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Parenteral oil-based drospirenone microcrystal suspensions—Evaluation of physicochemical stability and influence of stabilising agents

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ABSTRACT

Drospirenone (DRSP) is a contraceptive drug substance with challenging physicochemical properties, due to insufficient solubility in aqueous and oil-based vehicles as well as low chemical stability in aqueous fluids. Although it is one of the most popular orally used progestins, no parenteral long-acting contraceptive containing the drug substance is marketed. An oil-based DRSP microcrystal suspension (MCS) might be an attractive formulation option. The main focus of this study was to investigate the physicochemical stability of such preparations. Moreover, syringeability and injectability via autoinjector were analysed using a materials testing machine. A high chemical stability of DRSP was found in oil-based vehicles. Span® 83, cholesteryl oleate, lecithin, methyl cholate, Aerosil® R972 and 200 Pharma were tested for increasing the physical stability of DRSP dispersions. Changes in viscosity, rheological properties, and solubility were analysed. The intention was to show a stabilising effect of the excipients without increasing viscosity and solubility. To evaluate the physical stability of DRSP MCS with and without addition of stabilising agents, sedimentation and particle growth after storage were examined. Especially, the silica derivatives Aerosil® 200 and R972 Pharma influenced the physical stability positively.

1. Introduction

Parenteral injectable drug delivery systems allow a prolonged release of drug substance and avoid the first-pass effect (Wang et al., 2005; Burgess et al., 2004). Popular examples are long-acting parenteral contraceptives. They are effective, generally discreet, and reduce daily compliance challenges (Linn, 2003). DRSP is a progestin which is known to be used in contraceptives and for treatment of diseases, disorders and symptoms associated with deficient endogenous levels of oestrogen in women. As analogue to spironolactone, this unique contraceptive exhibits both anti-mineralocorticoid and anti-androgenic activities (Fachinformation, 2010; Nippe and General, 2010). Besides oral formulations, no parenteral formulation containing DRSP is on the market.

Long-acting parenteral contraceptives are mostly prepared as aqueous MCSs administered intramuscularly (i.m.) (Depot Provera®) or subcutaneously (s.c.) (Depot-subq Provera®). Or they are manufactured as oil-based i.m. solutions (Mesigyna®) (d'Arcangues and Snow, 1999; Gupta, 2006). Typical, parenteral applied oil-based vehicles are medium chain triglycerides (MCT) (e.g. in Ciatyl-Z® Depot), sesame oil (e.g. in Lyogen® Depot) or castor oil with addition of benzyl benzoate (e.g. in Noristerat®) (Fachinformation, 2010). Due to the challenging physicochemical properties of DRSP, an oil-based MCS might be a simple and reasonable formulation option for a once-a-month injection.

An ideal drug suspension for parenteral application should show no particle growth after storage, low sedimentation of suspended particles and easy application of homogeneous dosages (Akers et al., 1987; Rutz, 2007). Due to its physical instability, the preparation of suspensions is challenging (Boylan and Nail, 2002; Rutz, 2007). Sedimentation could be an issue during manufacturing and filling processes and may lead to inhomogeneous content uniformity (Rungseevijitprapa et al., 2009; Wong et al., 2008). Furthermore, dispersed particles tend to crystal growth and aggregation during storage. However, the particle size of drug substance is an important factor influencing bioavailability, injectability and syringeability, product appearance as well as overall stability of i.m. or s.c. injectable suspensions (Nash, 2006). For aqueous systems, numerous approaches are known to improve physical stability (Bowman et al., 2005; Rungseevijitprapa et al., 2009; Swabrick et al., 2005). In contrast, oil-based systems have been less investigated. Not all techniques used to stabilise aqueous suspensions can be translated to oil-based formulations, due to differences in properties of vehicle and in drug-vehicle-interaction. Typically, surface-active additives were added decreasing the interfacial tension between drug substance and surrounding liquid phase (Tadros, 2005). Moreover, the addition of thickening agents should increase

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the viscosity of the external liquid phase, thus slowing down sedimentation and agglomeration of solid particles (Ali et al., 2010). However, the rise in viscosity is possible up to the limit of injectability through a standard needle in a reasonable timeframe (Burgess et al., 2004; Dexter and Schott, 1979). Furthermore, the solubility of drug substance in the oil-based vehicle was assumed to influence e.g. the release behaviour (Martinez et al., 2008).

Considering these issues, six excipients are tested. In the following, they are referred to as stabilising agents. An increase in viscosity and solubility by addition of the excipients should be avoided. Lecithin is included in pharmaceutical products as dispersing, emulsifying, and stabilising agent. It is contained in i.m. injections in a concentration of 0.3–2.3%. Sorbitan ester derivatives are used as wetting agent for insoluble drug substances in lipophilic bases in a concentration of 0.1–3% (Rowe et al., 2003). Further test substances are cholesteryl oleate, a cholesteryl fatty acid ester and methyl cholate, a cholic acid ester. Both have a sterol structure. The hydroxyl group at C-3 of cholesteryl ester derivative is associated with fatty acid. The carboxyl group of the cholic acid derivative is methylated. The excipients are assumed to interact with the suspended steroidal drug substance as well as with the external lipid phase. Colloidal silicon dioxide is added as suspending and thickening agent in a concentration of 2.0–10.0% and as emulsion stabiliser in a concentration of 1.0–5.0% (w/w) (Rowe et al., 2003). In high enough concentrations, e.g. methyl cholate, cholesteryl oleate, Aerosil® 200 Pharma, and Aerosil® R972 Pharma have a thickening effect on some lipophilic vehicle. Thus, they have to be used in low enough concentrations.

The aim was to evaluate the chemical and physical stability as well as the applicability as parenteral injection of oil-based DRSP MCS. Furthermore, a possible stabilising effect of the chosen excipients on oil-based DRSP MCS without increasing the viscosity or affecting the solubility of drug substance should be investigated.

2. Materials and methods

2.1. Materials

Drospirenone (micronised, Bayer Schering Pharma AG, Berlin, Germany), medium chain triglycerides (Myritol® 318 PH) (kindly provided by Cognis GmbH, Düsseldorf, Germany), castor oil (Riedel-de-Haen, Seelze, Germany), sesame oil (Fluka Chemie AG, Buchs, Switzerland), benzyl benzoate (Symrise GmbH & Co. KG, Holzminden, Germany), lecithin (L-α-Lecithin from soybean) (Calbiochem, Darmstadt, Germany), methyl cholate (Alfa Aesar, Karlsruhe, Germany), hydrophobic colloidal anhydrous silica (Aerosil® R972 Pharma), colloidal silicon dioxide (Aerosil® 200 Pharma) (both kindly provided by Degussa, Essen, Germany), cholesteryl oleate (Alfa Aesar, Karlsruhe, Germany), sorbitan sesquioleate (Span® 83, Sigma–Aldrich Chemie GmbH, Steinheim, Germany) were used.

2.2. Chemical stability of DRSP in oil and aqueous medium

70 mg/g of DRSP was added to MCT, sesame oil, or castor oil. The samples were mixed using a vortex mixer (Heidolph, REAK 2000, Heidolph Instruments GmbH, Schwabach, Germany). Thereafter, the suspensions were blended at 39 rpm for 24 h using a roller mixer (Britze, DA II). Afterwards, the samples were stored tightly closed in wide-neck brown glass flasks in an environmental chamber at 25 °C and a relative humidity of 60%. After 1 year, the amount of DRSP as well as its degradation product isoDRSP was assayed as indicators for chemical stability. The suspensions were taken from storage and were blended using a roller mixer. Thereafter, 1 mL of the samples was centrifuged twice at 7500 rpm for 10 min (Sigma Laborzentrifugen ZK15, Sigma Laborzentrifugen GmbH, Osterode, Germany). 0.5 mL of supernatant was pipetted into a volumetric flask and was mixed with 25 mL of acetonitrile. Then, the samples were analysed by high performance liquid chromatography with UV detection (HPLC/UV). Furthermore, 70 mg/g of DRSP was dispersed in USP phosphate buffer at pH 6.8 under stirring at room temperature using a magnetic stirrer (RT 15 power IKAMAG, Ika-Werke GmbH & Co. KG, Staufen, Germany). After 2 d, 14 d and 42 d, 2 mL of samples was filtered using syringe filter (Whatman® Spartan®, pore size 0.45 μm, Whatman GmbH, Dassel, Germany).

The amounts of DRSP and isoDRSP were determined by using an Agilent 1100 series HPLC/UV system (Agilent Technologies Deutschland GmbH, Böblingen, Germany). 10 μL of samples was injected onto ODS Hypersil column (length 6 cm, inner diameter 4.6 mm, 3 μm; Agilent Technologies Deutschland GmbH, Böblingen, Germany) using as mobile phase a mixture of water and acetonitrile at a ratio of 60–40% (v/v) and a flow rate of 1 mL/min. The samples were detected at a wavelength of 270 nm. To calculate the amount of DRSP, a 6-point-calibration was performed using an external DRSP standard. The data were analysed by using the software Empower™ 2 (Waters GmbH, Eschborn, Germany). The relative ratio of isoDRSP and DRSP was determined by comparing the peak areas of both compounds. The retention times were known.

2.3. Solubility of DRSP with and without addition of stabilising agents

70 mg/g of DRSP was dispersed in sesame oil, castor oil or MCT with or without addition of a stabilising agent by sonication at 5× 10% cycle, 100% power for 5 min using an ultrasound device (Bandelin Sonopuls HD2070, Bandelin electronic GmbH&Co.KG, Berlin, Germany). As stabilising agents, 0.2% (w/w) methyl cholate, Aerosil® 200 Pharma and R972 Pharma, as well as 2% (w/w) Span® 83, lecithin, and cholesteryl oleate were used. Thereafter, the samples were blended for 3 d using a roller mixer. Furthermore, 70 mg/g of DRSP was suspended in USP phosphate buffer pH 6.8. The amount of DRSP was analysed like described in Section 2.2 using HPLC with UV detection.

2.4. Determination of viscosity and rheological properties by a parallel plate rheometer

A rheological determination of viscosity was performed by a parallel plate system (Gemini II, Malvern Instruments GmbH, Herrenberg, Germany). Stabilising agents and preparation of samples was described in Sections 2.2 and 2.3. Thereafter, suspensions were centrifuged at 2000 rpm for 30 min (Heraeus® Biofuge® Fresco, 4× 15 mL, DJB Labcare Ltd., Buckinghamshire, UK) (castor oil suspensions were centrifuged twice). The clear supernatants were analysed using a plate with a diameter of 40 mm, at a gap width of 1 mm and 20 °C. The samples were dropped on the tempered plate. After adjustment of the gap width and 15 min at rest without stressing, the measurements were started. Firstly, the viscosity of formulations was determined at low shear of 0.2 Pa and 1 Hz every 10 s over 1 min simulating resting state. Furthermore, the instantaneous viscosity under shear stress was investigated. The shear rate was increased from 1 to 20 s^{-1} and in the following decreased to 1 s^{-1} over 2× 180 s.

2.5. Evaluation of syringeability and injectability

The terms syringeability and injectability were defined in detail by Boylan and Nail (2002) and Crowder et al. (2003). 1 mL of sesame oil or MCT was filled in glass syringes with 27 G ½ staked-on needles ($n = 10$). The cylindrical tube had an inner diameter of 6.35 mm. The syringes were clamped into a materials testing machine (Type

Z010, Zwick Roell AG, Ulm, Germany). The liquid was expelled from syringes with a defined force. Time for depletion of formulation was determined. The syringes should be completely depleted within 10 s. Furthermore, 0.5 mL of MCT containing 70 mg DRSP was drawn into 1 mL plastic syringes (Tuberkulin 1x100 Soft-Ject, Henke Sass Wolf GmbH, Tuttlingen, Germany) ($n = 5$). The barrel had an inner diameter of 4.25 mm. Due to the smaller inner diameter, the force required to expel the suspensions from plastic syringe were assumed to be 2–2.5 times lower compared to glass syringes. The plastic syringes were applied with 23 G 25 mm or 27 G 13 mm needles. The prefilled syringes, inserted into the force tester, were emptied within 10 s. The force being necessary for complete depletion was recorded.

2.6. Particle size analysis of DRSP microcrystals by laser diffraction after storage

Changes in particle size during storage were determined exemplary on 1% (w/w) DRSP MCS. Prior to preparation, vehicles were saturated with DRSP to avoid any influence by drug solubility of vehicles and to achieve a constant amount of DRSP microcrystals in the suspensions. Thus, the vehicles were prepared by dispersing DRSP in castor oil, sesame oil, MCT, or water under stirring for 3 d using a roller mixer. Thereafter, the fluids were separated from undissolved drug substance by double suction filtration using filter papers (GF/B, Whatman®, Whatman GmbH, Dassel, Germany). For preparation of suspensions without stabilising agents, 200 mg of DRSP was added to 20 g of DRSP saturated vehicle. The mixtures were blended by magnetic stirrer and roller mixer. For preparation of suspensions with stabilising agents, the excipient was dispersed in 20 g of DRSP saturated vehicle by sonication at 5× 10% cycle, 100% power until getting a clear solution. The tested stabilising agents and their used concentrations were described in Section 2.3. After cooling, 200 mg of DRSP was added. All samples were stored tightly closed in wide-neck brown glass flasks in an environmental chamber at 25 °C and a relative humidity of 60% for 1 year.

The particle size of DRSP microcrystals was determined by laser diffraction (Sympatec GmbH System-Partikel-Technik, Clausthal-Zellerfeld, Germany) (sensor: Helos, dispersing unit: Cuvette; 50 mL, software: Windox 5). Before measurement, the samples were blended by a roller mixer for 12 h. A few drops of the suspensions were diluted in 50 mL of MCT saturated with DRSP under moderate stirring by a magnetic stirrer integrated in the measurement apparatus. All oil-based vehicles were soluble in the fluid. The dilution medium was prepared by dispersing an excess of DRSP in MCT under stirring for 24 h and filtration of the undissolved drug substance by vacuum filtration (Nalgene sterile bottle filters, Nalgene® Labware, Fisher Scientific GmbH, Schwerte, Germany). The initial particle size distribution of DRSP microcrystals was determined by suspending the drug substance in the dilution medium. Aqueous DRSP suspension were diluted in 50 mL of water saturated with DRSP, prepared analogous to DRSP saturated MCT. Particle sizes between 0.5 and 175 μm were measured over 10 s. The optical particle concentration was allowed to range between 5 and 50%. For all samples, blank values of the dilution media were determined before measuring. Furthermore, blank tests of the vehicles with and without addition of stabilising agents were performed. The median particle sizes of volume-weighted particle size distribution were recorded.

2.7. Measurement of contact angle using static sessile drop method

Sessile drop method was an optical contact angle method for estimation of the wetting behaviour of liquids on a solid surface (Fig. 1). During static contact angle measurement, the size of the drop was not changed. The measurement was carried out on a DSA 10 calculating with software version 1.80 (Kruess GmbH, Hamburg, Germany). Prior to contact angle determination, pellets of DRSP were prepared using a materials testing machine. Therefore, about 100 mg drug substance was mould by using pressing forces of 10 t for 30 min. The contact angles of sesame oil, castor oil and MCT were determined. For analysing the influence of stabilising agents on wetting behaviour, castor oil mixed with 0.2% (w/w) Aerosil® 200 or R972 Pharma, or 2% (w/w) cholesteryl oleate was used. To avoid dissolving of DRSP pellet during contact angle measurement, the liquids were saturated with DRSP. Preparation of samples was described in Sections 2.2 and 2.3. Suspensions were centrifuged like described in Section 2.4. The supernatants were dropped on the surface of the DRSP pellets. Therefore, the fluid was pumped through a capillary located above the solid and was dropped on the clean solid surface (see Fig. 1). The angle between baseline of the drop and the tangent at the drop boundary was plotted over time ($n = 5$).

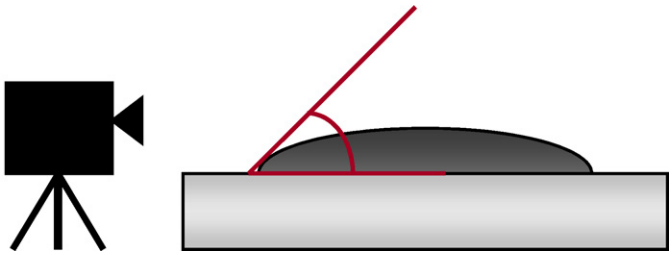

Fig. 1. Optical contact angle measurement by sessile drop method. Liquid was dropped on a solid surface. Thereafter, angles between baseline of the drop and tangent of the drop were determined.

2.8. Analysis of sedimentation using image analysis

Changes in particle size during storage were determined exemplary on 1% (w/w) DRSP MCS. Preparation of samples with or without addition of stabilising agents was described in Section 2.6. For analysing sedimentation, suspensions were photographed after 30 d. The level of sediment and supernatant was measured. Therefore the software Axio Vision 4.5, Carl Zeiss Imaging Solutions (Carl Zeiss AG, Oberkochen, Germany) was used. The relative ratio between height of sediment and total height of sample was calculated. For evaluation of the results, the sedimentation volume F, the ratio between the volume of sediment and the total volume of the suspensions was calculated (Kölling, 2007; Rungseevijitprapa et al., 2009).

2.9. Statistics

The experiments were conducted in triplicate, where not otherwise stated. Arithmetic mean values as well as standard deviations were calculated. The 95% confidence interval was computed to compare two different sample groups.

3. Results and discussion

3.1. Chemical stability of DRSP in aqueous and oil-based vehicles

With a solubility of 11.6 ± 0.3 μg/mL, DRSP was practically insoluble in USP phosphate buffer pH 6.8 and was furthermore chemical instable in aqueous medium, especially at acidic pH. During storage, the 15β,16β-methylen derivative of spironolactone isomerised due to a change in configuration at position 17 resulting in formation of the DRSP epimer isoDRSP (see Fig. 2).

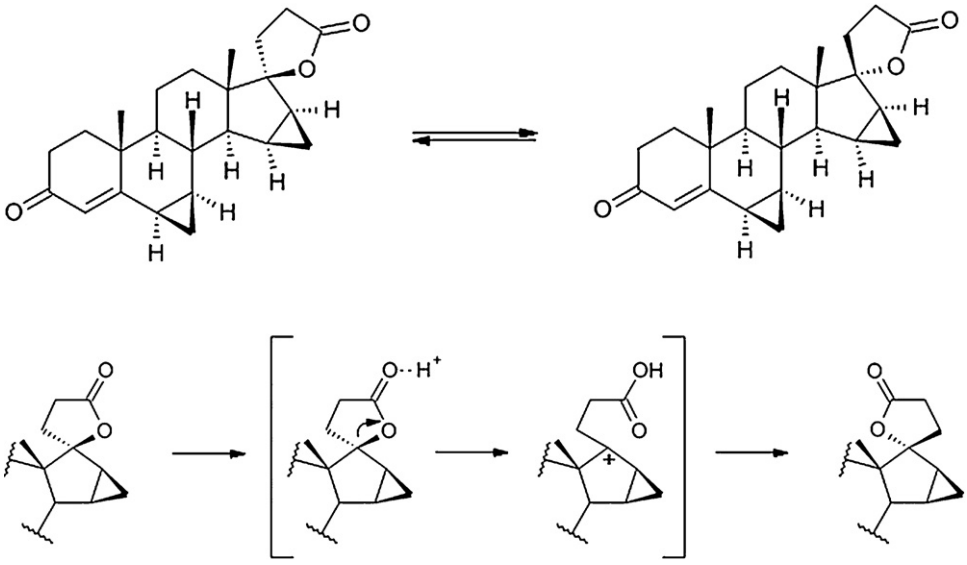

Fig. 2. DRSP and the epimer isoDRSP.

The extent of isomerisation in USP phosphate buffer pH 6.8 is presented in Table 1. After storage of 42 d, an obvious degradation of DRSP into isoDRSP was detected as measured by a relative ratio of 70% DRSP to 30% isoDRSP. On the other hand, no traces of isoDRSP were detected in MCT, sesame oil or castor oil after 1 year storage (see Fig. 3). Consequently, an oil-based solution or suspension would be an option for a chemically storage-stable formulation of DRSP.

3.2. Solubility of DRSP

First of all, an adequate DRSP dose for a once-a-month injection was calculated. Oral contraceptives contained 3 mg DRSP. 76% of the drug substance was bioavailable after oral administration (Fachinformation, 2010). A dose of at least 2 mg of DRSP per day was computed for i.m. or s.c. application, respectively. Therefore, at least 60 to 70 mg of DRSP should be necessary for a once-a-month injection. Depending on the injection site, the administered volume should be less than 5 mL for i.m. injection and less than 2 mL but optimally 0.5 mL for s.c. application (Rutz, 2007; Strickley, 2004). With respect to the calculated dose, the solubility of DRSP in oil-based vehicles was tested (see Table 2). Less than 10 mg/mL DRSP was soluble in MCT and sesame oil. Furthermore, about 12 mg/mL drug substance was dissolvable in castor oil.

3.3. Viscosity of oil-based vehicles

The viscosity of MCT, sesame oil and castor oil is shown in Table 2. With a dynamic viscosity of about 1000 mPa s, castor oil was not suitable for parenteral administration. Nevertheless, the liquid was used as model vehicle for an oil-based DRSP suspension regarding storage stability in dependence on viscosity. MCT had the lowest viscosity.

Table 1
Isomerisation of DRSP in USP phosphate buffer at pH 6.8 ($n = 3$).

Time of storage in d	Relative amount of isoDRSP in %
2	7.6 ± 0.7
14	10.9 ± 1.7
42	30.1 ± 5.2

3.4. Syringeability and injectability

Advantageously, using an autoinjector, the formulations could be injected s.c. by the patients themselves. It should be analysed if an injection of oil-based DRSP MCS via autoinjector was possible and which needle size should be used. Referring to (Rungseevijitprapa and Bodmeier, 2009), formulations were easy to inject using injection forces of up to 50 N. Furthermore, injection forces over 100 N were evaluated to increase the risk of glass barrel burst during administration via autoinjector. However, over 100 N were necessary to inject 1 mL of sesame oil with 27 G syringes. Although, 1 mL of MCT passed through 27 G needles within 10 s using a force of 45 N, 1 mL of DRSP MCT suspensions could not be ejected applying a force below 100 N.

Referring to the Hagen–Poiseuille equation (see Eq. (1)),[1] different approaches might be possible to reduce the injection force.

$$\frac{dV}{dt} = \frac{\pi \cdot r^4}{8 \cdot \eta} \cdot \frac{\Delta p}{l} \quad (1)$$

where dV/dt = volumetric flow rate, r = internal radius of the tube, l = length of the tube, Δp = pressure drop and η = dynamic fluid viscosity. One option was the prolongation of injection to 20–30 s. It was assumed that this would be less convenient for the patient's compliance and was therefore not followed up. Furthermore, the reduction of injection volume should decrease the injection force. However, about 30 N was still necessary to eject 0.5 mL of DRSP MCT dispersion from a plastic syringe through a 27 G needle. The force would be 2–2.5 times higher for injection with an autoinjector used with a glass barrel. Thus, this formulation might only be injected with difficulties.

As needle size was shown to have no significant influence on injection pain, another possibilities was to use needles with larger inner diameter (Montgomery et al., 2005). Most oil-based solutions and microparticulate formulations were administered with 19–25 G needles every 4–12 weeks (Rutz, 2007). For s.c. administration, 23 G or thinner needles were used (Montgomery et al., 2005; Ricci and Kyle, 2009). An average force of only about 10 N was necessary to expel 0.5 mL of DRSP MCT suspension from plastic

[1] Hagen–Poiseuille law gave the voluminal laminar stationary flow of a uniform viscous fluid being incompressible, through a long cylindrical pipe with a constant circular cross-section (Ali et al., 2010).

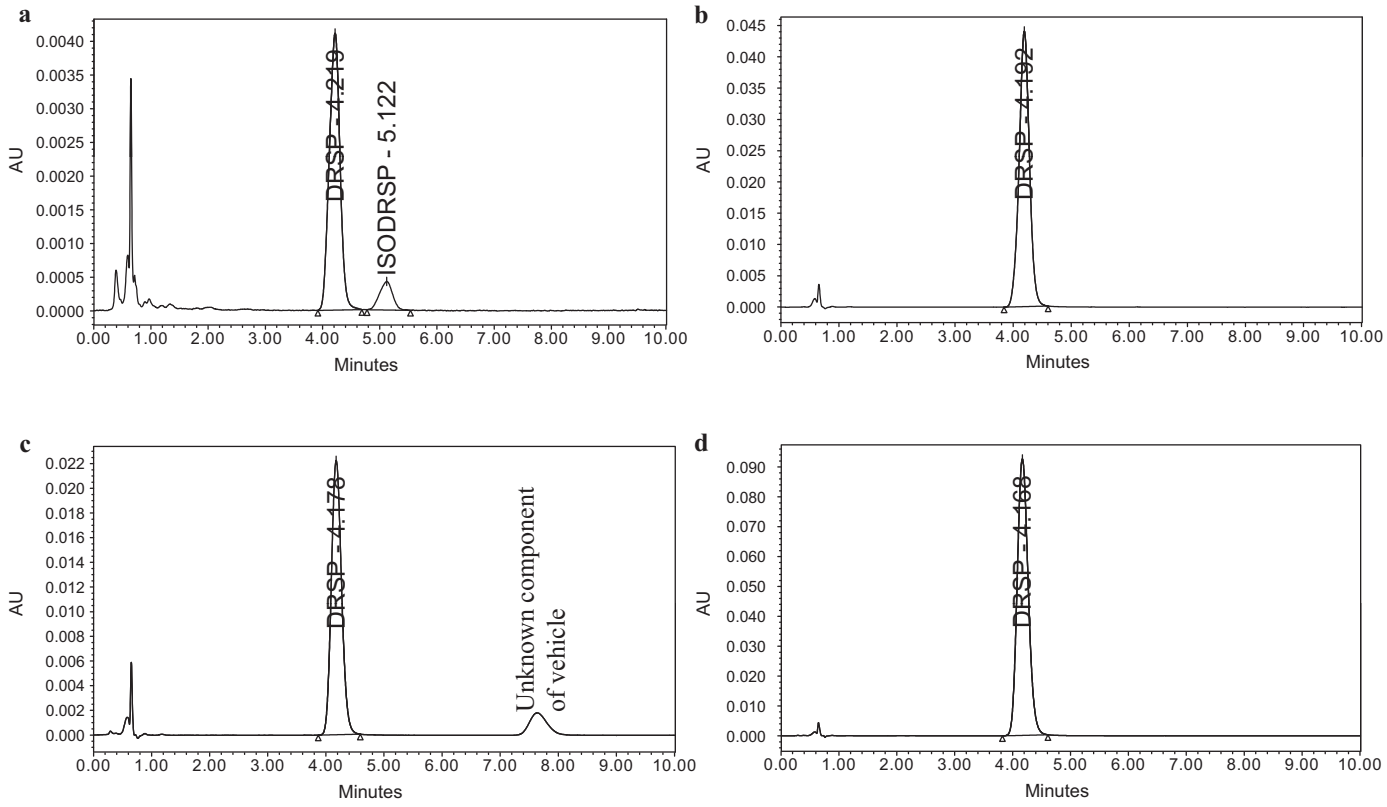

Fig. 3. HPLC chromatograms of DRSP suspended in (a) USP phosphate buffer pH 6.8 after storage of 14 d at room temperature, (b) MCT, (c) sesame oil, and (d) castor oil after storage of 1 year at 25 °C ($n = 3$). IsoDRSP was only found in aqueous suspension.

syringes with 23 G needles. Consequently, the formulation should be easy to inject via a conventional autoinjector used with 23 G needles. In general, DRSP sesame oil suspensions might not be s.c. applicable using an autoinjector with 23 G or smaller needles. Nevertheless, the formulations could be used for i.m. application.

3.5. Particle size analysis of DRSP MCS after storage

For micronised DRSP, an average median particle size of 11.6 ± 2.5 μm was determined. In general, microcrystals DRSP had a median particle size of about 5 μm after micronisation measured

Table 2
Viscosity and DRSP solubility of MCT, sesame oil, and castor oil without and with addition of stabilising agents. Viscosity was investigated at 0.2 Pa as well as at 20 s^{-1} and 20 °C using a rheometer ($n = 3$).

	Concentration in % (w/w)	Solubility of DRSP in mg/mL	Instantaneous viscosity at 0.2 Pa, 20 °C in mPa s	Instantaneous viscosity at 20 s^{-1}, 20 °C in mPa s
MCT + excipient				
–	–	6.9 ± 0.3	31.1 ± 1.1	30.2 ± 0.9
Lecithin	2	7.0 ± 0.1	–	–
Span® 83	2	6.7 ± 0.5	33.6 ± 0.0	32.2 ± 2.7
Cholesteryl oleate	2	6.5 ± 0.1	31.3 ± 0.9	30.3 ± 0.9
Methyl cholate	0.2	7.0 ± 0.5	31.9 ± 0.8	32.1 ± 1.5
Aerosil® 200 Pharma	0.2	7.0 ± 0.1	32.7 ± 1.9	27.6 ± 1.7
Aerosil® R972 Pharma	0.2	6.8 ± 0.1	31.3 ± 1.2	28.3 ± 3.6
Sesame oil + excipient				
–	–	3.9 ± 0.2	77.5 ± 0.3	79.6 ± 2.0
Lecithin	2	4.0 ± 0.4	–	–
Span® 83	2	3.9 ± 0.5	81.7 ± 1.1	79.5 ± 7.8
Cholesteryl oleate	2	3.7 ± 0.7	72.3 ± 1.2	73.3 ± 5.4
Methyl cholate	0.2	3.7 ± 0.4	75.1 ± 2.6	77.5 ± 2.7
Aerosil® 200 Pharma	0.2	3.8 ± 0.3	77.0 ± 0.8	76.1 ± 1.7
Aerosil® R972 Pharma	0.2	3.9 ± 0.7	79.5 ± 1.0	80.7 ± 5.9
Castor oil + excipient				
–	–	12.3 ± 0.4	1032.3 ± 34.1 1134.0 ± 54.4[a]	942.2 ± 15.1 1117.8 ± 29.3[a]
Lecithin	2	12.4 ± 0.4	–	–
Span® 83	2	12.1 ± 0.4	1080.6 ± 14.3[a]	1071.3 ± 25.1[a]
Cholesteryl oleate	2	12.5 ± 0.2	1048.4 ± 12.5	1045.0 ± 23.3
Methyl cholate	0.2	12.1 ± 0.2	1137.4 ± 11.4[a]	1126.8 ± 32.2[a]
Aerosil® 200 Pharma	0.2	12.1 ± 0.7	1003.6 ± 10.6	978.8 ± 9.3
Aerosil® R972 Pharma	0.2	12.3 ± 0.8	1157.7 ± 16.8[a]	1144.0 ± 32.5[a]

[a] Batch II of castor oil.

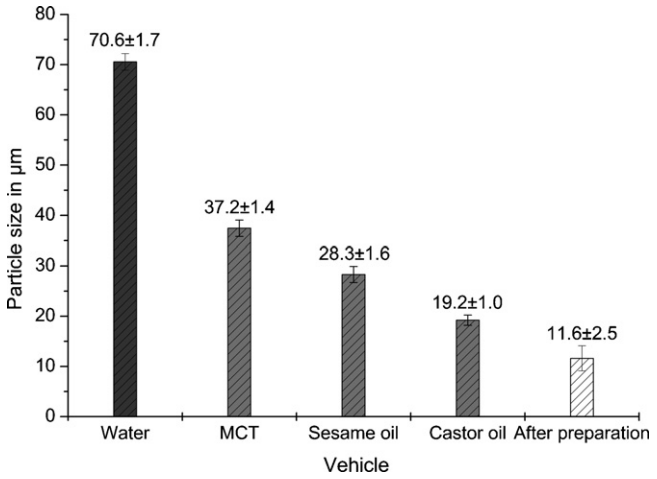

Fig. 4. Median particle sizes of DRSP suspended in water and different oils after storage of 1 year at 25 °C and of the raw material suspended in dilution medium ($n = 3$).

by laser diffraction of dry powder samples (unpublished results). The discrepancy between measurement results could be explained by particle growth during storage of DRSP powder or aggregation of microcrystals during sample preparation. Furthermore, particle size measurement could vary as a function of the type of instrument used for determination (Martinez et al., 2008; Wong et al., 2008).

As expected, a relatively large median particle size was measured after storage of 1 year in aqueous suspension (see Fig. 4). DRSP was a relatively hydrophobic substance tending to agglomeration in aqueous fluids without stabilising agents. Furthermore, crystal growth could cause an increase of particle sizes. In general, median particle sizes were smaller in lipophilic vehicles than in aqueous medium. As higher the viscosity of oil-based vehicle was as smaller was the median particle size of DRSP microcrystals after storage. Like assumed, it seemed that a higher viscosity prevented growth of particles. Nevertheless, the median particle size of DRSP increased significantly in all tested fluids without addition of stabilising agents.

The suspensions were also investigated by optical microscopy followed by determination via image analysis counting and sizing system (Nippe and General, 2010). No significant differences between number-weighted median particle sizes after storage of sesame oil, MCT, or castor oil suspensions were detected. For measurement, an object slide was pressed on the oil drop. Aggregated particles might be separated by the pressure indicating loose aggregation of particles.

3.6. Influence of stabilising agents on solubility of DRSP

The addition of stabilising agents could change the solubility due to solubilisation effects. No significant difference in solubility of DRSP with or without addition of stabilising agents is shown (Table 2).

3.7. Influence of stabilising agents on viscosity of vehicle

A possible thickening effect of stabilising agents was investigated (see Table 2). Even at a low shear, air bubbles were formed in samples containing lecithin (unpublished results). The rheometrical method did not seem to be suitable for this formulation. For MCT or sesame oil, the viscosity was slightly increased with addition of Span® 83. The excipient was a high viscous liquid being miscible with the vehicles. For MCT formulations including silica derivatives, viscosity at low shear was slightly higher compared to viscosity at high shear. Nevertheless, the differences were not significant. For preparation of castor oil DRSP suspensions, two different batches of the vegetable oil were used providing different viscosities. Stabilising agents caused no significant increase in viscosity.

3.8. Particle size analysis of DRSP MCS in addition of stabilising agents

The addition of the amphiphilic substances Span® 83 and lecithin did not affect the particle size of DRSP positively at storage, independently from the oil-based vehicle used (Fig. 5). In general, slight but no significant changes in median particle sizes could be observed for formulations containing methyl cholate. Interestingly, the addition of cholesteryl oleate to MCT and sesame oil suspensions led to median particle sizes being significantly smaller compared to non-stabilised suspensions indicating a stabilising effect. Due to just moderate particle growth in high viscous castor oil suspensions, only a slight influence on DRSP particle size was detected by addition of excipients.

Moreover, when Aerosil® 200 Pharma was added to sesame oil or castor oil suspensions, DRSP microcrystals had a significantly smaller median particle size after storage of 1 year in comparison to non-stabilised dispersions. The median particle size of DRSP in castor oil with addition of the silica derivative was even smaller than median particle size determined after preparation of suspensions. They were comparable to particle sizes of DRSP powder analysed by laser diffraction of dry powder samples after micronisation. The results indicated that a slight aggregation of microcrystals might already take place during sample preparation before storage. No significant disparities were detected between particles suspended in MCT with or without Aerosil® 200 Pharma. The added amount of Aerosil® 200 Pharma might be too low to cause an effect.

A significantly positive effect of Aerosil® R972 Pharma on reduction of particle growth was observed for all test fluids. Stabilisation of suspensions by addition of small amounts of highly dispersed silicon dioxide in aqueous medium without increasing viscosity was described by (Turck and Schmelmer, 2001). Here, this effect could be shown for the tested oil-based systems with addition of hydrophobic colloidal anhydrous silica and without addition of any other stabilising agents. Silica derivatives possibly formed stabilising structures, before they gelled the vehicle. Furthermore, an interaction between DRSP molecules and silica molecule by van der Waals force could prevent aggregation.

3.9. Contact angle between DRSP and castor oil with addition of stabilising agent

The contact angle between DRSP and MCT or sesame oil without addition of stabilising agents were very small, due to relatively rapid spreading over time ($11.8 \pm 2.9°$ for sesame oil, $2.1 \pm 2.2°$ for MCT determined at 1 s). Furthermore, the uniform deposition of the oil droplet was challenging, resulting in a relatively high standard deviation. Hence, slight changes in contact angle by addition of stabilising agents were hard to detect. A contact angle of $42.1 \pm 5.5°$ was determined between castor oil and DRSP, at 1 s. With addition of silica derivatives, influencing the particle sizes significantly, only slightly higher contact angles were determined (Aerosil® 200 Pharma $49.1 \pm 5.7°$, Aerosil® R972 Pharma $49.9 \pm 5.6°$). The difference was not significant. Cholesteryl oleate had an effect on DRSP particle sizes in sesame oil and MCT. The contact angles between castor oil with addition of cholesteryl oleate and DRSP were slightly lower in comparison to non-stabilised castor oil ($33.4 \pm 6.8°$ at 1 s). Again, the disparity was not significant. Oil-based vehicles did not show the high surface tension of water. Thus, a stabilising effect

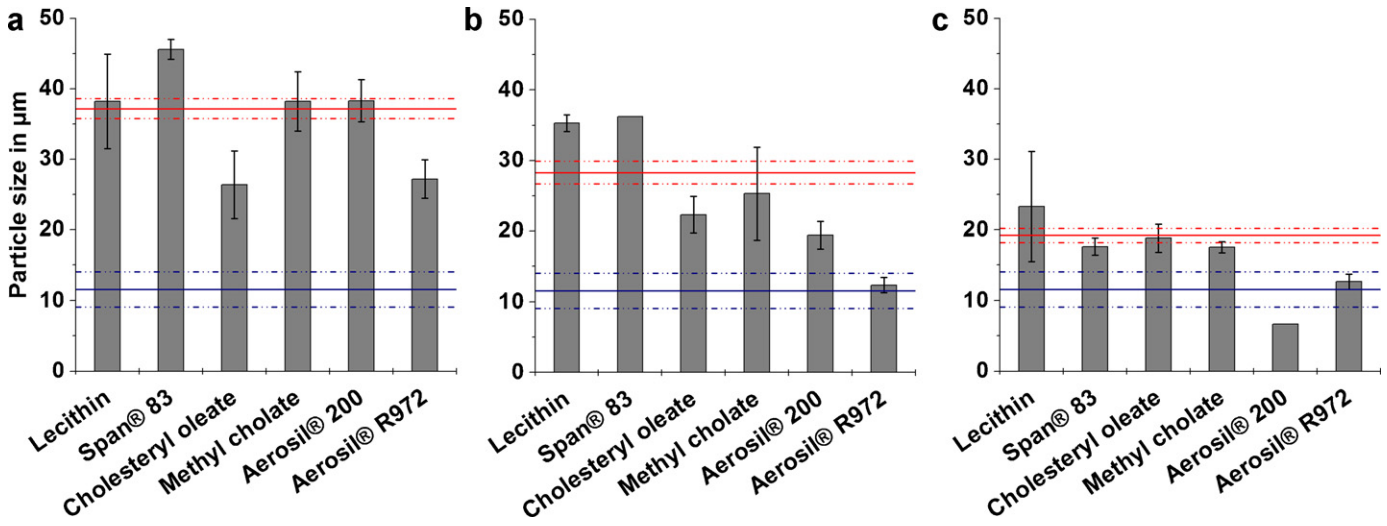

Fig. 5. Median particle size of DRSP microcrystals suspended in (a) MCT, (b) sesame oil, and (c) castor oil with addition of stabilising agents after storage of 1 year at 25 °C ($n = 3$). The median particle sizes of oil-based vehicles without addition of stabilising agents are shown as upper red line, the median particle size of DRSP in suspension before storage is shown as lower blue line (range of standard deviation in dotted lines). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

of excipients decreasing the surface tension might be lower on oil-based vehicles.

3.10. Influence of stabilising agents on sedimentation of DRSP microcrystals

After 1 month of storage neither a marked sedimentation nor a clear supernatant occurred in castor oil suspensions with or without addition of any stabilising agent. Caking was no issue for all oil-based suspension with or without addition of stabilising agents. Even after storage of 1 year, all suspensions were easily redispersible. Span® 83, lecithin, cholesteryl oleate, and methyl cholate had no influence on sedimentation of microcrystals suspended in sesame oil or MCT (see Fig. 6). Sediments of DRSP in MCT or sesame oil were fluffier by addition of Aerosil® 200 Pharma. Furthermore, the sedimentation was significantly slower compared to formulations without stabilising agent. The addition of 0.2% (w/w) Aerosil® R972 Pharma to MCT or sesame oil was found to have the highest impact on sedimentation; only low compact sediment was formed. The level of supernatant was relatively low ($F = 0.9$). As silica derivatives decreased sedimentation of DRSP microcrystals, this could result in decelerated particle growth and consequently in higher stability. Furthermore, sedimentation during manufacturing could be slowed down improving content uniformity. A possible explanation could be the formation of stabilising structures without significant increasing of vehicle viscosity. Interestingly, sedimentation was more reduced by addition of Aerosil® R972 Pharma, than by Aerosil® 200 Pharma although for complete immobilisation of the liquid, higher amounts of hydrophobic colloidal anhydrous silica were necessary (12% (w/w) Aerosil® R972 Pharma, 3% (w/w) Aerosil® 200 Pharma in MCT; unpublished results). Less self-aggregation of Aerosil® R972 Pharma was caused by the lower amount of hydroxyl groups (Evonik, 2011). On the other hand, a stronger affinity between molecules of hydrophobic colloidal anhy-

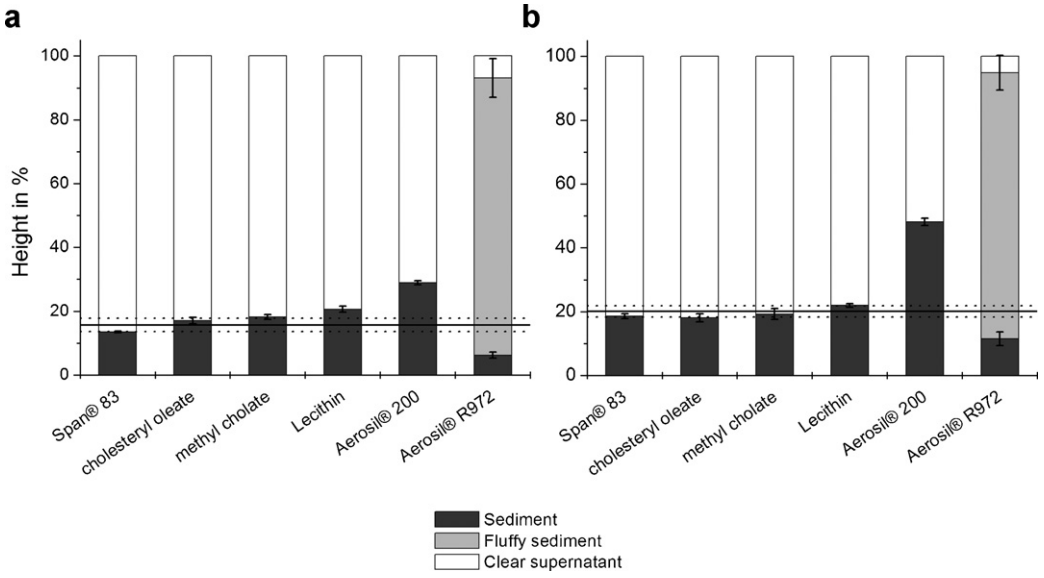

Fig. 6. Sedimentation of DRSP in (a) MCT and (b) sesame oil with or without addition of stabilising agents after storage of 1 month ($n = 3$). Sedimentation of MCT DRSP without addition of stabilising agents is shown as solid line (range of standard deviation in dotted lines).

drous silica and the steroidal drug substance via van der Waals bonds might be conceivable.

4. Conclusion

Oil-based DRSP suspensions showed a high chemical stability. Suspensions of the drug substance in MCT, sesame oil, and castor oil were investigated regarding their physical stability. High viscosity reduced sedimentation and particle growth. On the other hand, only low viscous MCT was suitable for s.c. injection via an autoinjector. Sesame oil was i.m. injectable. Thus, it should be shown if the addition of excipients improved the physical stability of oil-based suspensions without increasing viscosity.

All tested compounds did not influence the solubility of DRSP in the tested concentration. As desired, no thickening effect was observed with addition of any stabilising agent in the tested concentrations. The addition of Aerosil® 200 Pharma reduced particle growth of DRSP microcrystals in castor oil and sesame oil as well as sedimentation in all vehicles. Moreover, the addition of Aerosil® R972 Pharma even led to a decrease in particle growth and sedimentation in all tested DRSP formulations. It avoided an increase in median particle size completely in sesame oil and castor oil preparations in the tested timeframe.

As a consequence, the physical stability of oil-based DRSP MCS during manufacturing and storage could be significantly increased by addition of silica derivatives. In contrast to crystalline silicone dioxide, sol–gel derived glasses containing silica did not induce significant toxicity, showed biocompatibility, and was excreted in soluble form through the kidneys. The degradation rate could be controlled by synthesis (Hench and Wilson, 1986; Kortesuo, 2001). Therefore, a use in parenteral application might be possible.

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