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smartLipids® as third solid lipid nanoparticle generation – stabilization of retinol for dermal application

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smartLipids® as the 3rd lipid nanoparticle generation are made from a complex lipid mixture. The chaotic particle matrix structure provides higher loading with actives and a firmer inclusion inside the particle matrix being more protective for chemically labile molecules. Thus, these particles were used to develop an optimized retinol formulation. As a new approach, the old concept of the core-shell SLN particles was combined with the novel smartLipids® technology as new stabilization model. Particles were produced by hot high pressure homogenization, loaded with increasing amounts of retinol (5%, 15%, 20%), and both the physical (size, crystallinity) and chemical stability were monitored. According to the core-shell model, the retinol precipitates first, forming a core. Then, in the final solidification stage of the particles the retinol core gets surrounded by a shell of lipid-retinol eutectic mixture. With increasing retinol content, more retinol precipitates in the core and is chemically protected. The model was confirmed by the stability data obtained, e.g. with 5%, 15% and 20% retinol loading, after 60 days of storage 37%, 59% and 75% of retinol remained in the particle suspensions. Thus, chemical stability increased with loading. Size remained unchanged at about 200 nm. Crystallinity showed absence of polymorphic transitions, which can cause expulsion of active from the particle matrix, leading to degradation. After incorporation of the particles into a gel as dermal formulation, similar stability was observed. The developed concept can be transferred to other chemically labile dermal actives, in cosmetics and pharma.

1. Introduction

In 1991, solid lipid nanoparticles (SLN®) (Lucks and Müller 1991) were developed as a novel drug delivery system followed by a second generation of lipid nanoparticles, nanostructured lipid carriers (NLC®) (Müller et al. 2002). These lipid nanoparticles, composed of biocompatible and biodegradable lipids, possess low cytotoxicity. In the last two decades, low systemic toxicity has been of increasing interest in parenteral (Chinsriwongkul et al. 2012), oral (He et al. 2015), rectal (Din et al. 2015), ophthalmic (Pignatello et al. 2015) and dermal (Ghanbarzadeh et al. 2015) applications of lipid nanoparticles. Because they are nanosized, they provide maximum skin adhesion and occlusion by forming an invisible patch on the skin, which increases skin hydration and consequently drug penetration.

Recently, smartLipids® particles were developed as the third generation of lipid nanoparticles after SLN® and NLC®. SLN® typically produced from one solid lipid only tend to form a relatively ordered crystalline structure with little imperfections. This is limiting the loading capacity, and during storage and formation of more highly ordered β modification, drug expulsion can occur. To increase the loading, NLC® were developed being a mixture of typically 1 solid lipid and 1 liquid lipid (oil). Due to the higher solubility of drugs in oils, the overall loading in this mixture of solid and liquid lipid was increased. In the next step were smartLipid®s developed. Their particle matrix consists of up to 10 different lipids, forming a highly chaotic structure with many imperfections and thus much higher drug loading. Due to the very different spatial forms of the various lipid molecules, polymorphic transitions during storage are minimized or can be completely avoided (Ruick 2016). The smartLipids® are an improved carrier system when higher drug loadings and firm inclusion of the drug or cosmetic are required, as in the case of retinol. Higher drug loading is desirable to minimize the amount of particles to be admixed to gels or creams, to not affecting negatively the rheological properties and the skin feeling. Firm inclusion in the particle matrix is required to protect the chemically labile retinol.

In addition, the smartLipids® particles possess also the general properties of lipid nanoparticles, being advantageous for dermal application. These are e.g. formation of lipid film on skin, leading to occlusion and related enhanced skin penetration of incorporated actives, and controlled drug release minimizing skin irritation – all also being relevant for retinol.

Skin aging is induced by several factors including genetics, hormonal alterations, and UV radiation (Rittie and Fisher 2002). A combination of all these factors impacts skin appearance and function. A potential skin-aging treatment was found in all-trans-retinol by improving the mRNA and protein expression of CRABP II (cellular retinoic acid-binding protein) on normal human skin (Pallet et al. 1997). This observation was confirmed by another group (Kang et al. 2005), making retinol of much interest to dermal cosmetic and pharmaceutical studies. Nowadays, products with a retinol concentration of, in many cases, 0.05% are on the market. All-trans-retinol consists of a cyclohexenyl ring, a chain with four trans double bonds, and an alcohol group. The alcohol group in all-trans-retinol is easy to oxidize to carboxylic acid and makes retinol susceptible to degradation. Furthermore, pure retinol degrades within 2 days at 4°C and a pH of 7.0 (Lee et al. 2002), indicating that pure retinol is extremely unstable and challenging to formulate for cosmetic and pharmaceutical applications. Therefore, the type of delivery system used for retinol application plays a vital role in ensuring its chemical stability and efficacy.

Solid lipid nanoparticles have already been used for retinol encapsulation in 2000 (Jenning et al. 2000). Apart from a protective solid particle matrix, lipid nanoparticles have been shown to reflect UV radiation and thus protect encapsulated labile actives (Wissing and Müller 2003). Therefore, the stability of 5% (referring to lipid particle phase) retinol-loaded SLN and NLC particles was tested. After 1 week of storage at room temperature, the stability was

distinctly superior to solutions, but still 40% of the retinol in the nanoparticles had degraded (Jenning and Gohla 2001).
The objective of this study was to produce superior retinol-loaded particles by using the third generation technology smartLipids®, i.e. achieving a higher loading of the particle matrix (>> 5%) and improving distinctly the chemical stabilization by exploiting their firm inclusion properties. The chemical stability should also be investigated as function of loading with retinol. Stability of the produced particles was tested in a final dermal gel formulation, being the relevant parameter for potential use as dermal product. Chemical stabilizers such as antioxidants were not used to assess the pure stabilizing effect of the especially structured particle matrix.

2. Investigations, results and discussion

2.1. Production of retinol-loaded smartLipids® particles

2.1.1. Rational for selecting the lipid composition

The smartLipids® particles are described as a mixture of typically 5 to 10 different lipids, either all being solid or in mixture with liquid lipids (oils) (Müller et al. 2014a). To form a less ordered, complex and more imperfect lipid matrix for the retinol-loaded particles, five solid lipids with varying alkyl chain lengths (C16-C22) and a liquid lipid (C8-C12) were selected. It should be noted that the lipids used were mixtures of mono-, di- and triglycerides of the respective fatty acids, which even increases the polydispersity of this lipid mixture.
Compritol® 888 ATO is a conventional lipid used previously for stable lipid nanoparticles and has already been used in retinol-loaded SLN particles (Barthelemy et al. 1999; Roberts et al. 2015). It is a triglyceride composed of C22 hydrocarbons. Miglyol® 812 is a liquid lipid with C8-C12 alkyl chains. To fill the "gap" between C8-C12 and C22, the other 4 lipids with the carbon atom number of alkyl chains in between were selected for the lipid mixture (Dynasan 116® and 118, Precirol® ATO 5, Imwitor® 491).
The solubility of solid lipids for cosmetic actives and drugs is generally lower than of liquid lipids (oils). Thus oils are admixed to the solid lipid mixture to increase the loading capacity. In ideal lipid mixtures the solubility is additive according to the mole fraction of the respective lipids. It can be calculated based on solubility in single compounds and the mole fraction. In real mixtures the obtained solubility can show negative deviation (lower solubility) or a positive deviation (higher solubility than in ideal mixture), the latter being ideal for maximum loading. Preferentially an oil should be used which shows good solubility properties for the active to be incorporated. Miglyol® 812 has a solubility for retinol at room temperature of 26%. Thus it has the potential to increase the incorporation of retinol in the lipid mixture for particle production, that means achieving the envisaged 20% loading.

2.1.2. Stabilizer screening

The basic composition of the three particle formulations with increasing retinol content is given in Table 1. Retinol was added to the lipid mixture as retinol 50C from BASF, which is composed of 50% retinol dissolved in 50% Tween 20. That means by adding this product, the particle will contain a certain amount of Tween 20 automatically. This percentage of Tween 20 is also shown in Table 1 (left column, in brackets).

Table 1: Composition of retinol formulations with 5% to 20% retinol content.

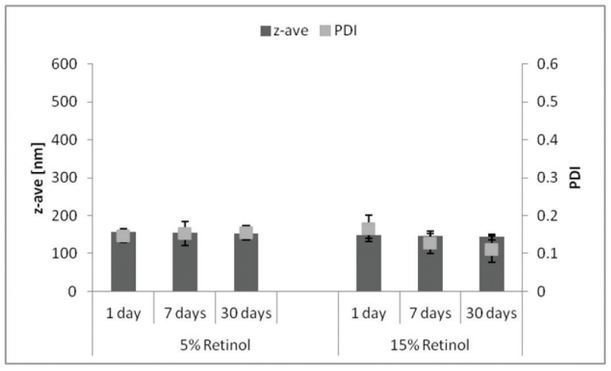
A. The composition of lipid mixture used for smartLipids® production

	solid lipids					liquid lipid
	Dynasan® 116	Dynasan® 118	Compritol® 888 ATO	Precirol® ATO 5	Imwitor® 491	Miglyol® 812
percentage [%] (w/w)	15	15	15	15	15	25

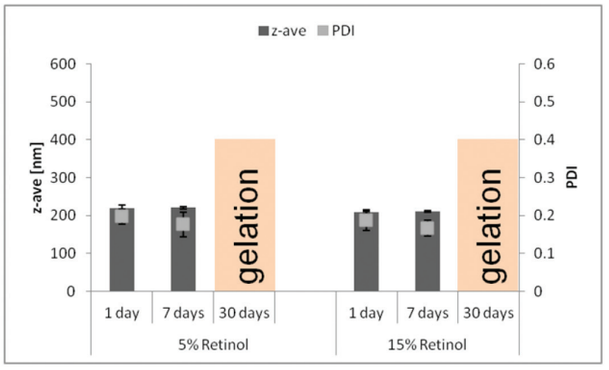
B. The composition of smartLipids® suspensions

	lipid phase		liquid phase	
	retinol (Tween 20)	lipid mixture	additional Tween 20	water
percentage [%] (w/w)	0.5 (0.5)	9.5	1.5	88
	1.5 (1.5)	8.5	0.5	88
	2.0 (2.0)	8.0	0	88

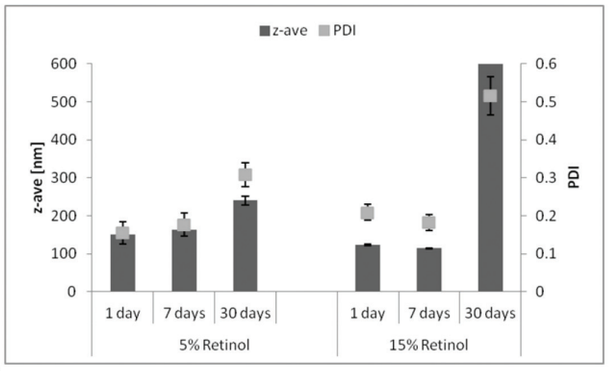
The percentage of Tween 20 results from the admixture of retinol in the form of retinol 50C from BASF, being a 50:50 solution of retinol and Tween 20.



A Tween 20



B Tween 80



C Poloxamer 407

Fig. 1: PCS diameters (z-ave: z-average diameter) and polydispersity indices (PDI) of smartLipids® particles produced with a composition of 10% lipid mixture, 5% and 15% Retinol 50C, 2% stabilizer (A: Tween 20, B: Tween 80, C: Poloxamer 407) and water. The formulations were stored at room temperature (RT) over a period of 30 days.

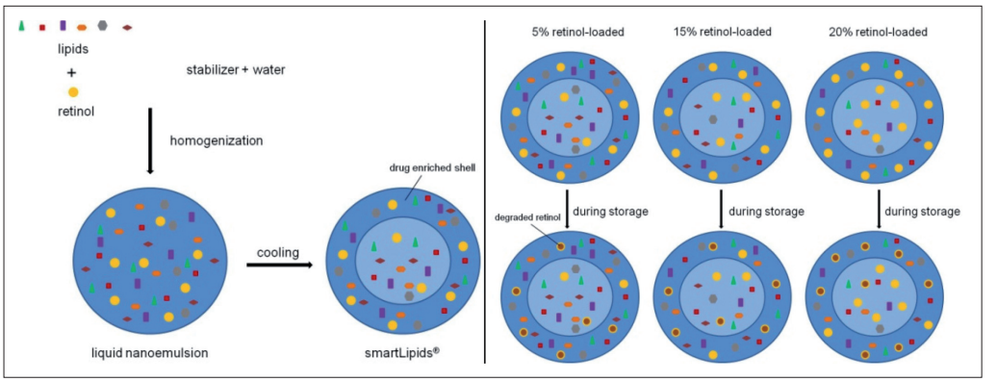


Fig. 2: Production and solidification process of smartLipids®: After homogenization the particles are liquid (liquid nanoemulsion), followed by forming a particle with enriched retinol core and outer shell (left). The distribution of retinol in smartLipids® particle matrix as function of retinol loading is shown on the right (for explanation cf. text).

Due to the fact that Tween 20 was contained anyway from the retinol addition, two Tween surfactants (20 and 80) were included in the stabilizer screening. Both stabilizers are very well tolerated by the skin, Tween 80 is even accepted for i.v. injection. A 2% (w/w) concentration of either Tween 20, Tween 80, Poloxamer 188, Poloxamer 407, Plantacare 818 UP or Plantacare 2000 UP were investigated for smartLipids® particle stabilization. The other stabilizers were selected based on previous experiences, when they lead to physically stable lipid particle suspensions (Dolatabadi et al. 2014; Luo et al. 2015; Ruick 2016).

Surprisingly, retinol-loaded smartLipids® particle dispersions stabilized by Poloxamer 188, Plantacare 818 UP and Plantacare 2000 UP congealed directly after production. This congealing phenomenon has been described previously, e.g. by Westesen and Bunjes (1995). Thus they had to be eliminated from the stabilizer list.

Fig. 1 shows the PCS diameters and polydispersity indices (PDI) of smartLipids® particles stabilized with Tween 20, Tween 80 and Poloxamer 407 over 30 days of storage. The mean particle size of all smartLipids® particles (5% and 15% retinol) stabilized with Tween 20 did not change significantly (150 nm, PCS z-average) and the PDI was less than 0.2 after 30 days of storage at RT (Fig. 1A). In contrast, the 5% and 15% retinol-loaded smartLipids® particles stabilized by Tween 80 remained practically unchanged both in PCS diameter (220 nm) and PDI (0.2) only during the first 7 days of storage. Then larger particles or lipid aggregates showed up and gelation was observed at day 30 (Fig. 1B).

For smartLipids® particles stabilized with Poloxamer 407, the 5% retinol-loaded smartLipids® particles showed a slight increase in z-ave and PDI during the 30 day storage time, from 150 to 200 nm and 0.16 to 0.30, respectively (Fig. 1C, left). The 15% retinol-loaded smartLipids® particles showed a pronounced increase in PCS size from 120 nm to more than 600 nm and in PDI from 0.2 to 0.5 over 30 days of storage (Fig. 1C, right). This increase in size was slighter than observed with Poloxamer 188. This might might be due to the higher sterically stabilizing capacity of Poloxamer 407 due to higher PEG content and larger molecular weight (molecular weight about 12,000 D). Based on the very good short term stability observed, Tween 20 was used as stabilizer in the further development of the retinol loaded particles, i.e. increasing the loading to 20% (Table 1B, lower line).

Regarding physical stability it should be kept in mind, when incorporating the particles into a hydroxypropyl cellulose (HPC) gel, the polymer network might further increase long-term stability. Increased physical stabilization of lipid nanoparticles in gel versus aqueous particle suspensions was reported previously (Wissing et al. 2000).

2.1.3. Theoretical model for chemical stabilization of retinol

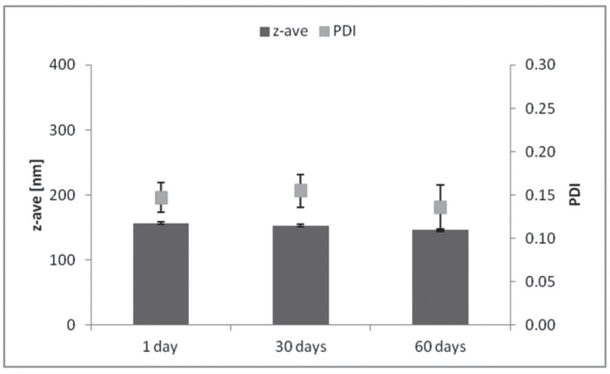
In this study smartLipids® particles were used to increase firstly the retinol-loading compared to SLN and NLC (e.g. previously reported 1% and 5%, resp.) (Jenning 1999). Secondly it was expected that the more complex lipid mixture with many imperfections would more firmly enclose the retinol and provide higher chemical stability as it has been proven in the case of the particles by Jenning and Gohla – NLC versus simple SLN (Jenning 1999). Long-term stability should additionally be higher because – in contrast to SLN and NLC – the smartLipids® show no or very little polymorphic transform during storage (Müller et al. 2014b; Ruick 2016). Polymorphic transform to more ordered beta modification leads to drug expulsion from the particle, which then can be degraded in the water phase.

Determination of solubility in the lipid mixtures showed that 20% retinol were soluble in the lipid melt at 70 °C. Cooling to room temperature after particle production will of course reduce the solubility of retinol in the mixture. According to the TX diagrams, this leads to precipitating of the retinol first, forming a retinol core, followed by solidification of a lipid retinol mixture forming the particle shell, as described by zur Mühlen et al. (1998). Particles of the core shell type are formed with enriched drug/retinol core (Fig. 2, left). The location inside the particle core should increase protection and chemical stability.

When increasing the retinol percentage in the lipid mixture from 5% to 15% and finally 20% will lead to an increased precipitated amount of retinol before the shell forms, that means the relative amount of retinol in the core increases with increasing retinol concentration (Fig. 2, right, upper). The relative distribution of active between shell localization and inside particle matrix/core is shifted to the inside. The retinol in the core is much better protected, thus relatively less of the loaded retinol should degrade. During storage mainly the retinol in the shell degrades, but the retinol in the core is less affected (Fig. 2, right, lower). This study should show, if the theoretical considerations and the developed model are correct.

2.2. Medium term stability of retinol-loaded smartLipids® particles: size and zeta potential

In the stabilizer screening, both 5% and 15% retinol-loaded smartLipids® particles stabilized by Tween 20 remained practically unchanged after 30 days of storage. This indicated that smartLipids® particles were able to incorporate a higher percentage of retinol without destabilizing the suspension as it was the case with Poloxamer and Plantacare containing formulations. Thus Tween 20-stabilized smartLipids® particles loaded with 20% retinol were produced and investigated regarding their medium-term physical and chemical stability, in addition to the 5% and 15% formulations. All three retinol-loaded smartLipids® particle dispersions (5%, 15% and 20%) were stored at room temperature for 60 days and their mean particle size (z-ave) and polydispersity index (PDI) as measure for the width of distribution were determined (Fig. 3). During the observation period both the z-ave and PDI values showed no pronounced alterations for all formulations. For 5% and 15% retinol-loaded smartLipids® particles, the mean particle sizes and PDI after 60 days remained practically unchanged (z-ave



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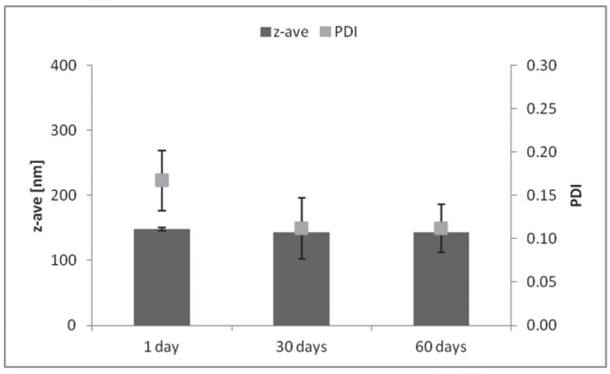

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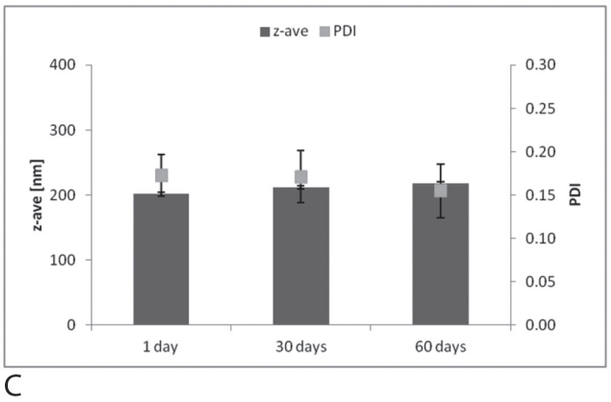

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Fig. 3: PCS diameters (z-ave: z-average diameter) and polydispersity indices (PDI) of smartLipids® particles with 5% (A), 15% (B), and 20% (C) retinol and stabilized by 2% Tween 20 over a period of 60 days.

approximately 150 nm, PDI below 0.2) (Fig. 3A, 3B). smartLipids® particles loaded with 20% retinol also showed no pronounced increase in particle size and PDI.

Table 2: Zeta potentials measured in conductivity water (adjusted to 50 µS/cm, pH 5.5) and in original medium

	conductivity water [mV]		original medium [mV]	
	1 day	60 days	1 day	60 days
5% Retinol	-17.9±1.3	-17.4±1.3	-6.8±0.5	-6.2±0.4
15% Retinol	-15.4±1.1	-15.7±1.2	-6.9±0.5	-6.9±0.4
20% Retinol	-16.1±1.2	-16.8±1.2	-7.5±0.6	-8.0±0.7

The zeta potentials were measured in the original dispersion medium (aqueous phase with the respective surfactant) and in "conductivity water", i.e. water adjusted to 50 µS/cm using a 0.9% (w/w) NaCl solution, pH of 5.5. The zeta potential can be used to predict the stability of nanoparticle suspensions. The zeta potential in conductivity water represents the Stern potential (indirect measure of repulsive surface charge, Nernst potential) and the zeta potential in the original dispersion medium reflects the thickness of the stabilizing diffuse layer. The zeta potentials for the three smartLipids® particle suspensions in conductivity water were just below -20 mV, and did not change over time (Table 2). This indicates a relatively low surface charge, when considering the zeta potential range from zero to a maximum of about 100 mV. The measured zeta potentials (Stern potentials) were practically identical for all three formulations – independent of the retinol loading. This is an indication that the surface is similarily composed – i.e. the particle shell consist of an identical mixture lipid to retinol. The increasing amount of retinol from 5% to 20% loaded particles is obviously located in the particle core – in agreement with the theory.

A stability determining factor is the zeta potential in the original dispersion medium, which is of course also related to the Nernst potential (the higher the Nernst potential, the higher the measured zeta potential). In their original dispersion medium, the zeta potentials of all smartLipids® dispersions were below -10 mV with practically no change over 60 days of storage (Table 2).

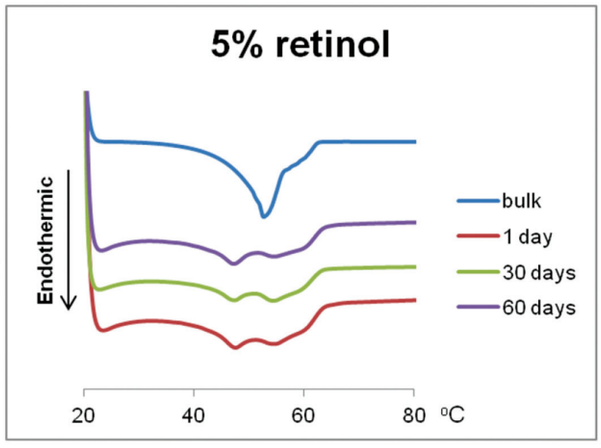
In general, a zeta potential greater than |30| mV is considered a stable suspension, above which a good physical stability can be predicted in case of electrostatic stabilization (Müller et al. 1996). This is valid for most low molecular weight and electrostatic surfactants, but not for high molecular weight, steric stabilizers (Kovacevic et al. 2014). The reason that the retinol-loaded smartLipids® dispersions remained stable can be that Tween 20 acts as a steric stabilizer in the system (Xin et al. 2013). In the original medium, the nonionic steric stabilizer adsorbs onto the particle surface forming a sterically stabilizing layer, this firmly adsorbed layer shifts the plane of shear during the zeta potential measurement and decreases the measured zeta potential value. The thicker this layer is, the lower is the zeta potential measured. The low zeta potentials measured prove the presence of this layer. Lipid nanoparticles with a zeta potential of below |20| mV were found to be physically stable in the case of steric stabilization. The tendency of particle aggregation increases with a decrease in the zeta potential value (Choi et al. 2014), which is not the case when an efficient steric stabilizer is used – as in this case Tween 20. The results (strong decrease in measured zeta potential) confirm that Tween 20 is adsorbed in a thick layer and has thus good stabilization efficacy for retinol-loaded smartLipids® particles. A recent study also confirmed that lipid nanoparticles even with low zeta potentials possessed high physical stabilities (Al Shaal et al. 2014).

2.3. Crystalline properties of matrix

Table 3: Differential Scanning Calorimetry (DSC) parameters of retinol-loaded smartLipids® particles over a period of 60 days

		onset temperature [°C]	enthalpy [J/g]	RI [%]
5% retinol	1 day	42.53	107.02	74.6
	30 days	41.46	107.80	74.6
	60 days	42.28	111.66	77.8
15% retinol	1 day	45.04	107.04	70.8
	30 days	44.84	106.45	70.2
	60 days	46.17	110.16	72.6
20% retinol	1 day	39.37	112.90	72.4
	30 days	39.32	112.22	71.9
	60 days	38.90	114.59	73.5

The melting characteristics and relative crystallization (crystallization index) of retinol-loaded smartLipids® particles over short-term storage were determined by DSC (Fig. 4 and Table 3). Figure 4 shows the DSC curves of retinol-loaded smartLipids® particle dispersions recorded from 20 °C to 80 °C at a heating rate of 5 K/min over a period of 60 days. Table 3 depicts the DSC parameters and recrystallization indices (RI) of smartLipids® particle dispersions. The DSC thermal behavior of the lipid mixture (bulk) was chosen as a reference. The heating curves of the bulk showed



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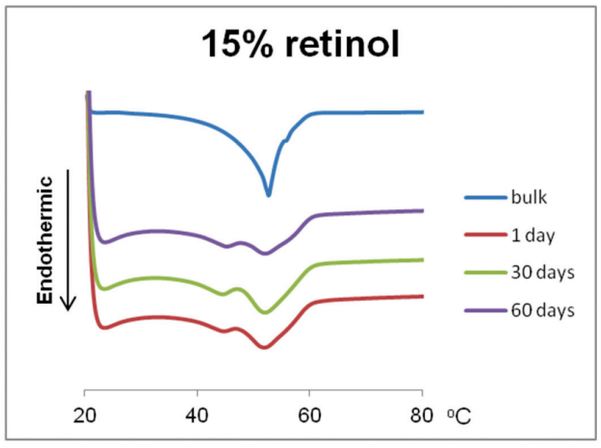

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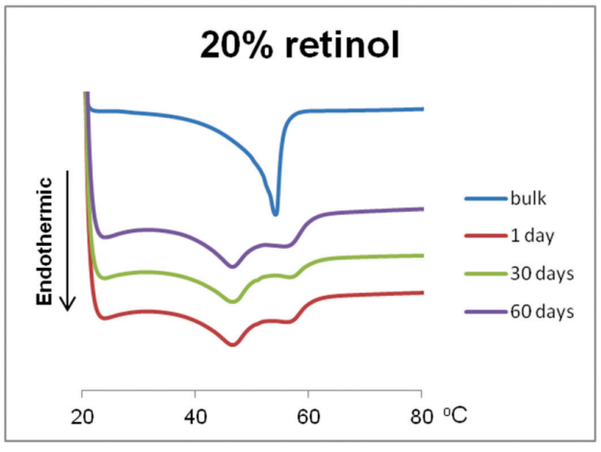

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Fig. 4. Differential Scanning Calorimetry (DSC) thermograms of 5% (A), 15% (B) and 20% (C) retinol-loaded smartLipids® particle dispersions stabilized with 2% Tween 20 over a period of 60 days.

different peaks in 5%, 15% and 20% retinol-loaded nanoparticles at 48.52 °C, 49.82 °C and 50.58 °C, respectively. This small difference occurred because the bulk lipids were mixed with different percentages of retinol (Fig. 4).

The thermograms in Fig. 4 show that the melting behavior of retinol-loaded smartLipids® particles differed distinctly from the bulk lipids. In the thermograms of retinol-loaded smartLipids® particles, the DSC heating peaks were broadened and the minimum melting temperatures were decreased. This phenomenon is common and can be attributed to the high surface area caused by forming nanoparticles, which prohibits the process of recrystallization of the lipid resulting in a lipid matrix in a metastable polymorph state (Westesen and Bunjes 1995). In addition, the broadened heating peak and the decreased melting point can be ascribed to an increased number of lattice defects (Westesen et al. 1993) and the interaction of the stabilizer with lipid molecules at the interface (Bunjes et al. 2000). The reduction in the melting points of lipid nanoparticle dispersions was reported recently in another study (Tronino et al. 2016). Based on the DSC thermograms, two endothermic melting events occurred in each retinol-loaded smartLipids® dispersion. The heating process detected the polymorphism of the lipids in the nanoparticles. According to the literature, the melting peak with lower temperature can be attributed to the α modification form, whereas the second corresponds to the β modification form for triacylglycerols, both α- and β- are conventional forms in lipid nanoparticles (Teeranachaideekul et al. 2008, 2007).

In DSC thermograms, the onset temperatures of the melting peaks was in the 38.9-46.2 °C range. This is relevant for dermal applications since human skin temperature is 32 °C and drug loaded particles need to stay solid. The melting enthalpies of all retinol-loaded smartLipids® particles were relatively lower than that of the bulk lipid. As expected, during 60 days of storage, the shape of the heating curves for each formulation remained unchanged, indicating a stable lipid matrix state of retinol-loaded smartLipids®. RI values were calculated from the melting enthalpies of the retinol-loaded smartLipids® particles against that of the bulk lipid (Table 3). No big differences were found between the enthalpy and RI values for each of the nanoparticle dispersions over the storage period. This is in agreement with the data from the melting curves, demonstrating that the internal structure of lipid nanoparticles is unchanged, the smartLipids® concepts worked in these formulations.

2.4. Chemical stability of retinol-loaded smartLipids® particles

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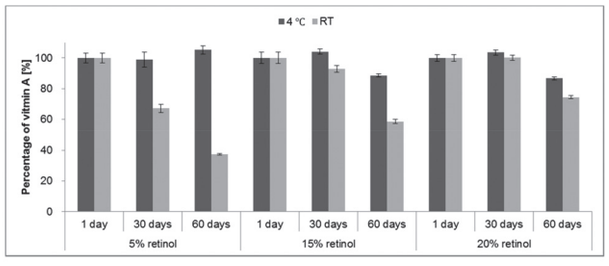

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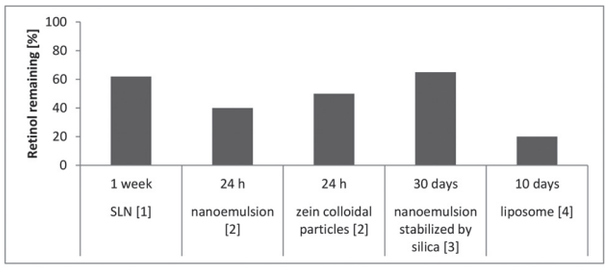

Fig. 5: Chemical stability of 5%, 15% and 20% retinol-loaded smartLipids® particles stabilized with 2% Tween 20 and stored at 4 °C and RT over a period of 60 days (A). Short-time stability of retinol in different kinds of retinol-loaded nanoparticles stored at RT. Data obtained from several other research groups ([1] (Jenning and Gohla 2001), [2] (Pan et al. 2015), [3] (Zhao et al. 2015), [4] (Ko and Lee 2010)) (B).

*indicates that data was taken from the literature.

In general, retinol is highly susceptible to degradation induced by light, heat, and oxygen exposure during storage. Therefore, maintaining the chemical stability of retinol over a period of time is a significant challenge. In this study, the chemical stability of retinol-loaded smartLipids® particles was investigated by measuring the content of intact retinol within the lipids using HPLC.

Figure 5 depicts the stability of encapsulated retinol in smartLipids® particles at 4 °C and RT over a period of 60 days, compared with several different forms of retinol-loaded nanoparticles published by other research groups in recent years. The encapsulated retinol degraded gradually during storage (Fig. 5A). At a storage temperature of 4 °C the 5%, 15% and 20% retinol-loaded smartLipids® dispersions degraded only slightly. The retinol content of the 5% retinol-loaded dispersion stored at 4 °C changed inconspicuously. The 15% and 20% retinol-loaded smartLipids® dispersions contained 89% and 87% of the original amount of retinol after 60 days at 4 °C, respectively.

Highly interesting, whereas at 4 °C the retinol content showed little change with increasing retinol loading, at room temperature the remaining retinol content increased with retinol loading from 5% via 15% to 20%. After 60 days at room temperature, the 5% retinol-loaded smartLipids® particle dispersion had 37% of the original retinol left, whereas the 15% and 20% retinol-loaded smartLipids® dispersions had 59% and 75% of the original retinol left, respectively.

As outlined above, with increasing retinol content, more retinol is located (and protected) inside the particle matrix. The specific surface area of the particles only increased a little with increasing loading. The surface area was similar, as can be seen for the PCS diameter being 157 nm, 148 nm, 201 nm for the 5%, 15% and 20% loaded particles, respectively. That means after maximum location of retinol in the surface layer (a preferential location of retinol already described by Jenning and Gohla (Jenning and Gohla 2001)), additional retinol is forced to locate inside the particle core. This relative amount is less susceptible to degradation, Fig. 2 (right) shows the principle mechanism, the theoretical model developed was confirmed by the HPLC data.

The relatively similar degradation at 4 °C can be explained by different degradation mechanisms. At lower temperature, primarily the surface located retinol degrades, whereas the retinol in the matrix shows very little degradation. The retinol amount in the outer particle shell is relatively similar, thus leading to similar degradation.

Figure 5B shows the chemical stability of different forms of retinol-loaded nanoparticles from other research groups in recent years. The degradation of 5% retinol-loaded SLN lipid nanoparticles was 38.0% within the first week (1st column in Fig. 5B) (Jenning and Gohla 2001). Based on the data from this study, an equation describing the relationship between retinol degradation and lipid particle loading was described, showing that the retinol degradation increased with the amount of retinol incorporated into the nanoparticles. This was explained by the expulsion of retinol from the particles (exceeding of loading capacity in more ordered matrix). This result is in contrast to smartLipids® particles, where 20% retinol-loaded particles retained the highest stability of retinol in the lipid particle core. This is well in agreement of the firmer incorporation of actives in the chaotic, highly unordered lipid matrix of smartLipids® (Müller et al. 2014a).

For an oil-in-water nanoemulsion system (2nd column in Fig. 5 B), the content of retinol after 24 hours was 40% (Pan et al. 2015). Furthermore, a novel nanoemulsion stabilized by silica was produced in order to improve the stability of retinol (Zhao et al. 2015). This formulation was effective and increased the percentage of retinol remaining in the nanoemulsion after 30 days of storage to around 65% (4th column in Fig. 5B). Another study prepared zein colloidal particles to encapsulate retinol (Pan et al. 2015). However, 50% of the retinol in these particles was degraded after 24 h at room temperature in this system (3rd column in Fig. 5B), indicating that zein colloidal particles are ineffective in decreasing the degradation of encapsulated retinol. In addition, the incorporation of retinol into liposomes was also performed (5th column of Fig. 5B) (Ko and Lee 2010). Approximately 20% of the initial retinol remained in the liposomes after 10 days of storage at room temperature. Liposomes were able, to some extent, to delay the degradation of retinol. However, retinol-loaded smartLipids® particles, especially those loaded with 20% retinol, demonstrated an improved capacity for enhancing the stability of retinol by preserving 75% of the initial retinol for 60 days.

2.5. Stability of a smartLipids® in particle-loaded hydrogel

A pre-requisite for the use of smartLipids® as carrier system for dermal application is the physical stability of the particles and the chemical stability of retinol in dermal formulations. The formulation with 20% retinol-loaded smartLipids® particles demonstrated highest drug capacity and chemical stability. Thus the 20% retinol-loaded smartLipids® particle suspension was incorporated into a HPC hydrogel to yield a dermal delivery system with a final retinol concentration of 0.05% (typical concentration in some products). A gel was preferred to a cream to avoid redistribution of the retinol to the oil droplets in which degradation takes place faster. Despite that this redistribution is extremely slow (very little effect on chemical stability), a gel is the optimal dermal formulation for retinol lipid nanoparticles.

Figure 6 shows the even distribution of smartLipids® particles and the absence of microscopically visible aggregates in a suspension-loaded hydrogel during 60 days of storage at room temperature. No particle size increases or obvious particle aggregations were observed over time. The incorporated nanoparticles showed a stable size distribution that was in concordance with the particle size analysis of the original retinol-loaded smartLipids® particle dispersion. This result confirmed that the semisolid gel base was able to retain the physical integrity of the incorporated smartLipids® nanoparticles.

Further, the chemical stability of the smartLipids® particle suspension-loaded hydrogel over 60 days storage time was also measured (Fig. 7). The degradation of retinol at 4 °C and RT concurred with the degradation of retinol in the original suspension. The percentage of retinol remaining in a smartLipids® particle suspension-loaded hydrogel at 4 °C at day 60 (88%) was practically identical to that of the original suspension (87%). However, the chemical stability of the hydrogel suspension stored at RT decreased slightly compared with the chemical stability of the original suspension (70% versus 74% in suspension). Possible explanation is a higher oxygen content of the water in the gel, which was not degassed by heating as in lipid nanoparticle production.

The DSC results proved the crystalline stability of the lipid matrix of the nanoparticles (no polymorphic transition) and the particle size distributions confirmed the physical stability of a smartLipids® particle-loaded hydrogel (data not shown). Therefore, a retinol-loaded smartLipids® particle hydrogel with both physical and chemical stability can be produced.

2.6. Conclusions

A theoretical model was developed that by increasing the loading of retinol, also the chemical stability can be increased. The model is based on the formation of a retinol enriched core. During cooling of the produced hot nanoemulsion, the retinol precipitates first, and then this core is being surrounded by a shell of an eutectic lipid-retinol mixture. With increasing retinol content, more retinol precipitates in the core and thus is more chemically protected. This principle can be exploited for other chemically labile drugs and cosmetic actives.

The concept of the chaotic smartLipids® worked efficiently for retinol, as seen from the DSC data. In short-term storage no polymorphic changes occurred, which supports the firm inclusion inside the particle matrix. Thus, ideal carriers for labile actives are lipid particles which are a) highly loaded and b) consist of a complex, chaotic lipid mixture. The stabilization also works in dermal formulations, as shown for gels.

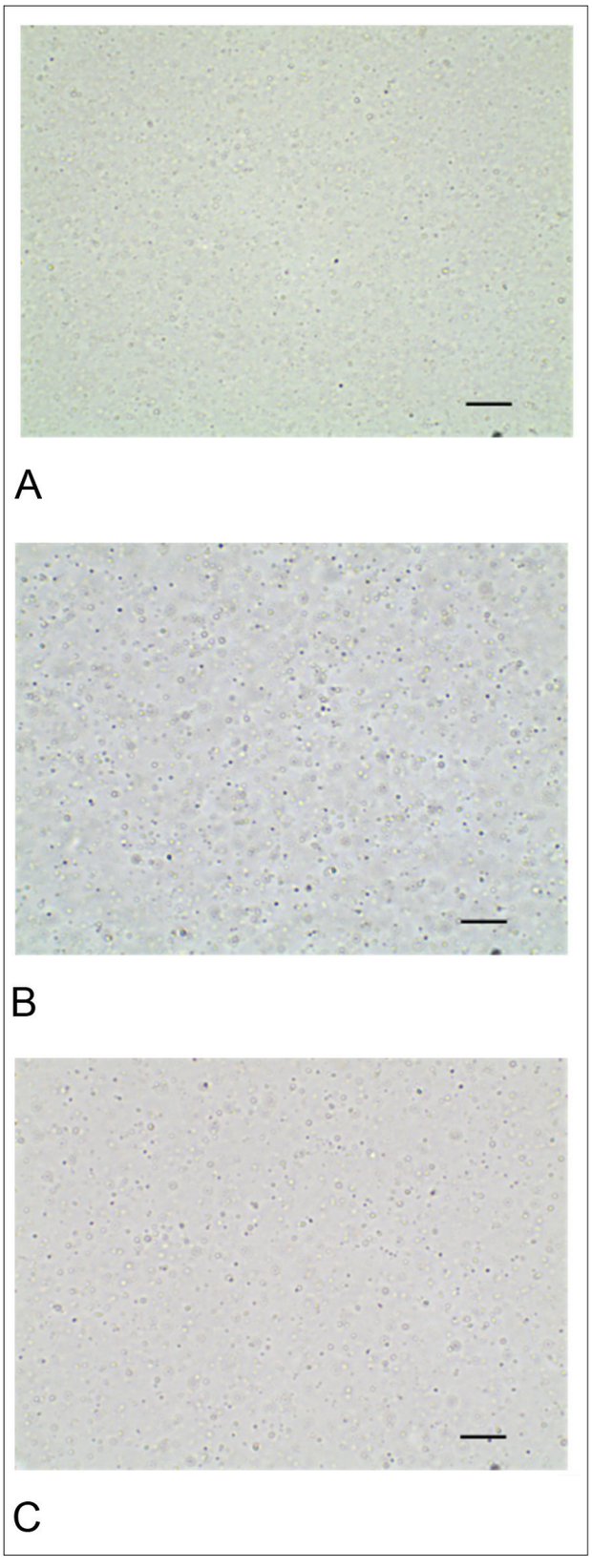

Fig. 6: Light microscopy (LM) of 20% retinol-loaded smartLipids® particles incorporated into an HPC gel on day 1 (A), 30 (B), and 60 (C) after preparation at 1000-fold magnification with a 10 µm scale bar.

Further increase in stability can be achieved by using optimized excipients (e.g. oxygen-free water, production under nitrogen, addition of anti-oxidants, optimal packaging etc.). This makes cosmetic long-term stable retinol products feasible without inconvenient storage in the fridge, and the principle can be transferred to dermal pharmaceuticals.

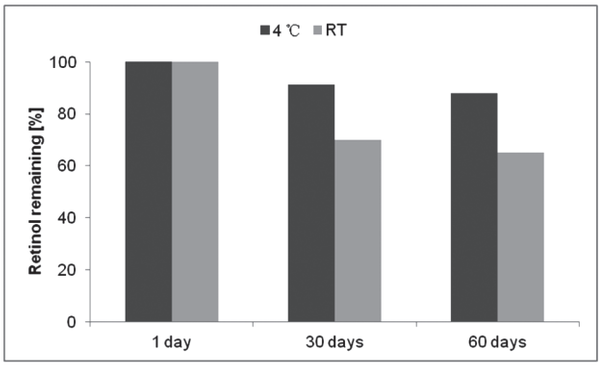

Fig. 7: Chemical stability of 20% retinol-loaded smartLipids® particles incorporated into an HPC gel on day 1, 30, and 60 at 4 °C and RT over a period of 60 days.

3. Experimental

3.1. Materials

As solid lipids, Dynasan® 116, Dynasan® 118, and Imwitor® 491 were donated from CREMER OLEO GmbH & Co. KG (Hamburg, Germany), and Compritol® 888 ATO and Precirol® ATO 5 as gifts from Gattefossé (Bad Krozingen, Germany). The liquid lipid, Miglyol® 812, was bought from Caesar & Loretz GmbH (Hilden, Germany). Tween 20 (AppliChem, Darmstadt, Germany), Poloxamer 407 (BASF, Ludwigshafen, Germany) and Tween 80 (AMRESCO, Solon, USA) were purchased and used as surfactants. Retinol 50C was a gift from BASF (Burgbernheim, Germany). Hydroxypropyl cellulose (HPC, 70 kD) was obtained from Caesar & Loretz GmbH (Hilden, Germany). Milli-Q water, purified by an ultrapure water system (Millipore GmbH, Darmstadt, Germany) was used as a dispersion medium.

3.2. Methods

3.2.1. Production of retinol smartLipids®

smartLipids® particles were produced by using a hot high pressure homogenization (Müller et al. 2000). Briefly, the solid and liquid lipids were mixed and melted at 85 °C and retinol was added (Table 1 A). The hot lipid mixture was then dispersed into a stabilizer solution at a 2% (w/w) final concentration (Table 1 B) and dispersed using an Ultra-Turrax (Janke & Kunkel GmbH, Germany) set to 8000 rpm for 30 s to yield a pre-suspension and then followed by 3 cycles of high pressure homogenization at 500 bars in 85 °C (HPH, Micron LAB 40, APV Deutschland GmbH, Germany). After homogenization, the produced hot nanosuspensions were cooled at room temperature.

3.2.2. Preparation of a smartLipids®-loaded hydrogel

The 20% (referring to lipid phase) retinol-loaded smartLipids® particles and extra water were mixed into 5.5% HPC gel to yield a formulation with a final retinol concentration of 0.05% and 5% HPC gel for dermal application. A 5.5% (w/w) hydrogel was prepared by dispersing HPC powder evenly on the surface of double distilled water, tightly sealing the mixture to prevent evaporation and storing it at 4 °C overnight. The smartLipids® particle suspension, water and hydrogel were mixed in a mortar and triturated thoroughly to yield a gel base with a final retinol and HPC concentration of 0.05% and 5%, respectively.

3.3. Characterization of smartLipids® particles

3.3.1. Particle size analysis

The size of the smartLipids® particles was determined by photon correlation spectroscopy (PCS, Zetasizer Nano ZS, Malvern Instruments, UK). PCS gives the hydrodynamic diameter (z-average, z-ave) of nanoparticles and the polydispersity index (PDI) as measure for the width of the particle size distribution. All samples were diluted with double distilled water and measured 10 times, a mean was calculated.

3.3.2. Zeta potential (ZP)

The zeta potential was measured with a Zetasizer Nano ZS (Malvern Instruments, UK) in conductivity adjusted water (double distilled water adjusted to 50 µS/cm with NaCl solution/conductivity at pH 5.5) and original dispersion medium (solution with the same concentration of surfactant as lipid particle suspension). The electrophoretic mobility conversion into the zeta potential was achieved using the Helmholtz–Smoluchowski equation.

3.3.3. Differential Scanning Calorimetry (DSC) measurements

The particle degree of crystallinity was analyzed by differential scanning calorimetry (DSC, Mettler Toledo, Germany). The smartLipids® particle suspension (approxi-

mately 1-2 mg of lipid) was weighed into a standard aluminum pan and was measured under nitrogen, with an empty pan as reference. The samples were heated from 20°C to 80°C applying a heating rate of 5 K/min and cooled down using liquid nitrogen. Furthermore, the recrystallization indices (RI), or the percentage of re-crystallized solid lipid related to the initial solid lipid concentration was calculated as follows (Freitas and Müller 1999):

$$RI[\%] = \frac{\Delta H_{\text{aqueous smartLipids}^{\circledR}}}{\Delta H_{\text{bulk material}} \times \text{Concentration}_{\text{lipid phase}}} \times 100$$

where $\Delta H_{\text{aqueous smartLipids®}}$ and $\Delta H_{\text{bulk material}}$ are the melting enthalpies (J/g) of the smartLipids® particles and bulk material, respectively. The concentration of the lipid phase for smartLipids® particles was 10%.

3.3.4. High performance liquid chromatography (HPLC) analysis

Retinol concentrations were determined by high performance liquid chromatography (HPLC) to investigate the chemical stability of retinol-loaded smartLipids® particles. For the HPLC measurement, a defined amount of sample was transferred into a glass vial. Acetone was used to dissolve the sample under ultra-sonic condition. Then, the clear solution was measured by HPLC to determine the amount of retinol.
All HPLC analysis were performed on a Kroma System 2000 version 1.7 (Kontron Instruments, Germany), a solvent delivery pump 420 (Kontron Instruments, Germany), an auto sampler model 360 (Kontron Instruments, Germany), and a UV detector model 430 (Kontron Instruments, Germany) measuring at 325 nm. The analytical column was a Lichrospher 60 RP select B (particle size 5 µm, 125 × 4 mm, Knauer, Germany) with a flow rate of 1.0 ml/min at 25 °C. The mobile phase consisted of acetonitrile and water in a ratio of 80:20 (v/v). The running time was 7 min and the retention time for retinol was 3.2 min.

3.3.5. Light microscopy

A light microscope equipped with a digital camera (Moticam 3.0 MP, Motic Deutschland GmbH, Germany) was used to analyze the particle size distribution of the smartLipids®-loaded hydrogel. Images were taken at a x1000 magnification in oil immersion to investigate the morphology of the smartLipids® particles after loading into the HPC hydrogel.

3.3.6. Stability testing

To analyze both the physical and chemical stability of retinol-loaded smartLipids®, each sample was stored at room temperature for 60 days. The particle size analysis, zeta potential and thermal property were measured at certain intervals to indicate the physical stability properties. The chemical stability of retinol-loaded smartLipids® was investigated by measuring the content of retinol at certain intervals with the help of HPLC. As for the physical stability of smartLipids®-loaded hydrogel, only light microscopy was used because the concentration of diluted smartLipids® by HPC gel was too small to be measured by PCS.

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