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International Journal of Pharmaceutics 305 (2005) 167–175
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Pharmaceutical Nanotechnology

Drug release and permeation studies of nanosuspensions based on solidified reverse micellar solutions (SRMS)

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Received 29 December 2004; received in revised form 5 August 2005; accepted 3 September 2005

Abstract

Solidified reverse micellar solutions (SRMS), i.e. mixtures of lecithin and triglycerides, offer high solubilisation capacities for different types of drugs in contrast to simple triglyceride systems [Friedrich, I., Müller-Goymann, C.C., 2003. Characterisation of SRMS and production development of SRMS-based nanosuspensions. Eur. J. Pharm. Biopharm. 56, 111–119]. Nanosuspensions based on SRMS were prepared by homogenisation close to the melting point of the SRMS matrix. In a first step the SRMS matrices of 1:1 (w/w) ratios of lecithin and triglycerides were loaded with 17β-estradiol-hemihydrate (EST), hydrocortisone (HC) or pilocarpine base (PB), respectively, and subsequently ground in liquid nitrogen to minimise drug diffusion later on. The powder was then dispersed in a polysorbate 80 solution using high pressure homogenisation. The drug loading capacities of the nanosuspensions were very high in the case of poorly water-soluble EST (99% of total 0.1%, w/w, EST) and HC (97% of total 0.5%, w/w, HC) but not sufficient with the more hydrophilic PB (37–40% of total 1.0%, w/w, PB). These findings suggest SRMS-based nanosuspensions to be promising aqueous drug carrier systems for poorly soluble drugs like EST and HC.

Furthermore, in vitro drug permeation from the different drug-loaded nanosuspensions was performed across human cornea construct (HCC) as an organotypical cell culture model. PB permeation did not differ from the nanosuspension and an aqueous solution whereas the permeation coefficients of HC-loaded nanosuspensions were reduced in comparison to aqueous and oily solutions of HC. However, the permeated amount was higher from the nanosuspensions due to a much lower HC concentration in the solution than that in the nanosuspension (solution 0.02%, w/w, versus nanosuspension 0.5%, w/w). The high drug load of the nanoparticles provides prolonged HC release. Permeated amounts of EST were reduced in comparison to HC and only detectable with an ELISA technique.

The EST release from nanosuspensions and different EST-loaded systems revealed a prolonged EST release from the nanoparticulate systems in contrast to a faster release of an oily solution of an equal EST concentration. With regard to an aqueous EST suspension of similar concentration which represents a depot system the release rate from the nanosuspensions revealed the same order of magnitude which points again to a prolonged release potential of the nanosuspensions.

Keywords: Nanosuspension; Solid lipid nanoparticles; Solidified reverse micellar solution; Drug delivery system; Drug release; Drug permeation

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doi:10.1016/j.ijpharm.2005.09.007

1. Introduction

Poorly water-soluble drugs such as 17β-estradiol-hemihydrate (EST) and hydrocortisone (HC) often have to be formulated as semisolid systems or oily solutions for topical administration (Rote Liste, 2004). Due to a low compliance of semisolid and oily formulations especially in ophthalmology, the development of an aqueous system of poorly soluble drugs with improved applicability is an interesting alternative. Furthermore, many ophthalmic drugs such as pilocarpine as an anti-glaucoma compound often have to be administered two to four times per day (Rote Liste, 2004). Frequent dosing also minimises patient's compliance. Therefore, prolonged release formulations are of major interest. Thus, drug release from the developed aqueous systems as well as drug permeation across the cornea should to be studied. For the latter purpose a human organotypical cornea construct according to Reichl et al. (2004) was used as an alternative in vitro permeation model.

17β-Estradiol-hemihydrate is a potent estrogen in the topical treatment of postmenopausal keratoconjunctivitis sicca (Sator et al., 1998). Usually, a sesame oil solution of 0.025% (w/w) EST (Fischer and Reimann, 1999) is administered for three to four times per day (Sator et al., 1998). Hydrocortisone is suspended in a concentration of 0.5–1.5% (w/v) in viscous aqueous eye drops (Rote Liste, 2004; Dolder and Skinner, 1990). Hydrocortisone acetate at concentrations of 0.5–2.5% (w/w) is prepared as lipophilic ointment in the therapy of inflammations of the eye such as (allergic) conjunctivitis, iritis and postoperative irritations of the eye (Rote Liste, 2004).

Aqueous suspensions of solid lipid nanoparticles show promise as colloidal drug delivery systems and have been described in a variety of previous studies (Siekmann and Westesen, 1992, 1994a,b; Schwarz et al., 1994; Müller et al., 1995; Müller and Lucks, 1996; Westesen et al., 1997), reviews are given in Müller et al. (2000) and Mehnert and Mäder (2001). In contrast to a simple triglyceride matrix which is mostly used to form the particles, the nanosuspensions of the present study are based on solidified reverse micellar solutions (SRMS) according to Friedrich and Müller-Goymann (2003). SRMS consist of a 1:1 (w/w) ratio of lecithin and triglycerides. The solidified lipids still remain as reverse micelles at least in part. The reverse micelles are likely to be frozen in the solid state and display a high solubilisation potential for different types of drugs (Friedrich and Müller-Goymann, 2003) in the same manner as liquid reverse micellar solutions do (Papantoniou and Müller-Goymann, 1995). The latter ones additionally enable prolonged release upon transformation into a lamellar mesophase due to water uptake (Müller-Goymann and Hamann, 1993; Schneeweis and Müller-Goymann, 1997). The SRMS-based nanosuspensions were manufactured with homogenisation technique which includes milling of the drug-loaded SRMS in liquid nitrogen and a subsequent high pressure homogenisation close to the melting point of the lipids (Friedrich and Müller-Goymann, 2003). These moderate conditions were applied to minimise drug diffusion into the aqueous phase of the systems.

2. Materials and methods

2.1. Materials

Softisan® 100 (*S100*) and *Softisan® 142* (*S142*), i.e. solid triglyceride mixtures, with mp of 35 and 43 °C, respectively, were supplied by Condea (Witten, Germany). *Phospholipon® 90 G* (*P90G*), a purified soybean lecithin of at least 90% (w/w) phosphatidylcholine, was provided by Nattermann Phospholipid GmbH (Köln, Germany), *polysorbate 80* (*PS80*) and *sorbitol* were purchased from Caesar & Loretz (Hilden, Germany), *thimerosal* was purchased from Merck KGaA (Darmstadt, Germany), *pilocarpine base* (*PB*) was supplied by Dr. Winzer Pharma (Olching, Germany), *17β-estradiol-hemihydrate* was provided by Jenapharm (Jena, Germany), *hydrocortisone* was purchased from Synopharm (Barsbüttel, Germany), *acetonitrile* (Acros Organics, Geel, Belgium), *methanol* (Fisher Scientific, Loughborough, Great Britain), *isopropanol* (Riedel-de Haën, Seelze, Germany) and *acetic acid* (J.T. Baker, Deventer, Netherlands) were used in HPLC grade, *diethylether* was purchased from Grüssing (Filsum, Germany), *silicone oil AK 350* was purchased from Wacker (Burghausen, Germany), *sesame oil* was purchased from Sigma–Aldrich (Schnelldorf, Germany), *water* was used in bidistilled quality.

2.2. Manufacturing of solidified reverse micellar solutions

A 1:1 (w/w) ratio of P90G and S100 or S142, respectively, was stirred with a teflon coated magnet at a temperature of 60 °C until a transparent yellow melt, i.e. a reverse micellar solution (Papantoniou and Müller-Goymann, 1995), was obtained. After drug solubilisation within the reverse micellar solution in concentrations of 6.5% (w/w) PB, 0.7% (w/w) EST or 3.0% (w/w) HC, respectively, the homogeneous systems were stirred at room temperature until solidification. Solidified matrices of 1:1 (w/w) ratio are abbreviated SRMS100 and SRMS142 depending on the respective triglycerides.

2.3. Manufacturing of nanosuspensions

The drug-loaded SRMS matrices were milled in liquid nitrogen (−196 °C) for 15–20 min. The resulting frozen fat powder was dispersed in an aqueous solution of PS80 under stirring for 90 s at 13,000 rpm with an Ultra-Turrax T25 basic (Ika, Staufen, Germany). The PS80/SRMS ratio was 1:5 (w/w) and the systems were preserved with 0.005% (w/w) thimerosal and isotonised with sorbitol.

An EmulsiFlex-C5 (Avestin, Ottawa, Canada) was used for high pressure homogenisation. The coarse suspension was passed through the apparatus first without pressure for 2 cycles and was then homogenised continuously for 20 cycles at a pressure of 1000 bar (SRMS100 systems) or 1500 bar (SRMS142 systems), respectively. After manufacturing, the suspensions were stored at a temperature of 4–8 °C overnight prior to physicochemical and technological characterisation.

The final drug concentrations of the nanosuspensions amounted to 0.1% (w/w) EST, 0.5% (w/w) HC and 1.0% (w/w) PB, respectively.

2.4. Photon correlation spectroscopy (PCS)

PCS measurements were performed with a Zetasizer 3 (Malvern, Herrenberg, Germany) modified with a He/Ne laser model 127 (Spectra Physics, Mt. View, CA, USA). After dilution with filtered bidistilled water to avoid multiscattering events the different suspensions were investigated at an angle of 90° in a measuring cell AZ 10 equilibrated at 20 °C.

2.5. Transmission electron microscopy (TEM) of freeze-fractured specimen

Samples were shock-frozen in melting nitrogen at −210 °C between two flat gold holders. The frozen systems were fractured at −100 °C in a BAF 400 instrument (Balzers, Wiesbaden, Germany). Specimens were shadowed with platinum/carbon (2 nm) at 45° and with pure carbon (20 nm) at 90° for replica preparation. After cleaning in conc. sulphuric acid and water the replicas were viewed on uncoated grids with a transmission electron microscope EM 300 (Philips, Kassel, Germany) at a voltage of 80 kV.

2.6. Drug loading capacity

The drug loading capacity was determined indirectly after centrifugation of the systems in a Vivaspin® 20 concentrator (Vivascience, Hanover, Germany) for 90 min (10 °C, acc. 6000 × g) in a Beckman Coulter Allegra 64R centrifuge (Beckman Coulter, Palo Alto, CA, USA). The drug concentration in the aqueous continuous phase was determined by HPLC as described below and afterwards the remaining content of drug in the nanoparticles was calculated.

2.7. Drug release studies

Release studies of EST were performed in modified Franz diffusion cells (Franz, 1975) over 7 h at a temperature of 37 °C if not mentioned otherwise. The diffusion barrier was a siliconised Spectrapore® membrane MWCO 6000–8000 Da (Spectrum Medical Industries, Los Angeles, CA, USA). EST-loaded nanoparticulate systems of SRMS100 and SRMS142 as described above were used as donors as well as solutions of 0.025 and 0.1% (w/w) EST in sesame oil and an aqueous dispersion of 0.122% (w/w) EST. Isotonic phosphate buffered saline pH 7.4 (PBS) was used as receiver which was stirred with a magnetic stirrer at 500 rpm during the experiment. Two hundred and fifty-microliter aliquots of the receiver were taken at fixed time intervals and replaced by the same amount of PBS. Released amounts of EST were determined by HPLC as described below. By plotting the released amounts versus the square root of time, the flux J [g/(cm^2 s$^{0.5}$)] was calculated from the linear slopes of the release curves. Considering the EST con-

centration c_0 the standardised flux J/c_0 (cm/s$^{0.5}$) was calculated.

2.8. Drug permeation studies

In vitro drug permeation across a human organotypical cornea construct according to Reichl et al. (2004) was examined using modified Franz diffusion cells over 7 h at a temperature of 37 °C. The aforementioned nanoparticulate systems loaded with EST, HC or PB, respectively, were used as donors as well as a solution of 0.02% (w/w) HC in sesame oil and an aqueous solution of 0.77% (w/w) PB which was buffered at the same pH of 6.8 as the PB-loaded nanosuspensions. Again, isotonic phosphate buffered saline pH 7.4 was used as receiver which was stirred with a magnetic stirrer at 500 rpm during the experiment. Two hundred and fifty-microliter aliquots were taken from the receiver every hour and replaced by the same amount of PBS. Permeated amounts of EST were determined with a 17β-estradiol ELISA Kit (IBL, Hamburg, Germany) by using a Wallac 1420 Victor^2 multilabel reader (Perkin-Elmer, Turku, Finland). HC and PB were quantified by HPLC as described below. The permeation parameters of the different drugs were calculated by plotting the permeated amounts versus the time. The permeation coefficient P (cm/s) was calculated as flux J/drug concentration c_0 from the linear slope of the permeation curves.

2.9. HPLC methodology

Concentrations of the drugs were determined using a Waters 515, 717 plus, 186 HPLC system (Waters, Eschborn, Germany) with data analysis by Waters Millenium 32 Chromatography Manager Software. PB: Gromsil 120 ODS-3 CP 5 μm, 125 mm × 4 mm column (Grom, Herrenberg, Germany), mobile phase acetonitrile/phosphate buffer (6.81 g/l KH_2PO_4, 1.66 g/l KOH) pH 7.0 (23:77, v/v, 1.0 ml/min, λ = 215 nm); EST: Hypersil ODS 5 μm 250 mm × 4 mm column (Grom), mobile phase acetic acid 2%/acetonitrile/isopropanol (45:45:10, v/v/v, 1.0 ml/min, λ = 280 nm) (Schicksnus and Müller-Goymann, 2002); HC: Hypersil ODS 5 μm 250 mm × 4 mm column (Grom), mobile phase methanol/water (60:40, v/v, 1.1 ml/min, λ = 250 nm) (Way and Hadgraft, 1991). The correlation coefficients of calibrations were at least 0.999 for all substances.

3. Results and discussion

3.1. Determination of the drug loading capacity

The particle size evaluation of the drug-loaded nanosuspensions resulted in mean particle sizes of 60–130 nm and polydispersity indices of less than 0.3. That means that the nanosupensions are fairly homogeneous in size and size distribution. The drug loading capacities were then calculated by subtraction of the drug concentration in the continuous aqueous phase from the total drug concentration (Table 1).

In the case of hydrophilic PB drug loading capacity amounts to 37% (SRMS100) and 40% (SRMS142) of total concentration of 1.0% (w/w) PB in the nanosuspensions, respectively. This is due to drug diffusion out of the nanoparticles into the outer phase during the homogenisation process. The homogenisation results in a temperature increase, and thus melting of the particles at least partially. A further decrease in drug content during storage was not detected. In contrast the drug loading capacity is very high for rather lipophilic EST and HC. These drugs are nearly completely encapsulated within the nanoparticles (99% of total 0.1%, w/w, EST; 97% of total 0.5%, w/w, HC). No precipitation of these poorly water-soluble drugs could be observed by electron microscopy which points again to an almost complete entrapment of EST and HC, respectively. Just the original anisometrical nanoparticles of platelet-like shape (compare Friedrich and Müller-Goymann, 2003) have been detected so far, when investigating the abovementioned drug concentrations. The drug concentra-

Table 1
Drug loading capacity ± S.D. (%) of the nanoparticles

Drug	Total drug concentration (%, w/w)	System	n	Loading capacity (percent of total drug concentration)
PB	1.0	SRMS100	4	36.7 ± 1.2
		SRMS142	4	40.3 ± 1.4
EST	0.1	SRMS100	7	99.0 ± 0.4
		SRMS142	7	99.3 ± 0.4
HC	0.5	SRMS100	5	96.7 ± 1.4
		SRMS142	3	96.5 ± 0.3

tions determined in the outer phase are similar to the saturation solubilities of the respective drugs in water, which amount to about 12 μg/ml EST and 200 μg/ml HC.

The SRMS-based nanosuspensions seem to be a suitable aqueous drug carrier systems for the poorly soluble drugs EST and HC. However, appropriateness of these colloidal systems is limited in the case of hydrophilic drugs like PB.

3.2. Permeation studies

In vitro permeation studies were performed using an established cell culture model (human cornea construct) (Reichl et al., 2004, 2005) to determine the transcorneal drug transport from nanosuspensions.

The PB permeation rate from drug-loaded nanosuspensions (1.0%, w/w, of PB) was compared with that from an aqueous solution of 0.77% (w/w) of PB which is the same concentration as in the outer phase of the nanosuspensions. The permeation profiles of these systems are presented in Fig. 1; Table 2 shows the calculated fluxes J and permeation coefficients P. All the systems show identical permeation behaviours resulting in comparable drug fluxes. The permeation rate is dependent on the PB concentration in the continuous phase only. The permeation coefficients are higher in comparison to an aqueous pilocarpine hydrochloride solution,

Table 2
Drug permeation across human organotypical cornea construct, flux $J \pm$ S.D. [$\times 10^{-8}$ g/(cm^2 s)] and permeation coefficient $P \pm$ S.D. ($\times 10^{-6}$ cm/s) from 1.0% (w/w) PB nanosuspensions ($n = 6$) and 0.77% (w/w) PB aqueous solution ($n = 5$)

System	J	P
SRMS100 nanosuspension	23.0 ± 5.0	22.6 ± 4.9
SRMS142 nanosuspension	19.7 ± 1.2	19.5 ± 1.2
Aqueous solution	23.9 ± 5.8	30.6 ± 7.4

i.e. Borocarpin® S eye drops (2%, w/w, pilocarpine hydrochloride). For this commercial formulation a permeation coefficient P of $13.4 \pm 3.0 \times 10^{-6}$ cm/s was determined also using human cornea construct as in vitro permeation model (Reichl et al., 2004). The difference could be explained in terms of different pH values (present systems: pH 6.8, Borocarpin® S: pH 5). The dissociation balance is shifted to the more lipophilic PB in the case of the higher pH of 6.8. PB can penetrate much easier than PB^+ (ionised form) into the epithelium of the cornea due to its higher partition coefficient (Suhonen et al., 1998).

The HC permeation from 0.5% (w/w) HC nanosuspensions is compared with that from an oily solution of 0.02% (w/w) HC only which is due to the low HC solubility in oily vehicles (Fig. 2; Table 3). The low HC concentration of the sesame oil solution is reflected in a low flux J, which is also in agreement with the high viscosity and thus a low diffusion coefficient in the vehicle. In

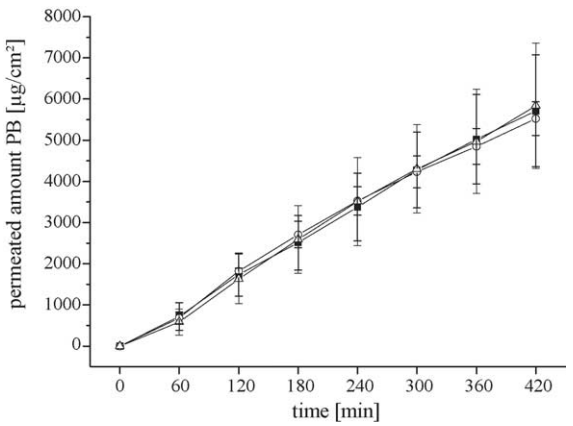

Fig. 1. PB permeation across human organotypical cornea construct from nanosuspensions (1.0%, w/w, PB, $n = 6$) and an aqueous solution (0.77%, w/w, PB, $n = 5$). SRMS100 nanosuspension (■), SRMS142 nanosuspension (◯) and aqueous solution (△). Bars represent ± standard deviation.

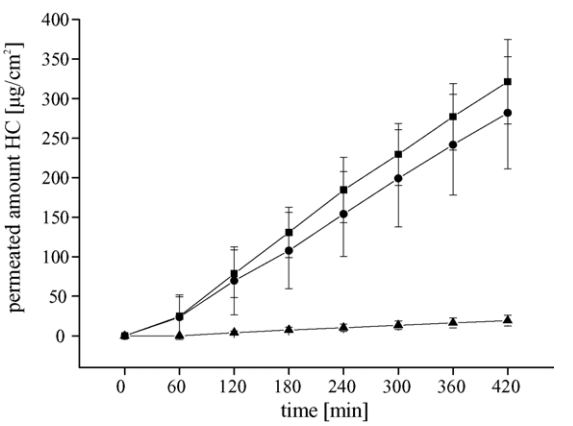

Fig. 2. HC permeation across human organotypical cornea construct from nanosuspensions (0.5%, w/w, HC) and an oily solution (0.02%, w/w, HC). SRMS100 nanosuspension (■, $n = 8$), SRMS142 nanosuspension (●, $n = 7$) and sesame oil (▲, $n = 8$). Bars represent ± standard deviation.

Table 3
Drug permeation across human organotypical cornea construct, flux $J \pm$ S.D. [$\times 10^{-9}$ g/(cm^2 s)] and permeation coefficient $P \pm$ S.D. ($\times 10^{-6}$ cm/s) from 0.5% (w/w) HC nanosuspensions (SRMS100: $n = 8$, SRMS142: $n = 7$) and 0.02% (w/w) HC solution in sesame oil ($n = 9$)

System	J	P
SRMS100 nanosuspension	13.6 ± 2.4	2.69 ± 0.47
SRMS142 nanosuspension	12.0 ± 2.9	2.34 ± 0.57
Sesame oil	0.87 ± 0.28	4.41 ± 1.44

contrast, the permeation rate from the nanosuspensions is more than 10 times higher. The same holds for previous data from the literature (Reichl et al., 2004) which display a flux J of $1.08 \pm 0.08 \times 10^{-9}$ g/(cm^2 s) and a permeation coefficient P of $5.41 \pm 0.40 \times 10^{-6}$ cm/s in the case of an aqueous solution of 0.02% (w/w) HC. The permeation rate is mainly dependent on drug solubility in the continuous phase of the systems. After homogenisation, the HC solubility in the outer phase of the nanosuspensions is in the same order of magnitude as that of both the aqueous and the oily solution (ca. 200 μg/ml). Since the permeation experiment was run at 37 °C, the particles are likely to be melted. Hence, lecithin leakage could occur and mixed micelles of lecithin and polysorbate 80 could arise. In this case, HC solubility in the outer phase of the nanosuspensions should rise as well as in consequence of a direct influence of the elevated temperature on HC solubility. In contrast to the investigated aqueous and oily HC solutions with a constant drug concentration, the HC concentration in the aqueous phase of the nanosuspensions increases further due to the possibility of subsequent HC delivery from the particles. HC permeation from the nanosuspensions does not reveal low permeation rates. However, the systems are suitable for a prolonged release due to continuous drug delivery from the particles into the outer phase. In comparison to the SRMS100 system the flux and permeation coefficient of SRMS142 nanosuspensions are slightly reduced. This tendency is in agreement with a higher viscosity of SRMS142 versus SRMS100 droplets at a temperature of 37 °C where both types of particles are melted. The higher viscosity is due to higher chain length of S142 fatty acids resulting in a decrease in HC diffusion coefficient within the liquid lipid droplets in agreement with the Stokes–Einstein equation.

Table 4
Drug permeation across human organotypical cornea construct, flux $J \pm$ S.D. [$\times 10^{-10}$ g/(cm^2 s)] and permeation coefficient $P \pm$ S.D. ($\times 10^{-7}$ cm/s) from 0.1% (w/w) EST nanosuspensions ($n = 4$)

System	J	P
SRMS100 nanosuspension	7.50 ± 1.98	7.31 ± 1.93
SRMS142 nanosuspension	4.89 ± 1.61	4.76 ± 1.57

In contrast to HC permeation EST permeation rate is rather low and could not be determined by HPLC. Using an ELISA Kit allowed for EST analysis during permeation across a human organotypical cornea construct (Fig. 3). EST fluxes from the nanosuspensions are reduced by factor 20 in comparison to those of HC, the permeation coefficients are lower by factor 4 (Table 4). Again, flux and permeation coefficient of the SRMS142 system are reduced versus the SRMS100 system and could be explained the same way as in the case of HC.

3.3. EST release studies

In addition to permeation studies, EST release experiments from nanosuspensions and oily solutions as well as aqueous suspension were performed. Fig. 4a–c shows the released EST amounts versus the square root of time; Table 5 represents the calculated fluxes of the systems.

Table 5
Estradiol release from nanosuspensions ($n = 6$) and oily solutions ($n = 6$) as well as aqueous suspension ($n = 3$), flux $J \pm$ S.D. [$\times 10^{-8}$ g/(cm^2 s$^{0.5}$)] and standardised flux $J/c_0 \pm$ S.D. ($\times 10^{-5}$ cm/s$^{0.5}$)

System	EST (%, w/w)	J	J/c_0
SRMS100 nanosuspension (37 °C)	0.1	4.42 ± 0.34	4.27 ± 0.33
SRMS142 nanosuspension (37 °C)	0.1	3.98 ± 0.37	3.95 ± 0.37
SRMS100 nanosuspension (20 °C)	0.1	2.47 ± 0.29	2.40 ± 0.28
SRMS142 nanosuspension (20 °C)	0.1	2.85 ± 0.11	2.77 ± 0.10
Sesame oil (0.1% EST, 37 °C)	0.1	9.42 ± 0.66	10.2 ± 0.72
Sesame oil (0.025% EST, 37 °C)	0.025	1.27 ± 0.09	5.54 ± 0.38
Aqueous suspension (37 °C)	0.122	4.70 ± 0.11	3.85 ± 0.09

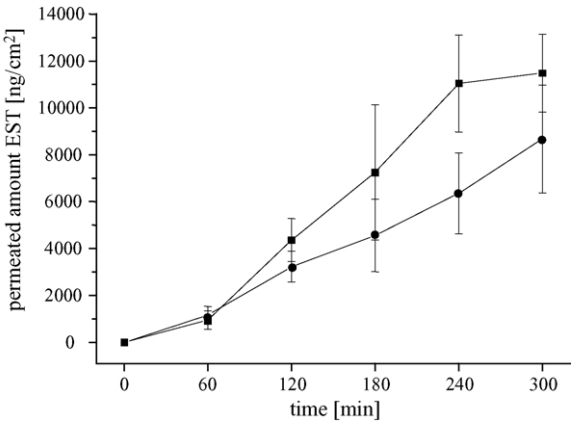

Fig. 3. EST permeation across human organotypical cornea construct from nanosuspensions (0.1%, w/w, EST, $n = 4$). SRMS100 nanosuspension (■) and SRMS142 nanosuspension (●). Bars represent ± standard deviation.

In contrast to an oily solution of the same drug concentration (0.1%, w/w, EST), the fluxes J of the nanosuspensions are smaller by factor 2.3 (Fig. 4a). EST release (flux) from the nanosuspensions was slightly higher in comparison with an oily solution of 0.025% (w/w) EST which is used in therapy of keratoconjunctivitis sicca (Sator et al., 1998; Fischer and Reimann, 1999), due to the higher EST concentration of nanosuspensions. However, standardisation of the flux J in terms of dividing by the total drug concentration c_0 demonstrates slower drug release from the nanosuspensions than from oily solutions (Table 5). The slight decrease in the flux of SRMS142 nanosuspensions in comparison to SRMS100 systems as mentioned above could also be observed during the release studies.

The temperature influence (20 or 37 °C) on the EST release is displayed in Fig. 4b. Due to infinite viscosity of solids and a nearly complete encapsulation of the drug within the particles, EST release rate from solid particles at 20 °C was distinctly reduced in comparison to the melted particles at 37 °C. Furthermore, a temperature decrease (37 to 20 °C) likewise caused a reduced diffusion coefficient resulting in a decreased drug release. At 20 °C the SRMS142 system revealed no difference compared to the SRMS100 system because infinite viscosity holds for both types of particles in the solid state.

Similar release profiles were obtained with both the nanosuspensions and an aqueous suspension of 0.122%

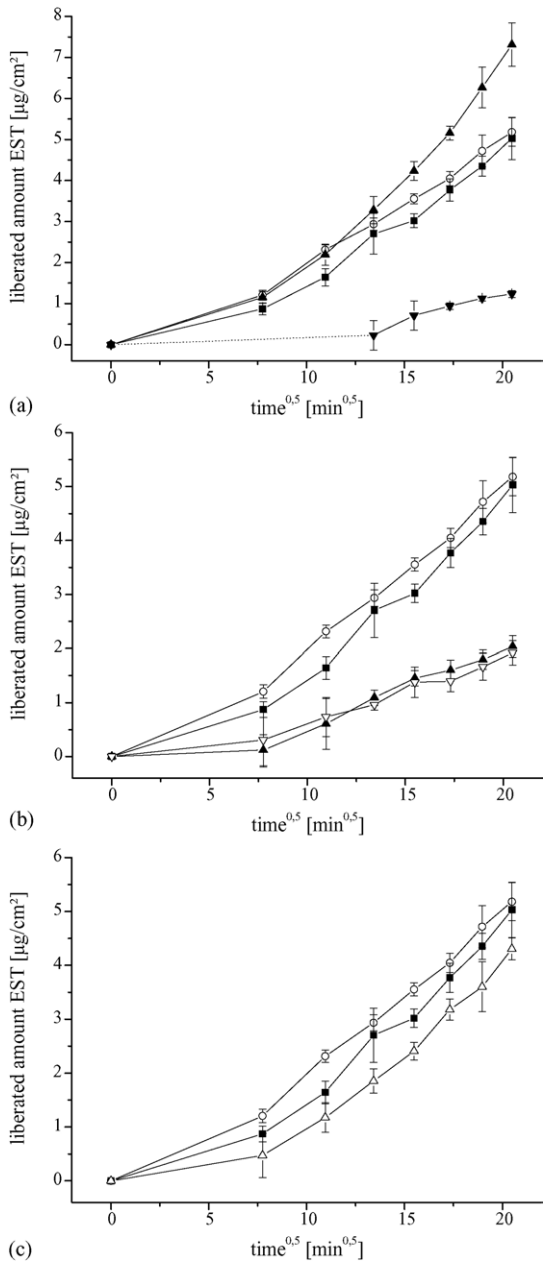

Fig. 4. EST release from nanosuspensions (0.1%, w/w, EST, $n = 6$) and oily solutions ($n = 6$) and an aqueous suspension ($n = 3$) at 37 °C. (a–c) SRMS100 nanosuspension (■) and SRMS142 nanosuspension (◯). Bars represent ± standard deviation. (a) Comparison with sesame oil solutions with 0.1% (▲) and 0.025% (w/w) EST (▼). (b) Release at 20 °C. SRMS100 nanosuspension at 20 °C (▲) and SRMS142 nanosuspension at 20 °C (▽). (c) Comparison with an aqueous suspension of 0.122% (w/w) EST (△).

(w/w) EST (Fig. 4c; Table 5). The latter system provided a depot revealing an EST solubility in the aqueous phase which was 10 times lower than that of the nanosuspensions (1.5 μg/ml versus 12–15 μg/ml). An explanation of this finding could be again the formation of mixed micelles of lecithin and polysorbate 80 after leaking of lecithin from melted particles as in the case of HC. In general, the drug solubility in the outer phase and thus the concentration gradient is the driving force of the release rate (Chen-Chow and Frank, 1981). Despite the higher EST solubility within the continuous aqueous phase of the nanosuspensions, obviously the release from the nanoparticles themselves seems to be rather slow. This finding again suggests prolonged EST release from the nanosuspensions because the sum of nanoparticles obviously provides a larger active surface for EST release than the sum of macrocrystals of the aqueous EST suspension. According to the Noyes–Whitney equation, a larger surface would lead to a faster dissolution in the case of similar diffusion coefficient *D*. The diffusion coefficients of the nanoparticles seem to be reduced.

4. Conclusions

Nanosuspensions based on solidified reverse micellar solutions are a suitable drug carrier system for poorly water-soluble drugs like estradiol and hydrocortisone and represent an alternative aqueous system to oily vehicles. The drug loading capacity for such substances depends on the phospholipid content in the lipid matrix and is high enough in a manner that the drugs are nearly completely incorporated within the nanoparticles. However, the applicability of nanosuspensions with regard to hydrophilic drugs is limited. Only 37–40% of total 1.0% (w/w) pilocarpine base is located within the particles although the drug could be solubilised to a high extent in the SRMS melt (Friedrich and Müller-Goymann, 2003).

Permeation studies reveal no differences between PB-loaded nanosuspensions and an aqueous solution due to equal drug solubility in the aqueous outer phase. The HC permeation rates from nanosuspensions are higher than those from an aqueous and an oily solution of a lower HC concentration. In contrast, considering total HC content yields decreased permeation coefficients of the nanoparticulate systems. Due to subsequent HC delivery from the nanoparticles, a prolonged release has to be considered. In comparison to HC, the permeation rate of EST is reduced. Permeated EST amounts could be detected by ELISA only.

EST release data from different systems again suggest a prolonged release from the nanosuspensions in relation to an oily solution of a similar drug concentration. Lowering of the temperature to 20 °C where the particles are in a solid state further decelerates EST release. This phenomenon enables modified release by choice of appropriate lipid matrices. A lipid of higher melting temperature would delay the release. The slight decrease in fluxes of the SRMS142 nanosuspensions in comparison to the SRMS100 systems also points to a modified release potential of the systems. Despite a higher EST solubility in the aqueous phase of the nanosuspensions versus an aqueous suspension EST release rate from nanosuspensions is similar to that from the aqueous EST suspension counteracting the influence of the higher concentration gradient on initial release. EST drug delivery from the nanoparticles themselves, however, seems to be slower than the dissolution of the EST crystals.

However, the usefulness of SRMS nanosuspensions as drug carrier for poorly water-soluble drugs suggested by drug release and in vitro permeation studies have to be further investigated by in vivo permeation studies.

Acknowledgements

We would like to thank Nattermann Phospholipid, Condea, Jenapharm and Dr. Winzer Pharma for kind support with materials. Furthermore, we would like to thank Nestec (Nestec Ltd., Nestlé Research Center Lausanne, P.O. Box 44, CH-1000 Lausanne 26) as the source of human corneal epithelial cell line CEPI 17 CL 4.

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