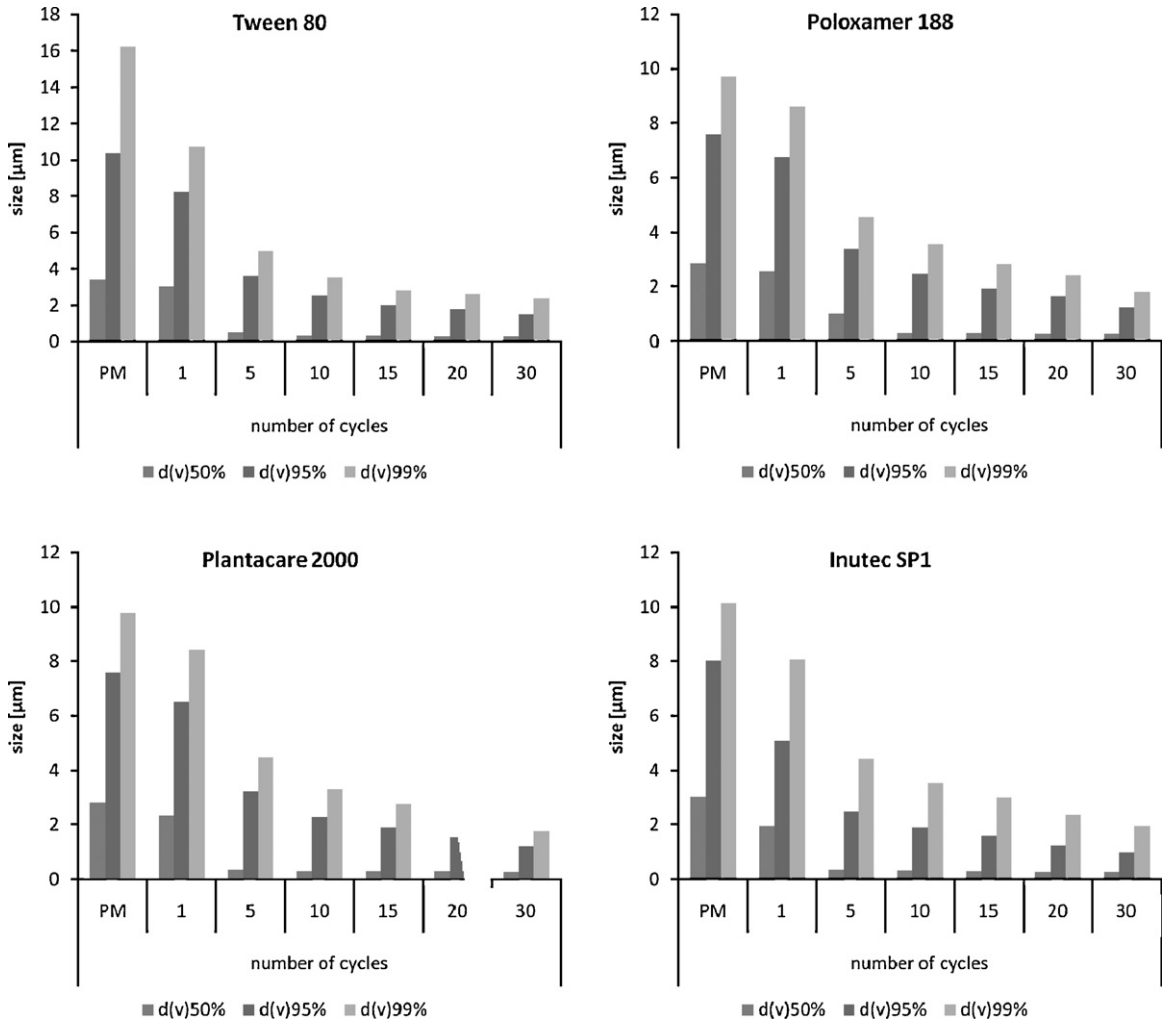

Fig. 4. Decrease in particle size (laser diffraction data) as a function of homogenization cycles for the four differently stabilized Hesperetin nanosuspensions (upper, left: Tween 80; upper, right: Poloxamer 188; lower, left: Plantacare 2000; lower, right: Inutec 2000, PM = values after pre-milling).

Such identical or similar final nanocrystal sizes are not obtained for each active. In case of resveratrol more pronounced differences were found (Kobierski et al., in press). The stabilising effect also depends on the affinity of the stabilizer to the surface, and the resulting packaging density and thickness of the stabilizer layer. For example, the thickness of the adsorption layer of Poloxamer polymers on polystyrene particles decreases with decreasing hydrophobicity of the particle surface (Müller, 1991). From this part of the study it can be concluded that in principle all stabilizers are suitable for the production of Hesperetin nanosuspensions. However, the question is, will they also to be able to stabilize the nanosuspensions during storage, especially under stress conditions?

3.3. Zeta potential (ZP) measurements

To fully characterize the charge conditions of particles, zeta potential measurements should be performed in distilled water and in the original dispersion medium of the suspension. Very often ZP measurements are performed in buffers of varying molarity, physiological salt solution (especially pharmacists think being physiological is a priori good) or other media. These measurements are rather meaningless to judge the surface potential (Nernst potential) or the physical long-term stability. Also one needs to be careful with the rule of thumb that a ZP of at least of an absolute value of 30 mV is required for a stable suspensions (Müller, 1996; Riddick, 1968).

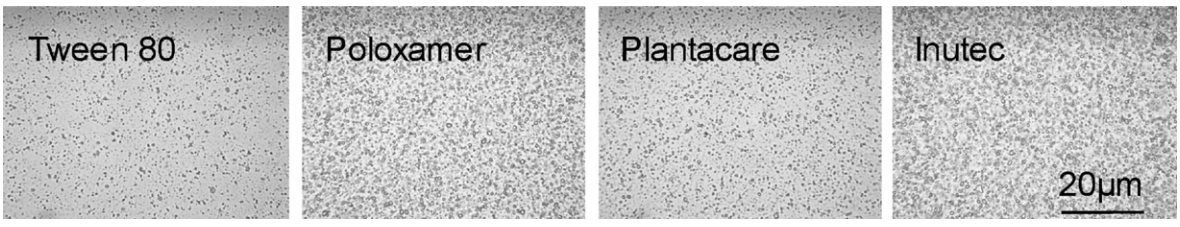

Fig. 5. Micrographs (magnification 1000×) of the nanosuspensions after production (30 cycles), no significant differences between the four suspensions are visible.

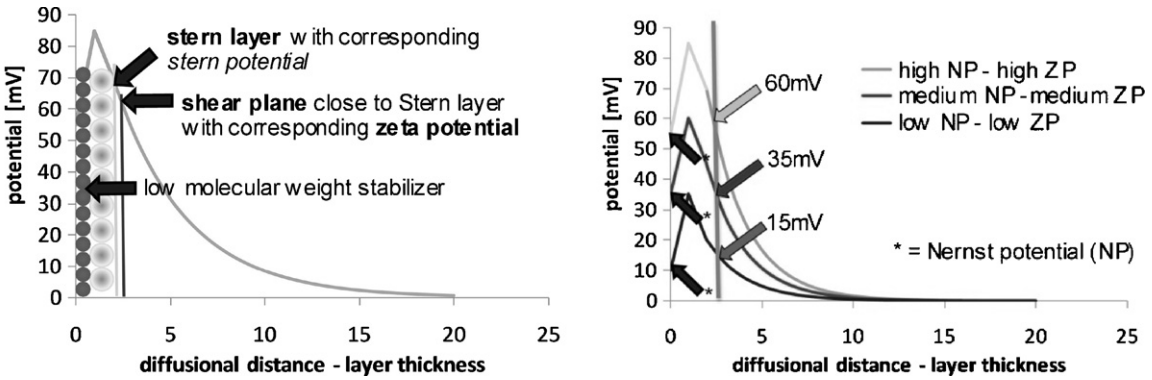

Fig. 6. Left: the surface charge of particles is analyzed as zeta potential, which is the potential at the shear plane. For measurements in water in praxis the zeta potential is set equal to the Stern potential, because the zeta potential measured is very close to the Stern layer, right: the Stern potential (and thus the zeta potential as well) is related to the Nernst potential. The higher the Nernst potential (NP), the higher is the Stern potential.

3.3.1. Zeta potential in distilled water

The zeta potential measured in distilled water is close to the Stern potential (Fig. 6, left). For theoretical calculations, the ZP measured in distilled water is set equal to the Stern potential. The Stern potential is related to the potential of the particle surface (Nernst potential). The higher the Nernst potential is, the higher is the Stern potential (Fig. 6, right). Having a high Nernst potential a priori promotes stability, because high Nernst potentials lead to high zeta potentials. By mechanical breaking of the particles similar or identical Nernst potentials should result.

Of course there is some interference by the adsorbed stabilizers. By diluting the suspension for the ZP measurement, not all of the stabilizer might be desorbed from the particle surface. Especially polymers such as Poloxamer and Inutec are more difficult to desorb, because for desorption all attached parts of the molecule need to detach from the particle surface. The ZP values measured in distilled water are in a similar range of −30 to −36 mV for the three stabilizers Poloxamer, Inutec and Tween, Plantacare is about −48 mV (Table 2, middle). An explanation might be that the non-ionic Plantacare fully desorbs, the measured −48 mV reflect the correlation to the surface potential. The first three stabilizers do not or only partially desorb, adsorbed stabilizer layers shift the plane of shear to larger distances from the particle surface, and lead consequently to a lower measured Stern potential (Müller, 1996). As a summary from these measurements, a Stern potential of around −50 mV indicates a well-charged surface, thus in principle promoting stability.

3.3.2. Zeta potential in the original dispersion medium

The measurement of the ZP in the original dispersion medium is a measure for the thickness of the diffuse layer. The higher the measured ZP, the thicker the diffuse layer and the more stable is the suspension (Fig. 7, left) (Müller, 1996). As a rule of thumb, absolute ZP values above 30 mV provide good, above 60 mV excellent stability. About 20 mV provide only a short-term stability, values in the range −5 mV to +5 mV indicate fast aggregation (Riddick, 1968). This is valid for low-molecular weight surfactants and pure electric stabilization, but not for higher or large molecular weight stabilizers, which act mainly by steric stabilisation. In this case ZP values of only 20 mV or much lower can provide sufficient stabilization. It has to be kept in mind that adsorbed layers of polymers/large molecules shift the plane of shear to a farer distance from the particle surface. This leads to a reduction of the measured zeta potential (Fig. 7, right). That means even in case of highly charged particle surfaces (high Nernst potential and high Stern potential) a relatively low ZP will be measured. Despite the low measured ZP, the suspensions are stable.

Present ionic compounds (e.g. electrolytes) in the dispersion medium reduce the thickness of the diffuse layer (detected by a measured reduced ZP) and hence the stability. Even when non-ionic stabilizers are used, very often they contain electrolytes, which lead to this effect. Plantacare possesses a ZP of about −30 mV in the original dispersion medium, indicating good physical stability during storage.

Poloxamer exhibits also a ZP of −30 mV (−34 mV in water, Table 2) which is relatively high for an adsorbed non-ionic polymer. Due to the shift of the plane of shear one would expect a lower ZP value. In case of Poloxamer stabilized polystyrene particle values in the range around −20 mV or lower were measured (Müller, 1991). The measured high value of −30 mV can only be explained that the adsorption layer of Poloxamer is relatively thin, providing potentially less stabilization.

In contrast to this, Inutec shows a reduction from −36 mV in water to about −17 mV in the original dispersion medium (Table 2). This indicates a thick adsorbed layer, and thus good stabilization.

Tween 80 exhibits a ZP value of −13 mV. With very well sterically stabilized systems even lower values were observed, being rather around −3 mV (Müller, 1991). From this, it can be concluded that the adsorbed layer is relatively thin, indicating that the stabilization with Tween might be a little bit more critical.

As a summary, Inutec and Plantacare promise good stabilization effects predicted on the measured ZP values being in agreement with the theories. Poloxamer might potentially more critical, but final judgement is not possible because of unknown factors such as adsorbed layer thickness.

Table 2
Zeta potential data for the four different nanosuspensions; left: analyzed in water (conductivity 50 mS/cm, pH 5.8); right: analyzed in the original dispersion medium.

Stabilizer	Zeta potential (mV)	
	In water	In original dispersion medium
Tween 80	−30.6	−13.3
Poloxamer 188	−34.7	−30.6
Plantacare 2000	−48.3	−29.1
Inutec SP1	−36.2	−16.9

3.4. Short-term stability test at different temperatures

The samples were stored in the fridge (4 °C), room temperature and at 40 °C. From the previous experiences nanosuspensions exhibited best stability when stored at room temperature. As outlined above, the nanosuspensions possess an increased saturation solubility. Storing them in the fridge reduces the solubility for many actives, leading to re-crystallisation of the active in form of large crystals. Depending on the extend of solubility reduction, the size increase is not detectable, very limited or clearly detectable. Stor-

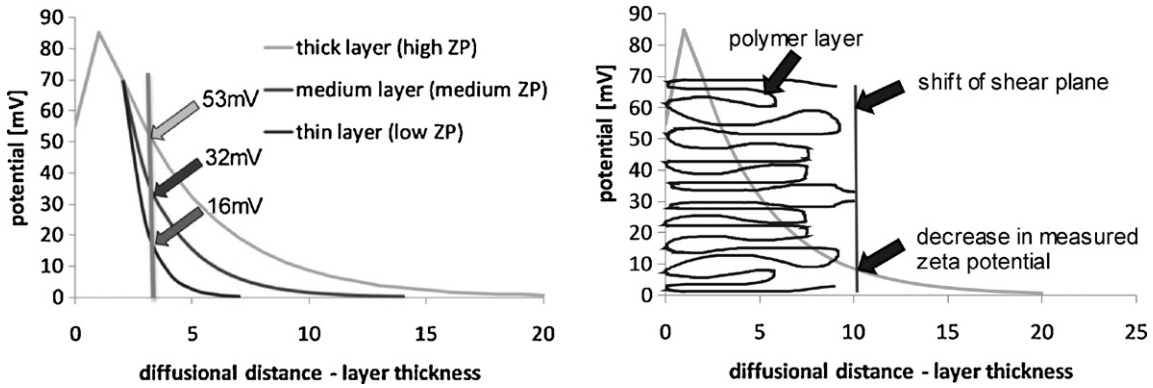

Fig. 7. Left: The measurement of the zeta potential in the dispersion medium is a measure of the thickness of the layer—the thicker the diffusional layer, the higher is the measured zeta potential; right: if large polymers are used for stabilization, the shear plane is shifted, resulting in decreased zeta potentials.

age at 40 °C increases for many actives the solubility; it can slightly reduce the nanocrystal size by dissolution of the drug nanocrystals. However, the smallest crystals dissolve best and disappear, larger ones remain which can also lead to an increase in the measured size (Keck, 2006). Most important is however the increase in kinetic energy of the diffusing particles which might overcome the energy barrier of electrostatic and/or steric repulsion leading to aggregation.

Based on the zeta potential measurements, the highest stability was expected for the nanosuspensions stabilized with Inutec and Plantacare. Figs. 8 and 9 show the PCS mean diameters and the LD diameters 99% of the four differently stabilized nanosuspensions at the three storage temperatures. The PCS diameter shows very sensitive changes in the bulk population, the d(v)99% is highly sensitive towards the occurrence of larger sized particles. Therefore, both parameters are useful tools to follow the physical stability. At all three storage temperatures, both the Inutec and Plantacare stabilized nanosuspensions show practically unchanged PCS and d99% diameters. The PCS values slightly fluctuate around 300 nm, within the reproducibility of the PCS measurements. In general, the d99% shows more fluctuations than, e.g. d90% or 50% in the measurements. Considering this, no increase took place over a period of 30 days, the nanosuspensions are stable at all three temperatures (Fig. 9).

The Poloxamer stabilized nanosuspensions are stable at room temperature (Fig. 8, lower left, bars), but show a slight but steady increase in d99% from day 3 to day 30 at 4 °C and 40 °C (Fig. 8, lower middle and right). Especially at 40 °C this is accompanied by a slight decrease in the PCS diameter of the bulk population. At the first glance this appears contradicting, but a potential explanation is that the larger sized nanocrystals of the bulk population are less stable, they aggregate, moving out of the measuring range

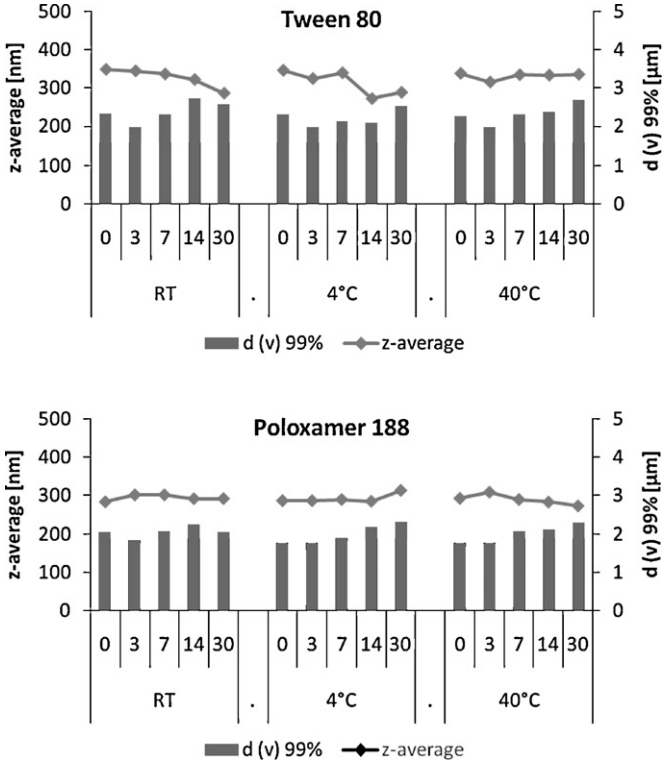

Fig. 8. Stability profile of Tween 80 (upper) and Poloxamer 188 (lower) stabilized Hesperetin nanosuspensions as function of days (0–30) stored at different temperatures (left: room temperature (RT); middle: 4 °C; right: 40 °C).

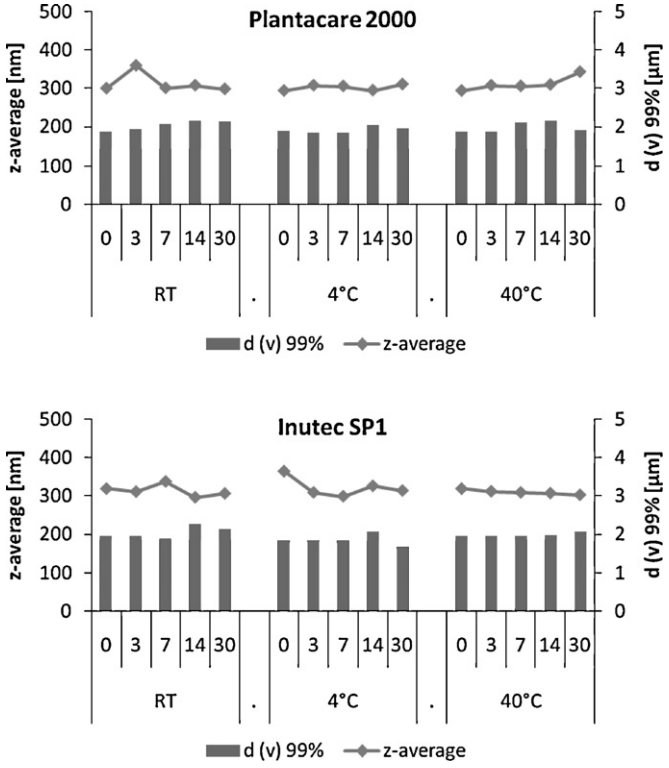

Fig. 9. Stability profile of Plantacare 2000 (upper) and Inutec SP1 (lower) stabilized Hesperetin nanosuspensions as function of days (0–30) stored at different temperatures (left: room temperature (RT); middle: 4 °C; right: 40 °C).

of the PCS and consequently the PCS diameter decreases due to the remaining smaller, stable nanocrystals. This phenomenon is known from o/w emulsions. Larger sized droplets coalesce, continue to coalesce and a separate second population of larger sized droplets is formed, whereas the stable small droplets remain. This slight increase is not limiting the use of these nanocrystals in dermal formulations, in case it does not continue during the further months of storage. In addition, it needs to be considered that in creams and gels the viscosity η is higher. Thus, the diffusion velocity – quantified by the diffusion coefficient D – is, according to the Einstein equation, lower (D reverse proportional to η) and thus also the related kinetic energy of the particles. Therefore, the nanocrystals will be a priori more stable in these dermal formulations. Based on these considerations, adding the nanocrystals to the dermal formulation immediately after production might potentially avoid any change in size of the Poloxamer stabilized nanocrystals.

The Tween stabilized nanosuspensions show an increase of d99% at all three storage temperatures (Fig. 8). At 4 °C and at room temperature this is accompanied by a slight decrease in PCS size of the bulk population. From these the Tween stabilized nanosuspensions were classified as the least stable ones. However, the considerations made for incorporation in more viscous dermal formulations are also valid for the Tween stabilized nanosuspensions.

4. Conclusions

Hesperetin nanosuspensions with a mean PCS diameter of 300 nm could be produced with the three stabilizers Poloxamer, Plantacare 2000 and Inutec. Obtaining the same size independent on the stabilizers used is in line with the theory that the final crystal size depends only on the power density (pressure) during the homogenization process and on the hardness of the crystals but not on the stabilizer. Tween 80 yielded slightly larger PCS diameters (around 350 nm) indicating that this stabilizer is not able to similarly efficiently stabilize the produced crystals at the end of the homogenization process, very slight aggregation seems to occur.

Interpretation of the zeta potential data predicted Inutec and Plantacare stabilized nanosuspensions as the most stable ones, which was confirmed by the short-term stability study. However, for dermal products also the Poloxamer and Tween stabilized nanosuspensions appear still usable, presumed that the slight aggregation does not continue. A long-term stability study over a period of up to 2 years is presently going on to finally assess the stabilizing ability of the stabilizers. After addition of the nanocrystals to dermal formulations, the nanocrystals are additionally stabilized by the high viscosity of the formulation. Despite this additional stabilizing effect, it is advantageous to incorporate a priori the most stable nanocrystals, therefore also the long-term stability of the nanosuspensions themselves is important. Nanosuspensions are also sold as concentrates for admixture to cosmetic and pharmaceutical products. Of course these products need to possess a sufficient shelf live. In addition, nanosuspensions are also of interest as oral pharmaceutical suspensions (e.g. product Megace ES).

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