Free University of Berlin, Germany; Department of Pharmaceutics[2], Kwame Nkrumah University of Science & Technology, Kumasi, Ghana[2], Institute of Bioscience, University Putra Malaysia, Serdang, Malaysia[3], University of Applied Sciences Kaiserslautern, Department of Applied Logistics and Polymer Science, Pirmasens, Germany[4]

Resveratrol nanosuspensions: interaction of preservatives with nanocrystal production

S. Kobierski[1], K. Ofori-Kwakye[1,2], R. H. Müller[1], C. M. Keck[3,4]

Received March 21, 2011, accepted May 2, 2011

Prof. Dr. Cornelia M. Keck, University of Applied Sciences Kaiserslautern, Department of Applied Logistics and Polymer Science, Applied Pharmacy Division, Carl-Schurz-Str. 10-16, 66953 Pirmasens, Germany
cornelia.keck@fh-kl.de

Pharmazie 66: 942–947 (2011) *doi: 10.1691/ph.2011.1038*

The effect of six different preservatives on the production process and stability of resveratrol nanosuspensions was investigated. Nanosuspensions of the anti-oxidant resveratrol were prepared by high pressure homogenization (1,500 bar, 20 homogenization cycles). The preservatives used were: caprylyl glycol (0.75%), Euxyl PE 9010 (1.0%), Hydrolite-5 (2.0), Phenonip (0.75%), Rokonsal PB-5 (0.5%) and MultiEx Naturotics (2.0%). Preservation is essential for oral and dermal nanosuspensions, but can impair the stability. The effect of the preservatives on stability as a function of cycle numbers was determined by size measurements (photon correlation spectroscopy (PCS), laser diffraction (LD) and light microscopy). Zeta potential measurements were performed for determination of the Stern potential (measurements in water) and as stability criterion (measurements in original dispersion medium), to elucidate the mechanism of destabilization. The preservatives could be placed into three groups. Hydrolite-5 did not affect the production process and the short term stability, sizes were practically identical to the preservative-free nanosuspension (e.g. PCS diameters 196 nm and 184 nm, respectively). All other preservatives impaired the stability medium to pronounced, being most pronounced for MultiEx Naturotics. Hydrolite-5 is recommended as preservative of choice. A mechanistic model was developed to explain the absence and the different degrees of destabilization. In general, when screening for suitable preservatives, suspensions are produced, different preservatives added and the size changes are monitored over long-term. The destabilizing effect of the preservatives on nanosuspensions became evident when added in the production process immediately, thus this can be used as a screening tool for optimal, non-destabilizing preservatives, replacing or minimizing time-consuming long-term stability studies.

1. Introduction

Dermal use of nanocrystals of poorly soluble actives is a novel application since 2005 (Keck and Müller 2008; Petersen 2008). It exploits the special properties of nanocrystals to increase the saturation solubility of poorly soluble pharmaceutical and cosmetic actives and consequently penetration into the skin resulting in an increased bioactivity (Fig. 1).

An increase in bioactivity by a factor of 1,000 was reported for the poorly water soluble anti-oxidative rutin compared to its water soluble derivative rutin-glycoside (Petersen 2008). In a human study, the rutin nanocrystals were 2 times more active at a 500 fold lower concentration. The antioxidant capacity was assessed by measuring the increase in the sun protection factor (SPF). Nanocrystals possess an increased saturation solubility c_s, in addition an increased dissolution velocity dc/dt. Based on the Noyes-Whitney equation, the increased dc/dt is due to the increase in solubility c_s, consequently the increased difference c_s-c_x (c_x - bulk solubility), and the increased surface area A.

Fig. 2 shows the mechanism of action of dermal nanocrystals, explained by using rutin as original plant molecule and its synthetic water-soluble derivative rutin-glucoside as examples. Due to the higher solubility of active rutin as a nanocrystal, there is an increased concentration gradient between dermal formulation and skin, compared to having μm-sized rutin crystals in the cream. This leads to a higher penetration of rutin into the skin. Rutin diffused into the skin is immediately replaced by fast dissolving molecules from the nanocrystals. Compared to the hydrophilic rutin-glucoside, the original lipophilic molecule rutin can better penetrate into the skin. In the skin, the rutin is believed to have a higher affinity to the relevant sites of action than the synthetically modified version. That means there is up to a total of four superimposed potential effects leading to the increased bioactivity of molecules in nanocrystalline form:

1. Higher solubility and resulting concentration gradient cream/skin
2. Depot of fast dissolving molecules from the nanocrystals in the cream
3. Better skin penetration compared to water-soluble derivatives
4. Higher activity in the cell of original molecule compared to derivative

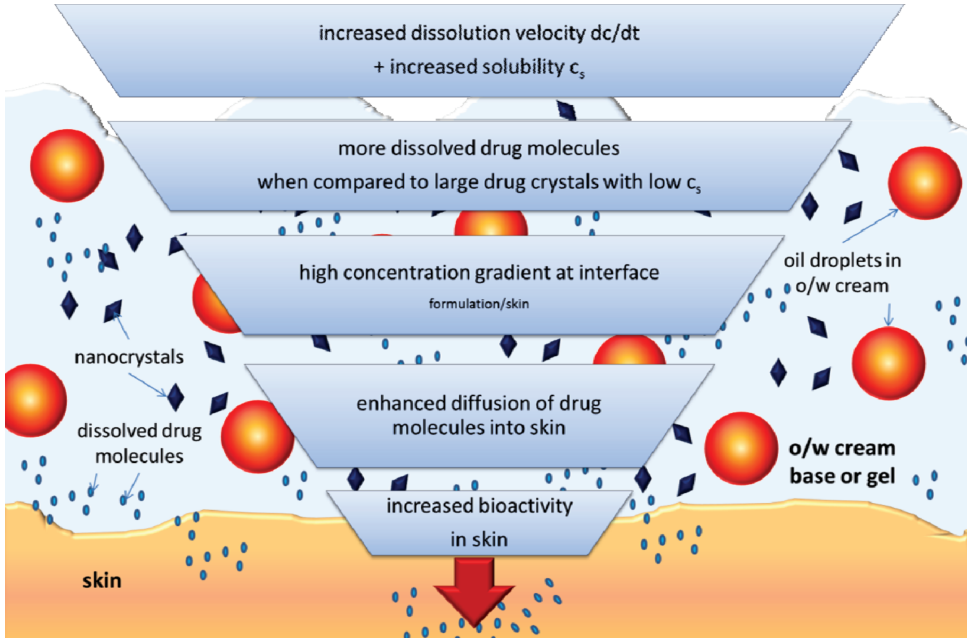

Fig. 1: Mechanism of action of dermal nanocrystals: The nanocrystals are dispersed in the water phase of an o/w cream, a part of the active is dissolved with a kinetic solubility being higher than the thermodynamic equilibrium solubility leading to an increase bioactivity in skin

In this study resveratrol was used as anti-oxidant for dermal application. Resveratrol is very well known from the French paradoxon (Renaud and de Lorgeril 1992, 1993). It was described to be contained in different concentrations in red wines (Gu et al. 1999), peanuts and mulberry skins (Stewart et al. 2003). Dermally applied it protects against e.g. UV irradiation (increased SPF), and acts therefore anti-aging (Chan 2002; Bowers et al. 1991). The thermodynamic solubility of resveratrol is low (approx. 3 mg/100 ml) (Sigma). Therefore, it is an ideal candidate to be used as nanocrystal in dermal cosmetic formulations. Pharmaceutical use appears sensible e.g. in combination with phototoxic compounds to avoid/minimize erythema formation, or as protective agent against skin cancer (Afaq et al. 2003). Nanocrystals are produced as aqueous concentrates, i.e. nanosuspensions with high drug content, and sold to cosmetic industry for admixture during the production of creams, lotions and dermal gels. Thus, for microbial safety reasons, these nanosuspensions need to be preserved. Ideally preservation is made prior to production of the nanosuspension, i.e. preserving the prepared macrosuspension prior to the wet milling or high pressure homogenisation step. This is sensible because the wet milling process lasts from 4–5 h up to several days, depending on the batch size and the physico-chemical properties (e.g. hardness) of the crystals. Ideally, bacterial growth should be excluded by preservatives being present.

However, preservatives can impair the physical stability of suspensions (e.g. zeta potential reduction, dehydration of stabilizer layer, displacement of stabilizer on particle surface by preservative) (Lucks et al. 1990). Therefore, preservatives can also affect the production process itself, e.g. impairing

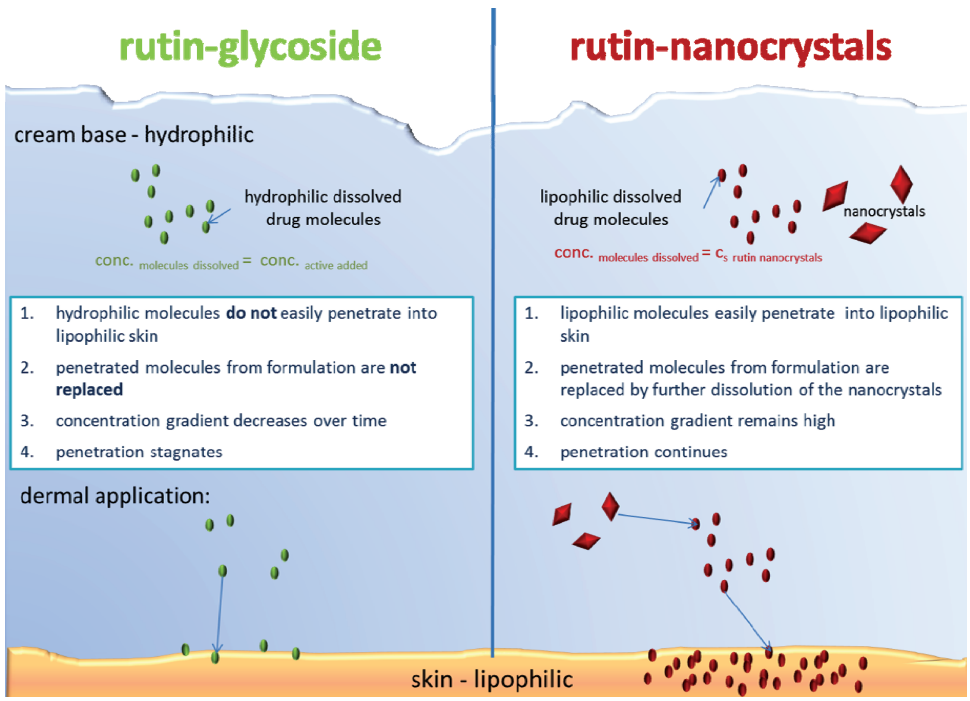

Fig. 2: Mechanism of action of dermal nanocrystals in comparison to a water-soluble derivative (explanation cf. text)

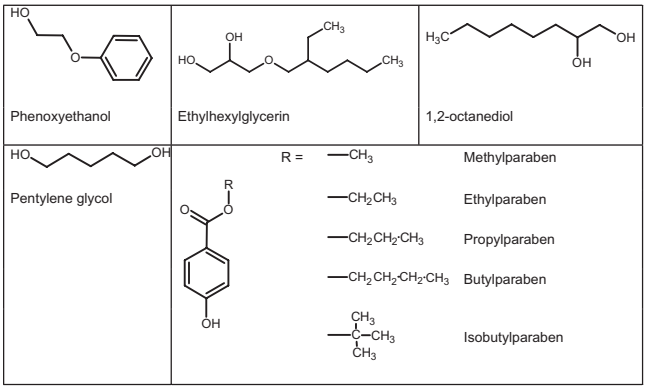

Fig. 3: Structure of preservatives listed in Table 1

the diminution efficacy due to agglomeration. In this study six preservatives were investigated regarding their suitability to be used. Preservatives were added to the respective resveratrol macrosuspension and subjected to high pressure homogenization to obtain nanocrystals. The resulting formulations were characterized in terms of size during the production process and by zeta potential measurements.

2. Investigations, results and discussion

2.1. Nanocrystal charge: zeta potentials

Suspensions can be stabilized by electrostatic repulsion, steric repulsion and ideally by a combination thereof. In this study Plantacare 2000 (C8-C16 fatty alcohol polyglycoside) was selected as stabilizer, because in a previous study with non-preserved Resveratrol nanosuspensions it proved to be superior to Tween 80, Poloxamer 188 and Inutec SP1 (Kobierski et al. 2009). Most electrolytes are known to reduce the particle charge, quantified as zeta potential (ZP). Therefore, in this study only non-ionic preservatives were selected to a priori minimize effects on the ZP. The preservatives used are listed in Table 1, the chemical structures are given in Fig. 3. Preservatives have a recommended concentration range. In this study concentrations in the medium range were selected to minimize interference with the nanosuspension, but still maintaining a microbial safety range. A medium concentration is regarded as sufficient, because the nanosuspension concentrates are typically opened only once in the production process, in contrast to e.g. eye drops, requiring higher preservative concentrations due to the multiple opening over up to 4 weeks usage period.

ZP measurements were performed in distilled water and in the original dispersion medium (water with surfactant and the respective preservative) at the day of production and after 14 days of storage. The results are shown in Table 2. The measured ZP in water is set equal to the Stern potential (Müller 1996). The Stern potential is related to the surface (Nernst) potential, it increases with increasing surface charge. The higher the surface charge of a particle, the higher is a priori the electrostatic stabilization. All the nanosuspensions were stabilized with the same stabilizer (Plantacare 2000), but preserved with different preservatives. For the measurement a small sample of nanosuspension is diluted in water. In case potentially adsorbed preservatives are completely or almost completely desorbed from the nanocrystal surface, an identical ZP should result for all nanosuspensions. The ZP in water is about −40 mV for the suspensions stabilized with Hydrolite-5 (−42.3 mV), Euxyl PE 9010 (−39.1 mV) and caprylyl glycol (−37.6 mV). It is about −33 mV for Phenonip and Rokonsal PB-5 and with -17 mV lowest for MultiEx Naturotics (Table 2). The medium and lower values indicate that obviously some preservatives remain adsorbed on the nanocrystal surface, when looking at the lower ZP values, especially also when comparing it to the −42.9 mV of the preservative-free nanosuspension (Kobierski et al. 2009). The zeta potentials measured in water indicate strongest interaction with the nanocrystals surface, and thus potential destabilization, for Phenonip, Rokonsal PB-5 and MultiEx Naturotics.

The ZP values in the original dispersion medium are an indication for the physical stability, for solely electrostatically stabilized systems about 30 mV (absolute value, independent on sign of charge) are considered to be required for a stable suspension. Slightly lower values are still sufficient, in case there is additionally a contribution by steric stabilization. In the original dispersion media the ZP values are drastically reduced, being still highest for the suspensions preserved with Hydrolite−5 and Euxyl PE 9010 (around −20 mV), medium for caprylyl glycol, Phenonip and Rokonsal PB-5 (about −14 mV), and with about +6 mV very low for MultiEx Naturotics. The reason for the observed charge reversal cannot be given, but the measurement was reproducible. The zeta potential data showed no change with storage time (Table 2), which is in agreement with the theory. Based on the ZP data stability problems were expected with most of the preservatives, with Hydrolite-5 and Euxyl PE 9010 being least impaired in stability. For the non-preserved nanosuspension the ZP in the original dispersion medium is < −5 mV, indicating full steric stabilization caused by a thick layer of the stabilizer (Kobierski et al. 2009). For the preserved nanosuspensions, the difference in ZP in the original dispersion is about |20 mV| compared to the ZP in water, hence the differences between the ZP in water and the original dispersion medium are much smaller than in the non-preserved suspension. These results indicate, that the preservatives change the adsorption pattern of the stabilizer, i.e. they influence the thickness of the stabilizing layer and its stabilizing mechanism. Pure steric stabilization as observed for the non-preserved suspension is not observed for the preserved nanosuspensions anymore, but a combination of steric and electrostatic stabilization.

2.2. Nanosuspensions - production and storage stability

The effect of an increasing number of homogenization cycles was monitored by laser diffractometry (LD) measurements. The diameter D50% was used as measure for the size of the bulk population, the diameters D95% and D99% to quantify the amount of larger particles present besides the bulk population. Fig. 4 shows the decrease in the D50% for all the nanosuspensions until cycle 15, no or little change occurs to cycle 20. However, the situation is different when looking at D95% and D99%.

Looking at the D95%, Hydrolite-5 seems not or little to affect the nanosuspension during the production. The values are similar to the preservative-free nanosuspension (Fig. 5). The other preservatives can be grouped into 2 groups:

Group A: Euxyl PE 9010 and Rokonsal PB-5 (Fig. 5, left): They show a continuous decrease until cycle 20. However the diameters are distinctly larger compared to the preservative-free nanosuspension. The preservatives lead to formation of aggregates, which can be reduced by increasing the number of homogenization cycles.

Group B: Phenonip shows very little or no decrease from cycle 10 to 20. The formed aggregates seem to be more stable, and consequently more difficult to remove. Caprylyl glycol and MultiEx Naturotics show even an increase in D95% up to cycle 20 (Fig. 5, right). The energy input even promotes aggregate formation, at least no aggregates are efficiently removed. The same tendency can be seen when looking at the diameters D99% (Fig. 6A and 6B), being most sensitive towards the presence of aggregates/large particles.

Table 1: List of preservatives used (trade names), chemical composition of preservative, concentration range typically applied in products, and concentrations used in this study

Preservative	Preservative code	Chemical composition	Recommended concentration range (%)	Conc. used (%)
Euxyl PE 9010	EPE	Phenoxyethanol, ethylhexylglycerin	1.0	1.0
Caprylyl glycol	CG	1, 2-Octanediol	0.5−1.0	0.75
Phenonip	Ph	Phenoxyethanol, methylparaben, ethylparaben, butylparaben, propylparaben, isobutylparaben	0.5−1.0	0.75
MultiEx Naturotics	ME	Magnolia bondii bark extract, propolis extract, grapefruit extract, chamomile extract, willow bark extract, camelia sinensis leaf extract, thujopsis dolabrata extract	1.0−3.0	2.0
Hydrolite-5	H-5	Pentylene glycol	1.0−3.0	2.0
Rokonsal PB-5	R	Phenoxyethanol, methylparaben, ethylparaben, butylparaben, propylparaben, isobutylparaben	0.3−1.2	0.5

Table 2: Zeta potentials of preserved nanosuspensions, measured in distilled water and in the original dispersion media consisting of Plantacare, the respective preservative and water (day 1 and day 14)

Formulation code	Zeta potential (mV)			
	In distilled water		In original dispersion medium	
	Day 1	Day 14	Day 1	Day 14
PL1 no preservative	−42.9 ± 1.3	n.a.	−4.3 ± 0.4	n.a.
PL1 + EPE	−39.1 ± 2.4	−36.8 ± 2.0	−19.1 ± 0.6	−20.2 ± 0.4
PL1 + CG	−37.6 ± 0.7	−35.2 ± 1.6	−13.3 ± 1.4	−11.9 ± 1.1
PL1 + Ph	−33.7 ± 0.3	−34.8 ± 1.2	−13.7 ± 0.2	−16.3 ± 0.2
PL1 + ME	−17.1 ± 2.3	−11.4 ± 0.1	+ 5.6 ± 0.2	+ 4.5 ± 0.1
PL1 + H-5	−42.3 ± 2.9	−39.8. ± 1.9	−21.3 ± 0.2	−23.9 ± 1.0
PL1 + R	−33.4 ± 0.5	−35.8 ± 0.2	−14.8 ± 0.1	−15.9 ± 0.1

These size results can be explained when considering the zeta potential data. Addition of a second component to a stabilizer film can increase the film stability (microviscosity), increasing consequently the stability of dispersions. The stabilizing effect depends not only on the chemical nature of the compound but also on its concentration. A classical example is the addition of cholesterol to liposomes (Vemuri and Rhodes 1995; Kirby et al. 1980). It increases the microviscosity (and thus rigidity) (Diederichs 1993; Ehlers et al. 1996) leading to an improved stability. The opposite effect is also possible if the compound does not sterically fit into the stabilizer layer, thus reducing the hydrophobic interactions between the hydrophobic surfactant parts and the film rigidity (microviscosity). Classical examples are sodium deoxycholate, nowadays used to formulate ultra flexible liposomes, and benzyl alcohol increasing the fluidity of surfactant layers in liposomes (Chen et al. 2009).

Based on these considerations a mechanism is proposed in Fig. 7. Hydrolite-5 seems to have very little affinity to the nanocrystal surface. The stabilizing Plantacare film on the nanocrystal surface contains very little Hydrolite-5 molecules, its stabilizing

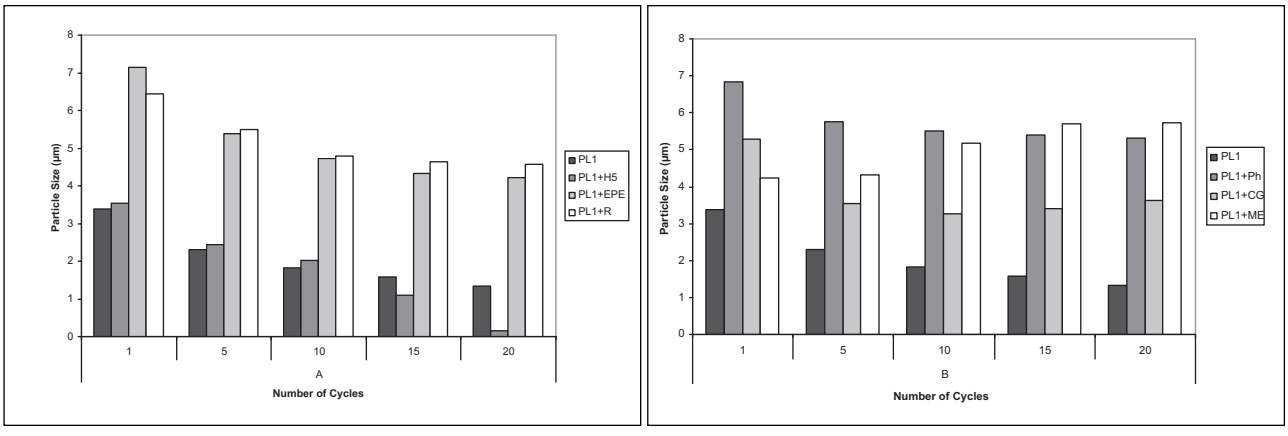

Fig. 4: LD diameters D50% of the preservative-free nanosuspension in comparison to the nanosuspensions preserved with: Left: Hydrolite-5, Euxyl PE 9010 and Rokonsal PB-5; Right: Phenonip, caprylyl glycol and MultiEx Naturotics

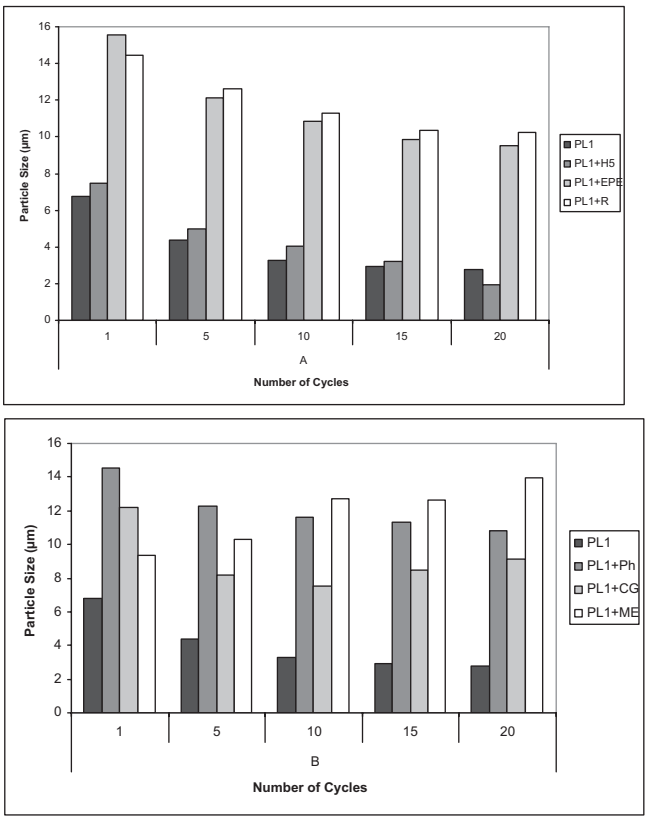

Fig. 5: LD diameters D95% of the preservative-free nanosuspension in comparison to the nanosuspensions preserved with: Left: Hydrolite-5, Euxyl PE 9010 and Rokonsal PB-5; Right: Phenonip, caprylyl glycol and MultiEx Naturotics

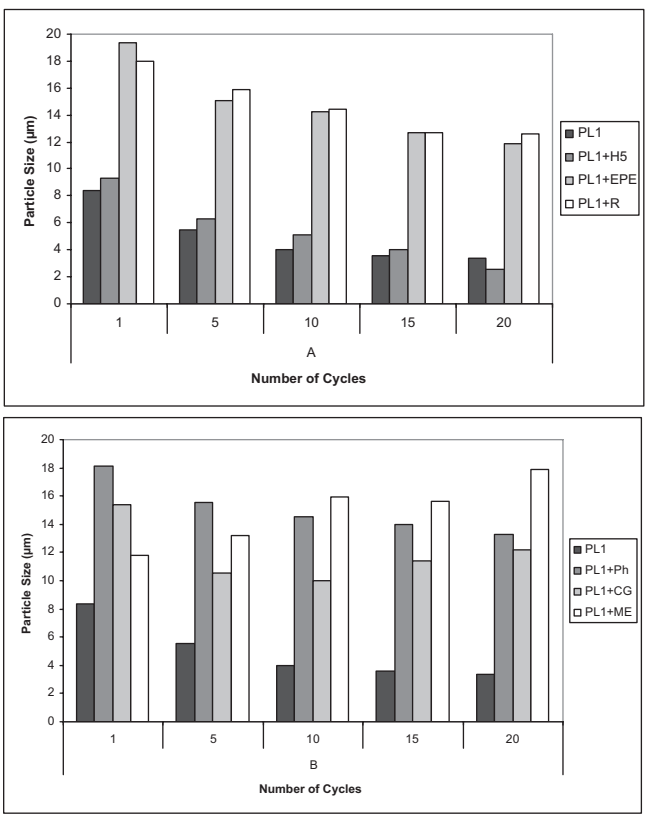

Fig. 6: LD diameters D99% of the preservative-free nanosuspension in comparison to the nanosuspensions preserved with: Left: Hydrolite-5, Euxyl PE 9010 and Rokonsal PB-5; Right: Phenonip, caprylyl glycol and MultiEx Naturotics

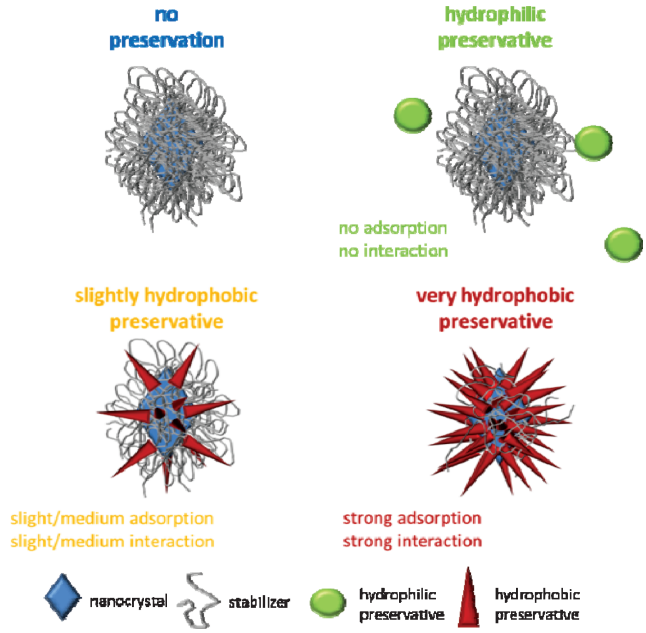

Fig. 7: Mechanism of interaction of the preservatives with the stabilizing adsorption layer of Plantacare on the nanocrystal surface (explanation cf. text)

ability is not affected. Preservatives of group A seem to interact stronger with the surfactant layer. They might have a higher concentration in the adsorption layer, or depending on the chemical structure despite a lower concentration in the layer, being more interfering. Group B, especially the hydrophobic MultiEx Naturotics seems to adsorb strongly on the surface, displacing Plantacare leading to a mixed adsorption layer. This layer has practically very little stabilizing ability. Energy input during the homogenisation breaks crystals, but at the same time it accelerates the crystals, which leads to aggregation in case of insufficient stabilization. This exactly takes place in the MultiEx Naturotics containing nanosuspensions. The diameters D95% and D99% increase (Fig. 5 and 6).

Due to the large sizes present in all preserved nanosuspensions – apart from the one with Hydrolite-5 – PCS measurements were only performed for this one. The large aggregates – as also identified by light microscopy – with diameters in the range 5 to 20 µm are outside the measuring range of PCS. PCS data would give even misleading results because the large particle faction is not "seen" by PCS.

PCS diameters were almost identical for the preservative-free and the Hydrolite-5 containing nanosuspension being 185 nm and 196 nm, respectively. The polydispersity indices were 0.41 and 0.42, respectively. Considering the reproducibility of PCS (1–2%, in this case 2–4 nm) and the batch-to-batch fluctuations in nanosuspension production, Hydrolite-5 does not to affect the stability during production of resveratrol nanosuspension.

This was confirmed by the storage data at room temperature. After 196 nm at the time of production, the PCS diameters measured were 187 nm, 194 nm and 201 nm after 7, 14 and 42 days of storage at room temperature, respectively. Little change occurred in the LD data (data not shown).

Hydrolite-5 is the preservative of choice not affecting the physical stability of the resveratrol nanosuspension during production and short term storage at room temperature. The zeta potentials in water indicate that there is no firm adsorption or little affinity to the nanocrystal surface, thus no destabilizing interaction with the stabilizer layer on the nanocrystals. This is opposite for the other preservatives, especially MultiEx Naturotics. A model was developed explaining the different degrees of destabilization by the three categories of preservatives (hydrolite-5, groups A and B).

Apart from the observed de-stabilization, pronounced adsorption to the large surface of nanosuspensions can lead to a loss of preservative activity. To compensate for this, in this study *a priori* not the lowest but a medium concentration within the recommended range of preservatives was used. Hydrolite-5 has a recommended range 1–3%, 2% were used in this study. If required, the use of distinctly higher concentrations (3% or above) seems feasible because of the lack of interference with the nanosuspension stability.
As conclusions from this:

1. Preservatives with lowest affinity to the nanocrystals surface should be used, because they show least destabilization and least loss of preservative action (= little loss to the nanocrystal surface).
2. Addition of preservatives in the production process can be used as a tool to screen for suitable, not interfering preservatives, because the destabilizing effect is clearly visible. This could replace long-term stability studies, or at least allows to limit the number of studies to the most promising preservatives.

3. Experimental

3.1. Materials

Resveratrol was used as a model active (antioxidant) for dermal application (E. Denk Feinchemie GmbH, München, Germany). Plantacare 2000 UP ((C8-C16 fatty alcohol polyglycoside)) kindly provided by Cognis GmbH (Düsseldorf, Germany) was used as a stabilizer for the nanosuspensions. Rokonsal PB-5 (ISP Biochema Schwaben GmbH, Memmingen, Germany), MultiEx Naturotics (Biospectrum Inc, GyunggiDo, Korea Republic), Euxyl PE 9010 (Schülke & Mayr GmbH, Nordestedt, Germany), Phenonip (Clariant UK Ltd, Leeds, UK), Hydrolite-5 (Symrise GmbH, Holzminden, Germany), and Caprylyl glycol (Sigma-Aldrich, Germany), were used as preservatives. 0.9% sodium chloride solution was provided by B. Braun Melsungen AG (Melsungen, Germany). Ultrapure water was obtained from a milliQ system (Millipore Corporation, Bedford, MA, USA).

3.2. Methods

3.2.1. Production of resveratrol nanosuspensions

Resveratrol nanosuspensions were produced by high pressure homogenization using a Micron LAB 40 (APV Deutschland, Germany). The resveratrol powder was dispersed in water containing Plantacare 2000 and the respective preservative. This led to a final concentration of 5.0% resveratrol, 1.2% Plantacare 2000, preservative and water, the concentration of the preservatives are listed in Table 1. The obtained macrosuspension was then homogenized applying a pre-milling followed by 20 cycles at 1,500 bar. Pre-milling was performed with 2 cycles at 300 bar, 2 cycles at 500 bar and 1 cycle at 1,000 bar. Pre-milling should disintegrate large crystals to avoid blockage of the homogenization gap. In between the cycles the nanosuspensions were cooled to room temperature in an ice bath.

3.2.2. Zeta potential measurements

The zeta potential (ZP) was measured in distilled water having the conductivity adjusted to 50 μS/cm by addition of 0.9% NaCl solution, and in the original dispersion medium. The conductivity of the distilled water was adjusted to avoid fluctuations in the conductivity due to differences in the day-to-day water quality. In distilled water, even a few μS/cm change can cause a shift in the ZP by about 5 mV (Müller 1996). Measurement in water yields the Stern potential. The measurement in the original dispersion medium yields the zeta potential for estimating the physical stability of dispersions. The preserved nanosuspensions were measured in aqueous solutions of Plantacare 2000 and preservative being identical in concentration to the water phase of the preserved nanosuspensions. Measurements were performed at 25 °C, in a disposable standard cuvette applying a field strength of 20 V/cm. The Helmholtz-Smoluchowski equation was used for calculating the ZP (mV).

3.2.3. Particle size analysis

Particle size analysis was performed by photon correlation spectroscopy (PCS) using a Malvern Zetasizer Nano ZS (Malvern Instruments, Malvern, UK). The measuring range is appr. 3 nm to 3/6 μm (depending on the density of the particles). It yields the z-average as a measure of the size of the bulk population and the polydispersity index (PDI) as measure for the width of the size distribution.
Size analysis of the particles in the lower μm range was performed by laser diffractometry (LD) using a Malvern Mastersizer 2000 (Malvern Instruments, Malvern, UK). Mie theory was applied fort he size analyis using 1.69 as real refractive index and 0.001 as imaginary refractive index. The real refractive index was determined applying the dn/dc method (measurement of refractive index of drug solutions of increasing concentration and extrapolating to 100% drug (Keck 2006)). The LD yields a volume distribution which was used to calculate the diameters 50% (D50%) for characterization of the bulk population and the diameters 95% (D95%) and 99% (D99%) as sensitive parameters for the presence of particles distinctly larger then the bulk population (i.e. remaining large crystals, formed aggregates).
Light microspcopy with and without polarized light (Orthoplan, Zeiss, Germany) was used to check whether the large particles detected by LD were large crystals or aggregates of nanocrystals.

References

Afaq F, Adhami VM, Ahmad N (2003) Prevention of short-term ultraviolet B radiation-mediated damages by resveratrol in SKH-1 hairless mice. Toxicol Appl Pharmacol 186: 28–37.

Bowers VD, Locker S, Ames S, Jennings W, Corry RJ (1991) The hemodynamic effects of Cremophor-EL. Transplantation 51: 847–850.

Chan MMY (2002) Antimicrobial effect of resveratrol on dermatophytes and bacterial pathogens of the skin. Biochem Pharmacol 63: 99–104.

Chen Y, Lu Y, Chen J, Lai J, Sun J, Hu F, Wu W (2009) Enhanced bioavailability of the poorly water-soluble drug fenofibrate by using liposomes containing a bile salt. Int J Pharm 376: 153–160.

Diederichs J (1993) PhD - thesis, Freie Universität Berlin.

Ehlers S, Bucke W, Leitzke S, Fortmann L, Smith D, Hansch H, Hahn H, Bancroff G, Müller R (1996) Liposomal amikacin for treatment of M. avium infections in clinically relevant experimental settings. Zentralbl Bakteriol 284: 218–231.

Gu X, Creasy L, Kester A, Zeece M (1999) Capillary electrophoretic determination of resveratrol in wines. J Agricult Food Chem 47: 3223–3227.

Keck CM (2006) PhD - thesis, Freie Universität Berlin.

Keck CM, Müller RH (2008) SmartCrystals® - Nanodiamanten zur Erhöhung der Bioaktivität. Labor&More 1: 64–65.

Kirby C, Clarke J, Gregoriadis G (1980) Effect of the cholesterol content of small unilamellar liposomes on their stability *in vivo* and *in vitro*. Biochem J 186: 591–598.

Kobierski S, Ofori-Kwakye K, Müller RH, Keck CM (2009) Resveratrol nanosuspensions for dermal application–production, characterization, and physical stability. Pharmazie 64: 741–747.

Lucks JS, Müller BW, Müller RH (1990) Inorganic suspensions – interaction with salts and ionic surfactants. Int J Pharm 58: 229–235.

Müller RH (1996) Zetapotential und Partikelladung, Wissenschaftliche Verlagsgesellschaft, Stuttgart.

Petersen RD (2008) PCT/EP2007/009943.

Renaud S, de Lorgeril M (1992) Wine, alcohol, platelets, and the French paradox for coronary heart disease. Lancet 339: 1523–1526.

Renaud S, de Lorgeril M (1993) The French paradox: dietary factors and cigarette smoking-related health risks. Ann N Y Acad Sci 686: 299–309.

Sigma, Resveratrol product information.

Stewart JR, Artime MC, O'Brian CA (2003) Resveratrol: a candidate nutritional substance for prostate cancer prevention. J Nutr 133: 2440S–2443S.

Vemuri S, Rhodes CT (1995) Preparation and characterization of liposomes as therapeutic delivery systems: a review. Pharm Acta Helv 70: 95–111.