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Formation of ion pairing as an alternative to improve encapsulation and stability and to reduce skin irritation of retinoic acid loaded in solid lipid nanoparticles

Gisele A. Castro[a], Anna Luíza L.R. Coelho[a], Cleida A. Oliveira[b], Germán A.B. Mahecha[b], Rodrigo L. Oréfice[c], Lucas A.M. Ferreira[a,*]

[a] Department of Pharmaceutics, Faculty of Pharmacy, Federal University Minas Gerais, Brazil
[b] Department of Morphology, Federal University Minas Gerais, Brazil
[c] Department of Metallurgical and Materials Engineering, Federal University Minas Gerais, Brazil

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ABSTRACT

This work aims to investigate the influence of the formation of ion pairing between all-*trans* retinoic acid (RA) and a lipophilic amine (stearylamine; STE) on the drug encapsulation efficiency (EE) and stability of solid lipid nanoparticles (SLNs). The SLNs were characterized for EE and size. The EE and particle size were significantly improved and reduced, respectively, when the surfactant or co-surfactant concentration increased. However, while the formulation without STE allowed only 13% of RA encapsulation, the EE for RA–STE-loaded SLNs was 94%. The stability studies showed a significant decrease in EE for the SLNs without STE, while, for SLNs loaded with RA and STE, the EE remained constant after 360 days. The interactions among ion pairing components and the lipid matrix were investigated through small-angle X-ray scattering (SAXS). The SAXS analysis revealed the presence of RA in the crystalline form in SLNs without ion pairing, while crystalline RA was not observed in SLNs loaded with RA/amine. Skin irritation studies showed that the SLNs loaded with the ion pairing were significantly less irritating when compared to the marketed RA-cream. This novel SLN formulation represents a promising alternative for topical treatment of acne with RA.

1. Introduction

Acne vulgaris is a frequent condition affecting millions of people and according to the evolution, acne is classified as mild, moderate or severe. Topical treatment is the first choice in mild and moderate acne, whereas systemic therapy is used to treat severe and moderate cases (Gollnick et al., 2003; Leyden, 2003).

Topical treatment of mild to moderate acne with all-*trans* retinoic acid (RA) has shown to be efficient and occupies a prominent position among the alternatives of treatment (Mills and Berger, 1998; Webster, 1998). Nevertheless, topical application of RA is followed by a high incidence of side-effects such as: sensitiveness to sunlight, eczematous irritation, erythema varying from mild to severe, as well as dryness and scaling (Ellis et al., 1998; Webster, 1998). These occurrences diminish the patient compliance, compromising the efficacy of the therapy (Gollnick and Schramm, 1998). To reduce these side-effects the use of novel drug delivery systems, which present potential to reduce such occurrences without reducing the efficacy, has been proposed (Castro and Ferreira, 2008).

Strategy for the new formulations includes modifying local bioavailability, controlling RA release through polymers or polymeric microspheres (Lucky et al., 1998; Rolland et al., 1993) or enhancing drug skin penetration with liposomes (Contreras et al., 2005). Nevertheless, problems related to physical and chemical stability of liposomes constitute obstacles to their wider therapeutic use. In addition, no satisfactory and accepted scaling-up method exists for microspheres (Dingler and Gohla, 2002). An interesting alternative for RA encapsulation could be the use of solid lipid nanoparticles (SLNs).

SLNs have gained attention as particulate system to improve the delivery and stability of drugs. These systems may be easily transposed to industrial scale as they do not require the use of organic solvents (Jenning et al., 2002; Müller et al., 2000). However, the RA encapsulation in SLNs is usually low (Jenning and Gohla, 2001; Shah et al., 2007) unless a high surfactant/lipid ratio is used (Lim and Kim, 2002; Hu et al., 2004). This favors the drug location at water–oil interface, because of the RA amphiphilicity (Kayali et al., 1991), consequently diminishing the benefits obtained by the encapsulation in lipid matrix (increased stability, controlled release, targeting effect).

* Corresponding author at: Av Antônio Carlos, 6627, Faculty of Pharmacy, Department of Pharmaceutics, Campus Pampulha - UFMG, CEP 31270-901, Belo Horizonte (MG), Brazil. Tel.: +55 31 34996939; fax: +55 31 3499 6830.
E-mail addresses: lucas@farmacia.ufmg.br, lucas.ferreira@pq.cnpq.br (L.A.M. Ferreira).

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The present research group recently reported that the formation of an ion pairing between RA and amines is a suitable alternative to increase drug encapsulation in SLNs (Castro et al., 2007). Thus, this step of our research aims to investigate the influence of ion pairing between RA and a lipophilic amine on the drug encapsulation and stability of SLNs, as well as on interactions with the lipid matrix, through small-angle X-ray scattering (SAXS). Moreover, the potential skin irritation of SLNs loaded with ion pairing (RA/amine) was compared to the marketed RA-cream when applied to a well-known animal model (rhino mice)—a mutant strain of hairless mouse widely used for the evaluation of the comedolytic activity of anti-acne drugs or formulations (Sakuta and Kanayama, 2005).

2. Materials and methods

2.1. Materials

Retinoic acid (RA) and propyleneglycol were provided by BASF (Ludwigehafen, Germany). The cholesterol (CHO), polyoxyl 20 cetyl ether (hydrophilic surfactant, HLB = 15.7; Brij 58), stearylamine (octadecylamine; STE), ethylenediaminetetraacetic acid (EDTA) and butylated hydroxytoluene (BHT) were purchased from Sigma Chemical Co. (St. Louis, USA). Compritol 888 ATO [glyceryl behenate, mixture of mono, di and triacylglycerols of behenic acid (C22)] was supplied by Gattefossé (Saint Priest, France). Hydroxyethylcellulose (Natrosol 250 HR) was obtained from Aqualon (Sao Paulo, Brazil). Vitanol A® cream (Stieffel, Brazil) was purchased from local market. All chemicals were of analytical grade.

2.2. Methods

2.2.1. Preparation of SLNs

SLNs were prepared by the hot melt homogenization method using an emulsification-ultrasound (Ultra-cell 750 W, Sonics Materials Inc., USA). First, oily (OP) and aqueous (AP) phases were weighed separately and heated to 85 °C. Keeping the temperature at 85 °C, AP was gently dropped into the OP with constant agitation, at 8000 rpm in an Ultra Turrax T-25 homogenizer (Ika Labortechnik, Germany). This emulsion was immediately introduced to the high intensity probe sonication (20% amplitude), for 5 min, using a high intensity ultrasonic processor (Microprocessor controlled, 750 W model; Sonics Materials Inc., USA). For the preparation of RA-loaded SLNs, Compritol 888 ATO was selected as a solid lipid component that will constitute the core of SLNs. The influence of surfactant (polyoxyl 20 cetyl ether) and co-surfactant (CHO) concentration as well as of the formation of an ion pairing between RA, a lipophilic acid, and a lipophilic amine (STE) was investigated (Table 1). The pH of the SLNs containing the ion pairing (RA and STE) was adjusted to 7.5 with a solution of 0.01 M HCl (Digimed DM 20, Brazil). Amine:RA molar ratio was of 1:1. However, the amine concentration in the SLN was enough to eliminate the free acidity from solid lipid (Maximum acidity: 4 mg KOH/g of lipid).

2.2.2. Particle size analysis

The mean particle diameter of SLNs in the dispersion was determined by photon correlation spectroscopy (PCS) using a Zetasizer 3000HSA (Malvern Instruments, UK), at a fixed angle of 90° and 25 °C. The SLN dispersions were diluted in distilled and filtered (cellulose ester membrane, 0.45 μm, Millipore, USA) water up to a counting of 50–300 Kcps (1000 counts per second). The RA-loaded SLNs were analyzed after filtration (cellulose ester membrane, 1.2 μm, Millipore, USA) to remove drug crystals. The accumulated data on particle size, assumed as spheres, were assessed and expressed as effective diameter evaluated through intensity. The maximum value acceptable for polydispersity index (PI) was 0.7. All measurements were performed in triplicate.

2.2.3. Zeta potential

Zeta potential measurements were carried out using a Zetasizer 3000HSA (Malvern Instruments, UK) at temperature of 25 °C. Before the measurements, SLN dispersions were diluted in filtered 1 mM NaCl solution (cellulose ester membrane, 0.45 μm, Millipore, USA) up to a counting of 100–1000 Kcps. All measurements were performed in triplicate.

2.2.4. Drug encapsulation efficiency

Encapsulation efficiency (EE) for RA was evaluated through the determination of RA concentration in SLNs before (total RA) and after filtration (cellulose ester membrane, 1.2 μm, Millipore, USA), as previously described (Castro et al., 2007). The influence of the RA concentration in the external aqueous phase of the SLNs was considered non-significant. Our previous data, obtained through ultrafiltration of RA-loaded SLN, confirmed these findings. The absence of RA crystals after filtration was confirmed by polarized light microscopy (Leica DML Microscope, Germany). The RA crystals present characteristic forms and can be easily distinguished from SLNs (Jenning and Gohla, 2001). RA concentration in SLNs (before and after filtration) was determined according to the method described by The United States Pharmacopeia (US Pharmacopoeia, 2003). Briefly, an aliquot of the SLN dispersion was dissolved in tetrahydrofuran (THF) and, later, diluted in a mixture of acetonitrile, distilled water and phosphoric acid (80:19.9:0.1). This mixture keeps the RA in solution (dissolved), but causes lipid precipitation. This dispersion was filtered in a 0.45 μm Millex HV filter (Millipore, USA) and analyzed by HPLC. Total RA concentration in SLNs was always over 90% of the total added. EE was calculated using the formula:

$$\mathrm{EE}\,(\%) = \left(\frac{\text{filtered RA}}{\text{total RA}}\right) \times 100$$

HPLC consisted of a Waters 515 HPLC Pump (Milford, USA), a Waters 717 Plus Auto-sampler (Milford, USA) and a detector UV–vis Waters TM 481 (Milford, USA). A reverse-phase column (C_{18}) with 125 mm of length and particles of 4 μm (LichroCart 125-4, Merck, Germany) was used. The mobile phase was constituted of a mixture of acetonitrile, distilled water and phosphoric acid (80:19.9:0.1). The detection was carried out at 340 nm, with a flow rate of 1.0 mL/min and 20 μl of sample. The retention time obtained was about 7.5 min. The five-point linear regression analysis resulted in the linear equation: $y = -2116 + 143{,}277x$ ($r = 0.99946$).

2.2.5. Synchrotron small-angle X-ray scattering (SAXS)

To investigate the influence of ion pairing on the structure of the SLN lipid matrix, the SAXS measurements were performed using a beam line from the National Synchrotron Light Laboratory (LNLS, Campinas, Brazil). The white beam was monochromatized ($\lambda = 1.608$ Å) and horizontally focused by a cylindrically bent and asymmetrically cut, single silicon crystal. A position sensitive X-ray detector (PSD) and a multichannel analyzer were used to determine the SAXS intensity. The X-ray scattering intensity, $I(q)$, was experimentally determined as a function of the scattering vector "q", whose modulus is given by $q = (4\pi/\lambda)\sin(\theta)$, where λ is the X-ray wavelength and θ represents half the scattering angle. Each SAXS pattern corresponds to a data collection time of 900 s. The parasitic scattering intensity produced by the collimating slits was subtracted from the experimental scattering intensity produced by all the studied samples. All SAXS patterns were corrected for the non-constant sensitivity of the PSD, for the time varying intensity of the direct synchrotron beam, and for differences in sample thickness. Because of the normalization procedure, the SAXS intensity

Table 1
Composition (% w/w) of solid lipid nanoparticle (SLN) formulations.

Ingredient	SLN A	SLN B	SLN C	SLN D	SLN E	SLN F
Oil phase						
Compritol 888 ATO	10	10	10	10	10	10
Retinoic acid (RA)	0.1	0.1	0.1	0.1	0.1	0.1
Polyoxyl 20 cetyl ether	1	2	4	2	2	2
Cholesterol	–	–	–	0.5	1.0	–
Stearylamine (STE)	–	–	–	–	–	0.3
BHT	0.01	0.01	0.01	0.01	0.01	0.01
Water phase						
Propyleneglycol	10	10	10	10	10	10
EDTA	0.01	0.01	0.01	0.01	0.01	0.01
Distilled water qs	100	100	100	100	100	100

was determined for all samples in the same arbitrary units so that they could be directly compared. The sample–detector distance of 551.6 mm was used during the measurements. Pure materials (RA, Compritol, STE), as well as the lyophilized SLN samples (72 h at a temperature of −45 °C), were placed between two sheets of mica. The results were subtracted from the obtained background using only the layers of mica in the system.

2.2.6. Stability studies

To investigate the influence of ion pairing on stability, two SLN formulations, with or without STE, which was used for formation of an ion pairing with RA, were prepared. The two SLN formulations with (RA–STE-loaded SLNs) and without STE were injected into 15 ml Falcon tubes within a nitrogen atmosphere and storage at 25 °C in the dark. At intervals of specified time, the particle size, zeta potential, and EE of SLNs were determined.

2.2.7. RA-induced skin irritation

The skin irritation potential of RA–STE-loaded SLNs was compared to a marketed RA-cream (Vitanol®, Stieffel) when applied to a well-known animal model (rhino mice)—a mutant strain of hairless mouse widely used for the evaluation of the comedolytic activity of anti-acne drugs or formulations. Female mice (7–9 weeks old) were divided into three groups of four animals and treated daily by topically applying 100 μL of RA–STE-loaded SLNs or marketed RA-cream, both containing 0.05% RA, to the back (6 cm^2) for 10 days. Hydroxylethylcellulose (0.8%) was used to gel the SLN dispersions loaded with RA to facilitate the application. Mice from the control group were covered with a placebo gel. Animals were examined for signs of skin irritation each day before RA treatment throughout the treatment period and given an overall "irritation score" of 0 to 4: 0 = no difference between the RA-treated animals and control, 1 = light erythema, 2 = well defined erythema, 3 = strong erythema, and 4 = very strong erythema with presence of a scar. Experiments followed an approved protocol by the Animal Ethics Committee from the Federal University of Minas Gerais (UFMG).

3. Results and discussion

3.1. Influence of concentration of surfactant

Since the surfactant concentration in the SLN formulation is very important to obtain high EE and long term physical stability, the influence of polyoxyl 20 cetyl ether concentration was evaluated. Table 2 shows the influence of surfactant concentration (or surfactant/lipid ratio) on the size and EE of RA in SLNs. The mean particle size of RA-loaded SLNs drastically decreased (682 ± 26, 374 ± 1 and 228 ± 3 nm) when the surfactant concentration increased (1, 2 and 4%, respectively). On the other hand, the EE was significantly improved (6.1 ± 0.8, 13.1 ± 0.7 and 34.7 ± 3%, respectively) when the surfactant concentration (or surfactant/lipid ratio) increased. These findings are consistent with previous observations, which showed that the increased amount of surfactant improved the loading capacity of RA in SLNs (Lim and Kim, 2002). The augmented loading of RA through an increase in surfactant concentration suggests that RA may be embedded in the surfactant layer rather than be incorporated into the lipid matrix. Therefore, this favors the interfacial location of RA (Kayali et al., 1991), consequently diminishing the benefits obtained by the encapsulation in lipid matrix (increased stability, controlled release, targeting effect). Furthermore, it could be observed that if the lipid concentration is gradually reduced, but with a constant surfactant concentration (increased surfactant/lipid ratio), the EE is gradually improved (data not shown).

3.2. Influence of cholesterol concentration

The addition of a co-surfactant, such as CHO, can be an interesting alternative in obtaining SLNs with smaller sizes and higher EE. As can be observed in Fig. 1, the EE for RA was improved by the increase in CHO concentration. The EE for RA-loaded SLNs containing 0.0%, 0.5%, and 1.0% of CHO was of 13 ± 1.4%, 15.9 ± 1.2%, and 21.7 ± 2.3%, respectively. The mean particle size of RA-loaded SLNs

Table 2
Influence of surfactant concentration (or surfactant/lipid ratio) on particle size and the encapsulation efficiency (EE) for RA in SLNs.

Surfactant (%)	Surfactant/lipid ratio	EE (%)	Size (nm)
1	0.1	6.1 ± 0.8	682 ± 26
2	0.2	13.1 ± 0.7	374 ± 1
4	0.4	34.7 ± 2.9	228 ± 3

Data are shown as means ± SD (n = 3).

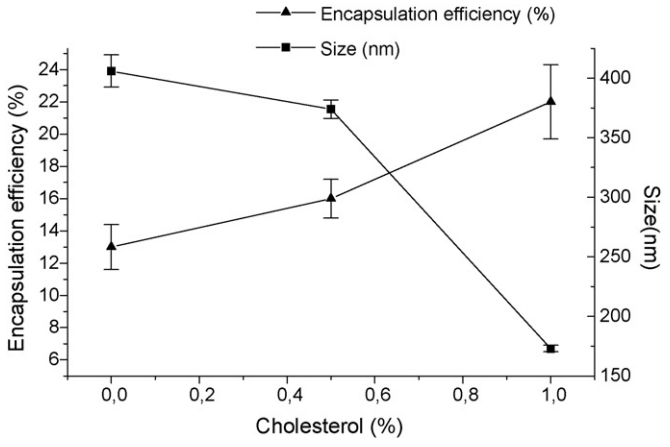

Fig. 1. Influence of cholesterol concentration on particle size and EE for RA in SLNs. Data are shown as means ± SD (n = 3).

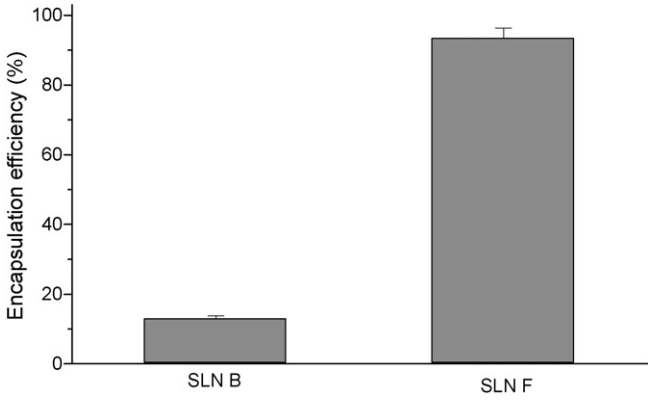

Fig. 2. Influence of RA-stearylamine ion pairing (RA–STE) on the EE for RA in SLNs. Data are shown as means ± SD ($n = 3$).

drastically decreased (406 ± 14; 374 ± 8 and 173 ± 3 nm, respectively). Therefore, the EE for RA was also improved and the size reduced, when the CHO concentration increased. In this case, the CHO adsorption into the interface, in association with the surfactant, appears to be the most plausible explanation for the reduced particle size. On the other hand, this phenomenon provides a higher superficial area and favors the interfacial adsorption of RA, thus explaining the increase in EE. An additional explanation for the improved EE would be the increased number of lattice defects triggered by the introduction of CHO in the lipid matrix structure, which is related to easier drug incorporation in SLNs.

3.3. The ion pairing influence

Fig. 2 shows the influence of ion paring (RA–STE) on RA encapsulation in SLN formulations. The data clearly show that the ion pairing incorporation in the SLN formulation led to a spectacular improvement on the EE. While the formulation without ion paring (SLN B) allowed only 13.1 ± 0.7% of RA encapsulation, the SLN loaded with the RA–STE ion pairing (SLN F) significantly improved the EE (93.5 ± 2.8%). A plausible explanation for EE increase for RA in SLN could be the formation of an ion pairing between RA and the lipophilic amine. The ion pairing increases the lipophilic properties of the drug making easier its incorporation into the lipid matrix. These findings are in agreement with previous observations which showed that loading capacity in SLN is related to drug lipophilicity (Münster et al., 2005; Schäfer-Korting et al., 2007). The STE improved the RA interactions with the SLN lipid matrix and to better understand these interactions, the SLN F was compared to the SLN B using the SAXS analysis.

3.4. SAXS

Systems and materials can scatter X-rays at low angles if they are composed of multiples phases with distinct electron densities and nanometric dimensions. Therefore, SAXS (small-angle X-ray scattering) is a useful analytical technique to study phase-separated nanomaterials. Scattering peaks in SAXS data can most commonly be associated with the presence of distinct phases. Their "q" value can be converted to the distance between the scattering sites by applying Bragg's law ($d = 2\pi/q_{max}$, where q_{max} = value of "q" at the scattering peak).

The SAXS patterns (Fig. 3A) clearly show the presence of a scattering peak at $q = 3.7\ nm^{-1}$ due to the crystalline form of RA in the SLNs without ion pairing (SLN B). However, crystalline RA was not observed in SLNs loaded with the ion pairing (SLN F). The intense interaction of STE with the SLN lipid matrix can be observed in Fig. 3B, considering that it was impossible to observe the presence of the lipophilic amine when dissociated from the lipid matrix in SLN F (scattering peaks, due both to STE and RA, were not observed in SAXS data associated with SLN F). In this case, this amine was associated with RA, forming the ion pairing. These findings are consistent with our previous studies (Castro et al., 2007), where the differential scanning calorimetry (DSC) techniques showed an extensive combination among the constituents of the ion pairing and the SLNs.

The SAXS patterns also provided information about how the crystalline units of SLNs are internally organized within an amorphous matrix (Fig. 4). Crystalline regions separated by distances (d; $d = 2\pi/q_{max}$), within a range of 2–6 nm, were observed when comparing the patterns of Compritol (Fig. 4A) with that of the two SLN formulations (Fig. 4B and C). However, reflections at 7.85 nm are visible only in the SLN B (Fig. 4B), suggesting the presence of phase-separated RA between the crystalline regions and not only inside them. The RA localization between the crystalline regions of the SLN lipid matrix may well represent another evidence of the weak interaction between the drug and the lipid matrix. This can favor the displacement of RA to the external phase of SLN suspension, consequently decreasing the EE over time.

In contrast, for SLN F, where the ion pairing triggered an intense interaction with the lipid matrix, RA (in association with STE) was completely inserted within the crystalline regions of the lipid, and reflections at 7.85 nm were not observed (Fig. 4C). In this case, only the Compritol long spacing at 6.28 nm could be observed (see also Fig. 4A). Moreover, the width of the scattering peaks indicates differences among the nanostructures. Thus, the narrower peaks, observed for SLN B (Fig. 4B), could be attributed to the presence of symmetrical and organized scattering regions with uniform distances among them. In contrast, the wider peaks, observed for SLN

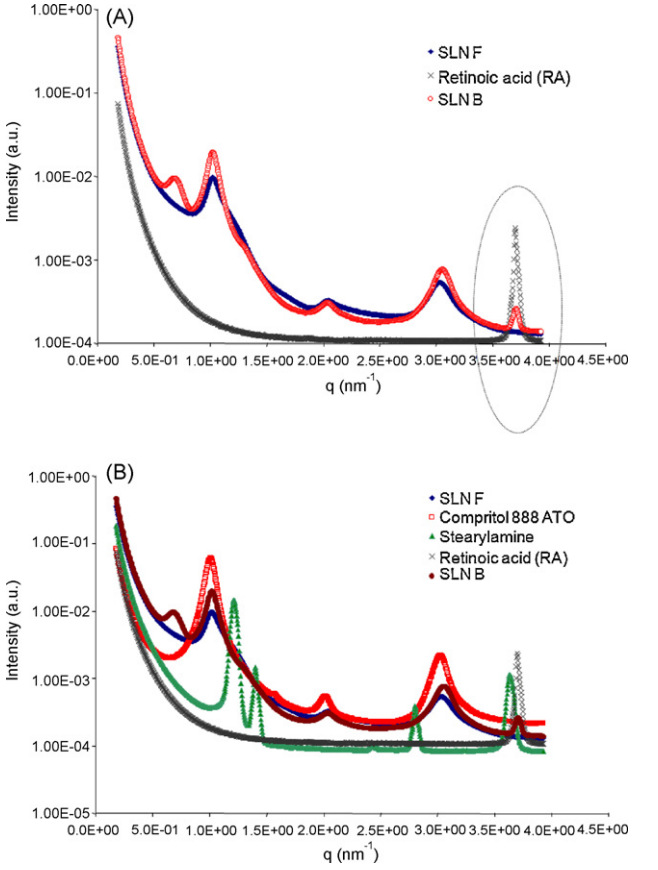

Fig. 3. (A) SAXS patterns for retinoic acid (RA), SLN B and SLN F. (B) SAXS patterns for SLN B, SLN F and its main components (RA, STE and solid lipid).

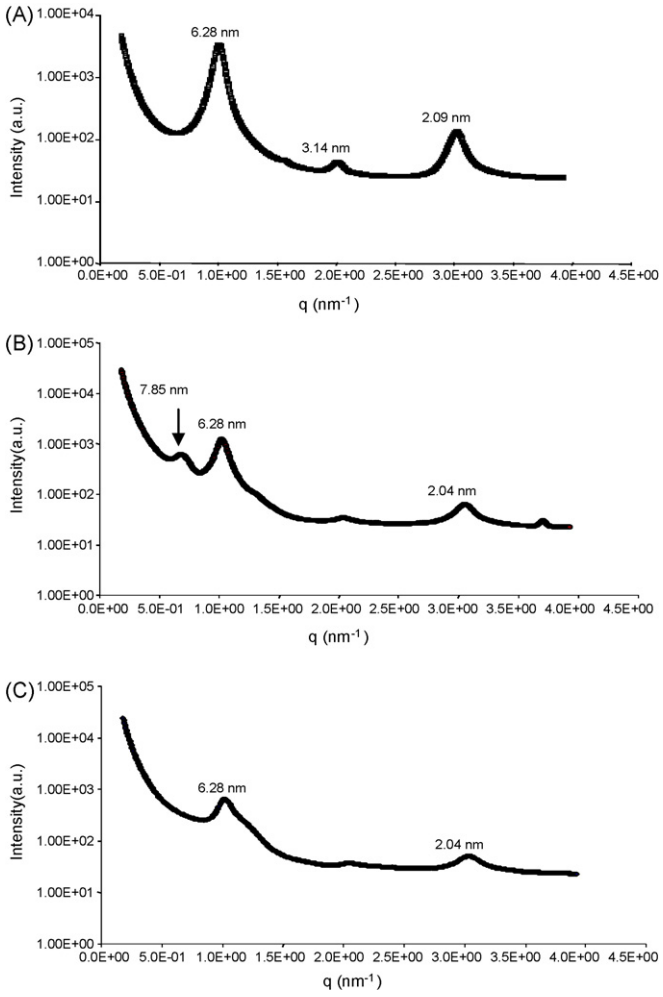

Fig. 4. SAXS patterns for solid lipid (A), SLN B (B) and SLN F (C).

F (Fig. 4C), indicate a disorganized lipid structure, most likely due to the presence of ion pairing, which in turn contributes to the higher drug loading capacity (Westesen et al., 1997; Jenning et al., 2000; Jenning and Gohla, 2001).

The analysis of the scattering center orientation from SLN B and SLN F provided information about the differences in particle arrangements between the two formulations (data not shown). For SLN B, an isotropic particle arrangement was visible, which could also be observed for solid lipid, STE, and RA. However, for RA–STE-loaded SLNs (SLN F), the SAXS analysis showed an anisotropic particle arrangement with preferential orientation in the y direction. According to Bunjes and Unruh (2007), the strong radial anisotropy of all interference maxima indicates the formation of large liquid crystalline domains of particles placed in parallel. Therefore, the presence of the ion pairing (AR + STE) in SLN F contributes to enhance the anisotropy of the solid lipid crystal structures. These findings confirm our previous data (Castro et al., 2008), where the scattering pattern of ion pairing (RA + STE), obtained by WAXS analysis, showed an amorphous-type pattern, with no similarity to those observed for the SLN individual components (RA and STE).

3.5. Stability studies of SLNs with and without RA–STE ion pairing

To compare the influence of the ion pairing on stability, two SLN formulations, with and without STE, both with a high EE for RA, were prepared. Our previous studies showed that the RA encapsulation in SLNs can be improved by increasing the surfactant (or high surfactant/lipid ratio) and CHO concentration or, alternatively, through the addition of a lipophilic amine (STE). Thus, RA-loaded SLNs without STE, with high EE (~90%), were obtained using a high surfactant/lipid ratio and CHO, while RA–STE-loaded SLNs (with ion pairing) were prepared by adding STE. Subsequently, the stability studies of these formulations were conducted.

As shown in Table 3, after 90 days of storage at 25 °C, no dramatic change in particle size was observed for both SLNs (with and without STE). In addition, SLN were also characterized for EE and zeta potential. It is important to note that the zeta potential for SLNs without STE was negative, while the incorporation of STE in the lipid composition increased the zeta potential values, which in turn rendered a positive outcome for RA–STE-loaded SLNs. A progressive decrease in zeta potential was observed for both formulations, which was more evident for SLNs without STE when compared to RA–STE-loaded SLNs.

Marked differences between formulations were observed when the data concerning the RA encapsulation in SLNs were evaluated. A significant decrease in EE was observed for the SLNs without STE, since the EE had fallen by about 52% only 30 days after storage at 25 °C. In this point, RA orange crystals were clearly observed in the SLN suspension. Therefore, our data showed that although a high EE (89 ± 2%) was obtained using an increased surfactant (or high surfactant/lipid ratio) and co-surfactant (CHO) concentration, it could not be maintained over the time. These findings suggest that a high surfactant/lipid ratio favored the interfacial localization of RA, which was moved to the external phase of SLN suspension and precipitated over the time.

In significant contrast, the EE for RA–STE-loaded SLNs was high (100 ± 1%) and remained constant after 90 days of storage at 25 °C (97 ± 5%). Thus, the stability studies of this formulation were prolonged and the parameters (size, zeta potential, and EE) were evaluated over 360 days (data not shown). At the end of one year, the size and EE remained constant. These exciting results showed that it was possible to produce RA-loaded SLNs with high EE and stability by employing RA–STE ion pairing. Therefore, the RA–STE-loaded SLNs offer a promising and stable delivery system for poorly soluble drugs, such as RA.

Table 3
Stability (at 25 °C) of the SLN formulations with (RA–STE-loaded SLNs) and without STE.

Parameters	SLNs without STE			RA–STE-loaded SLNs		
	T (days)			*T* (days)		
	0	30	90	0	30	90
Size (nm)	202 ± 16	154 ± 4	155 ± 9	180 ± 2	209 ± 21	178 ± 12
Zeta potential (mV)	−23 ± 1	−18 ± 1	−10 ± 2	47 ± 4	43 ± 1	39 ± 3
EE (%)	89 ± 2	52 ± 2	55 ± 2	100 ± 1	96 ± 4	97 ± 5

Data are shown as means ± SD ($n = 3$). Composition of SLN formulations was 1% Compritol 888 ATO, 0.5% polyoxyl 20 cetyl ether, 0.1% CHO, 10% propylenoglycol, 0.01% EDTA, 0.01% BHT, 0.01% RA, 0.1% STE (only for RA–STE-loaded SLNs) and distilled water.

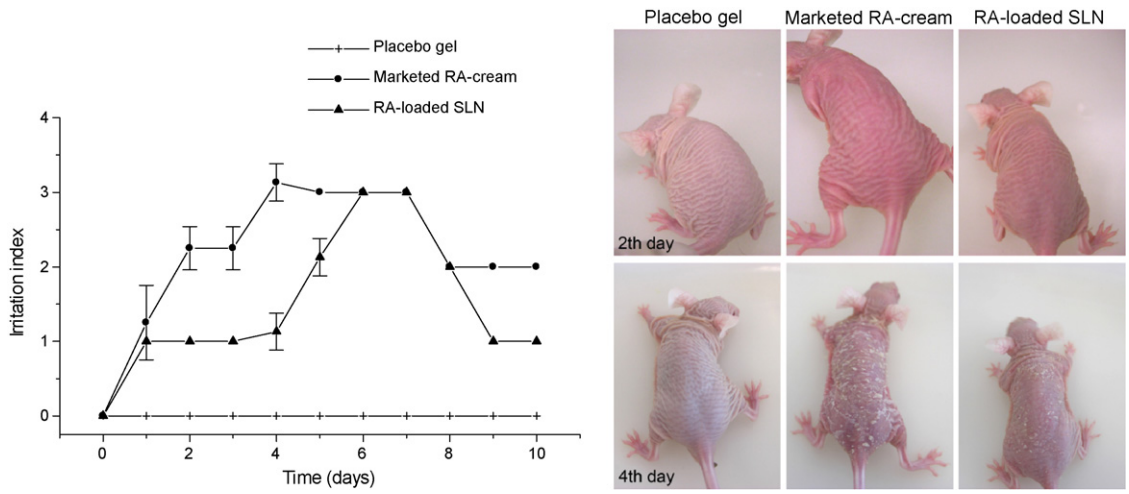

Fig. 5. RA-induced skin irritation in female rhino mice during treatment with the placebo gel and marketed RA-cream and RA–STE-loaded SLN, both containing 0.05% RA. Each animal was given a score of 1–4 based on four criteria indicating skin irritation as described in Section 2. Data are shown as means ± SD ($n = 4$). Insert: skin appearance during treatment with the placebo and formulations on the 2nd and 4th day after initiation of treatment.

3.6. RA-induced skin irritation

Although the RA therapy presents several beneficial physiological effects (regulation of epithelial cell growth and differentiation, sebum production, and collagen synthesis), the skin irritation provided by topical application of RA strongly limits its utility and acceptability by patients. Controlled drug delivery systems, like SLNs, can be an interesting alternative to protecting the skin from direct contact with the drug, which is encapsulated in the lipid matrix. This allows for a gradual drug delivery and presents the potential to reduce RA-induced skin irritation. To test this hypothesis, the skin irritation of SLN formulation containing the ion pairing (RA–STE-loaded SLNs) was evaluated in comparison with the marketed RA-cream, both containing 0.05% RA. The data obtained from this investigation, carried out on rhino mouse, are illustrated in Fig. 5. The data showed that cumulative applications of RA–STE-loaded SLNs and the placebo gel, when compared to marketed RA-cream, resulted in a considerably reduced skin irritation. The irritation index was significantly lower ($P < 0.05$) for animals treated with the RA–STE-loaded SLNs when compared to those treated with the marketed RA-cream on days 2, 3, 4, 5, 9, and 10 after the beginning of treatment (Fig. 5). Furthermore, the animals treated with marketed RA-cream, as compared to the animals treated with the RA–STE-loaded SLNs, presented a higher level of epidermal flaking. Therefore, RA–STE-loaded SLNs were significantly less irritating to the skin as compared to the effects produced by the marketed RA-cream. These data clearly indicated its potential in improving the skin tolerability of RA.

The insert in Fig. 5 demonstrates the typical skin appearance of one treated animal in each group (placebo gel, marketed RA-cream, and RA–STE-loaded SLNs) on days 2 and 4 after the beginning of treatment. Throughout the treatment, erythema and epidermal flakings were more intense in animals treated with the marketed RA-cream when compared to those treated with placebo and RA–STE-loaded SLNs. On the 2nd day, the animals treated with the marketed RA-cream showed a well-defined erythema, while in those treated with RA–STE-loaded SLNs only a mild erythema could be observed. The animals treated with the marketed RA-cream showed intense scaling on the 4th day, while the intensity of epidermal flaking was lower in those treated with RA–STE-loaded SLNs. In animals treated with the placebo gel, no level of skin irritation or epidermal flaking could be observed.

The reduced skin irritation observed in animals treated with RA–STE-loaded SLNs can be attributed to several factors. SLNs have shown a potential for controlled release, allowing for a gradual distribution of the drug in the skin (Jenning et al., 2000; Müller et al., 2002), as well as to reduce the contact of the acidic function of RA (–COOH) with the skin, thus resulting in reduced irritation (Yamaguchi et al., 2005). The findings described here provide statistical evidence for this phenomenon (reduced irritation) and are consistent with previous observations, which showed that the topical application of SLNs loaded with RA reduced drug-induced skin irritation in rabbits (Shah et al., 2007; Mandawagade and Patravale, 2008).

Finally, the SLNs developed in the present study could show more advantages as carriers of RA for the topical treatment of acne than those previously described, due mainly to high drug encapsulation efficiency (almost 100%) and stability. The incorporation of the lipophilic amine represents an exciting alternative to improve encapsulation and stability as compared to well-known methods, such as the selection of surfactant (or its concentration) and the lipid matrix.

4. Conclusion

In summary, a novel SLN formulation loaded with RA, a lipid acid, and a lipophillic amine (STE) has been designed and evaluated herein. Using an inexpensive, quick, and simple method, which does not require the application of organic solvents, it became possible to obtain high loading capacity (almost 100%) for RA in SLNs. Furthermore, developed SLNs were stable over 360 days at room temperature. This formulation provides intense interactions between the drug and the SLN lipid matrix. Moreover, a reduced RA-induced skin irritation produced by the RA–STE-loaded SLNs could be demonstrated when compared to that produced by the conventional marketed RA-cream, when both formulations were evaluated *in vivo* in an animal model. These findings suggest that this novel RA–STE-loaded SLN formulation represents a promising alternative for the topical treatment of acne using an all-*trans* retinoic acid.

Disclosure Statement

The authors declare that they have a patent application (no PCT/BR2009/000072) of the SLN formulation.

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