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Research Article

Solid Lipid Nanoparticles as Effective Reservoir Systems for Long-Term Preservation of Multidose Formulations

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Abstract. Cosmetic multidose preparations, as well as pharmaceutical ones, are at risk of contamination by microorganisms, due to their high water content. Besides the risk of contamination during manufacturing, multidose cosmetic preparations may be contaminated by consumers during their use. In this paper, the results of the utilization of nanoparticles as reservoir systems of parabens, the most used class of preservatives, were reported. Two different systems, solid lipid nanoparticles (SLN) made of pure precirol and nanostructured lipid carriers (NLC) made of precirol and almond oil, containing three parabens as single molecules or as a mixture, were prepared and tested. All the systems were characterized for size, polydispersion index, zeta potential and encapsulation efficiency. Release experiments, carried out in steady state and sink conditions, allowed to evidence that both SLN and NLC were able to act as reservoir systems. The antimicrobial activity of the systems was tested against *Candida albicans* ATCC 10231 with repeat insult tests. The results of the release experiments and the antimicrobial tests showed very low water concentration of parabens still maintaining their antimicrobial activity.

KEY WORDS: microbial contamination; multidose formulations; parabens preservatives; solid lipid nanoparticles; sustained release.

INTRODUCTION

The risk of contamination of cosmetic products by microorganisms is a problem not yet solved. Cosmetics are highly prone to this risk because of their usually high water content (1). Manufacturing processes can represent the first source of contamination and, in this sense, over the last years the application of the GMPs guidelines helped preventing contamination during production. On the other hand, multidose formulations have an intrinsic problem arising from their use and storage after opening. In fact, they can be turned contaminated after every single use, and they are often stored for long periods of time after opening at not suitable conditions and used carelessly. For these reasons, cosmetic formulations need to be always protected against microbial contamination with the addition of preservatives (2,3).

Notwithstanding the high number of allowed preservatives [56 different preservatives are currently permitted in cosmetic products in the European Community (4)], parabens, esters of 4-hydroxybenzoic acid, are the most used preservatives in cosmetics (5,6). They are found in most of the U.S. and European cosmetic formulations (1,7) because they are generally recognized as safe. Anyway, the presence of an additive always represents a problem for safety. From this point of view, the label of cosmetics with the descending order of ingredient concentration should represent the first level of protection for consumers, together with the limits imposed for each single additive by laws (4,8). However, it has been shown that sometimes there is no complete conformity between labels of cosmetics and their actual composition; in addition, most consumers may not fully understand the meaning of labels (3). Moreover, most of the used preservatives, parabens included, can cause concentration-dependent contact dermatitis (9), and there is an alert for overpreservation of cosmetics (1). As reported by Lundov *et al.* (1), "Research on the antimicrobial efficiency of preservatives in cosmetic products has not been given much attention and currently only few published studies have investigated the concentration-dependent effect of preservatives in cosmetic products".

Keeping all this in mind, this work investigated the possibility of exploiting the reservoir characteristics of solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) for sustained release of preservatives. SLN represent a very useful colloidal carrier system for the controlled release of lipophilic molecules (10,11), and they also proved to be very useful in the cosmetic field (12). By application of the SLN technology to lipophilic preservatives, their concentration in the aqueous phase should be taken low, therefore reducing the risk of allergic contact dermatitis while ensuring the microbiological safety of

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the product. In fact, should a contamination of the product occur, preservatives would be consumed by microorganisms, then they would be promptly released from the reservoir system and the antimicrobial activity restored. With this aim, SLN, made of pure precirol, and NLC, made of mixtures precirol/almond oil, were prepared, and three parabens of different lipophilicity (methyl, propyl and butyl paraben) were encapsulated within them. All these formulations were characterized for size, polydispersion index, zeta potential and encapsulation efficiency. Release studies of the three parabens from the nanoparticles were carried out in steady state as well as in sink conditions. Mixtures of propyl and butyl paraben in different ratios were also co-encapsulated inside SLN systems, and their behaviour was investigated. Finally, the capacity of these formulations of controlling and preventing contamination by repeated microbial insults was studied.

MATERIALS AND METHODS

Materials

All the reagents were of analytical grade. Butyl 4-hydroxybenzoate (butyl paraben, BP), Sephadex G-75, and sodium cholate were purchased from Sigma-Aldrich (Germany). Methyl 4-hydroxybenzoate (methyl paraben, MP) and propyl 4-hydroxybenzoate (propyl paraben, PP) were obtained from Fluka (Switzerland). Pure almond oil was purchased from Agrar (Italy). Precirol ATO 5 (a mixture of 40% tri-, 45% di- and 14% monoglycerides of palmitic and stearic acids) was kindly gifted by Gattefossé (France). Lutrol F68 (poloxamer 188) was from BASF (Germany). Dialysis membranes (cutoff, 12,000–14,000) were from Medicell International (UK). *Candida albicans* ATCC 10231 was obtained from the American Type Culture Collection (ATCC, Rockville, MD, USA), Sabouraud dextrose agar and RPMI 1640 medium from Sigma-Aldrich (St. Louis, Missouri, USA).

Preparation of SLN and NLC

SLN and NLC were prepared with the hot homogenization technique (13,14). Ten grams of Precirol ATO 5 (m.p.= 55.2°C) or a mixture Precirol ATO 5/almond oil 85:15 (*w*/*w*) was heated at 70°C. After complete melting, an aqueous solution (95 ml) of the surfactants (sodium cholate, 1.2 g and poloxamer 188, 3.1 g), heated at the same temperature, was added to the lipids. The mixture was hot homogenized with an Ultra-Turrax T18 Basic (IKA-WERK, Germany) for 10 min at 24,000 rpm and then left to cool down at room temperature. Paraben loaded SLN and NLC were prepared by adding 0.60 g of MP, PP, BP or their mixtures to the melted lipids and following the previously described procedure.

Particle Size Analysis and Zeta Potential Measurements

Particle size analysis of all the SLN and NLC samples was performed by photon correlation spectroscopy (PCS). The PCS analysis gave the mean diameter of the particles (*Z*-average) and the polidispersity index (PDI) as a measure of the width of the particle size distribution. The samples were diluted with twice distilled water and analysed with a Malvern Zetasizer nano ZS90 (Malvern Instruments, UK). The surface charge of all the samples was determined by measurements of the zeta potential carried out with the same instrument.

Encapsulation Efficiency

The amount of encapsulated parabens was determined by extracting the molecule from the nanoparticles, after separation of the un-encapsulated fraction by size-exclusion chromatography. To this aim, aliquots (5 ml) of each nanoparticles suspension were eluted through a column of Sephadex G-75, employing distilled water as eluant. The recovered SLN and NLC were freeze-dried, and then the solid (400 mg) was extracted with methanol (4×10 ml). All the extracts were filtered on paper, poured into a volumetric flask, raised to volume and submitted to UV/Vis spectrophotometric analysis (λ=258 nm). In order to test the accuracy of the extraction method, portions (5 ml) of whole nanoparticles (not passed through Sephadex G-75) were freeze-dried and extracted according to the procedure described above. All the experiments were carried out in triplicate.

Release Studies

The release rate of parabens from SLN and NLC was studied using a dialysis method in both steady and sink conditions. In both cases, dialysis tubes were filled with aliquots (50 ml) of paraben-loaded SLN and NLC, and release studies in distilled water (600 ml) were carried out. The tubes were maintained under stirring (100 rpm) at room temperature. Samples of the release medium were withdrawn according to the methodologies described in sections "Release Studies: Steady Conditions" or "Release Studies: Sink Conditions".

Release Studies: Steady Conditions

At predetermined time points (0, 2, 4, 6, 8, 24, 48, 72, 96 h), samples of the release medium (20 μl) were withdrawn and analysed by HPLC, monitoring parabens at 258 nm. The HPLC system consisted of a Perkin-Elmer Series 200 LC pump, equipped with a 235 Diode Array Detector (USA). The analyses were carried out using a Merck Hibar LiChrocart (250–4, 5 μm) RP-18 column, H_2O/CH_3OH mixture (3:7) as eluant (flow 0.6 ml/min). All the experiments were carried out in triplicate.

Release Studies: Sink Conditions

At predetermined time points (0, 2, 4, 6, 12, 24 h and then once a day up to 9 days), half of the volume of the release medium (300 ml) was withdrawn and replaced with an equal volume of distilled water. Samples were analysed for parabens content by HPLC, according to the previously described procedure. The experiments were carried out in triplicate.

Antifungal Susceptibility Assays

For the antifungal evaluation, *C. albicans* ATCC 10231 (ATCC, Rockville, Maryland, USA) was tested. *In vitro* antifungal activity of methyl, propyl and butyl paraben was evaluated in accordance with the CLSI broth microdilution

methods (15,16). The parabens were first dissolved in dimethyl sulfoxide at concentrations 100 times higher than the highest desired test concentration and then diluted in test medium, in accordance with the procedures of the CLSI. The final concentrations ranged from 1 to 1,000 μg ml^{-1}. Microdilution trays containing 100 μl of serial twofold dilutions of each substance in RPMI 1640 were inoculated with an organism suspension adjusted to attain a final inoculum concentration of 1.0×10^3–1.5×10^3 cells ml^{-1}. The panels were incubated at 35°C and observed for the presence of growth at 48 h. The minimal inhibitory concentration (MIC100) was defined as the lowest concentration that prevented any discernible growth with respect to the control.

Evaluation of the Efficacy of the Antimicrobial Preservation

The antifungal activity of parabens-loaded SLN was evaluated against *C. albicans* ATCC 10231. The tests were conducted in accordance with the EP guidelines (17). The preparations were inoculated with a standardized suspension of *C. albicans* ATCC 10231, corresponding to 10^5–10^6 CFU ml^{-1}. Blank nanoparticles (without preservatives) were inoculated and included in each challenge test as a positive control. Samples were kept at room temperature (20–25°C) protected from light throughout the study. Aliquots (1 ml) of the inoculated nanoparticles were removed immediately (0 day) and after 2, 7, 14 and 28 days for determination of viable cells by plate count. For sample SLN-PP:BP_2:1, aliquots (1 ml) were taken after 0, 2, 7, 14, 21, 28, 35, 42, 49 and 56 days. After sampling at 7, 14, 21, 28 and 35 days, the nanoparticle suspensions were re-challenged by using an inoculum level of 10^5–10^6 cells ml^{-1}. Neutralization conditions, as described in EP protocols, were used to ensure that any residual antimicrobial activity was eliminated (17,18). The number of viable cells (colony-forming units per milliliter of preparation) was determined on Sabouraud dextrose agar in triplicate.

RESULTS AND DISCUSSION

Preservation of multidose formulations from microbial contamination still represents a challenge, for two principal reasons: firstly, notwithstanding production under good manufacturing processes, this kind of products can be turned contaminated after every single use; moreover, all known preservatives can represent a risk for formulation safety. Among the different molecules and strategies allowed, encapsulation of parabens, the most common used preservatives for cosmetics, within lipid nanoparticles, which can act as reservoir systems and release them in a sustained way, was taken into consideration. Parabens are generally recognized as safe molecules, even if, like other preservatives, they are considered responsible for allergic contact dermatitis, a problem that affects around 7% of the world population (19). So, the behaviour of methyl, propyl and butyl paraben loaded, alone or as mixtures, inside SLN, made of precirol, and NLC, made of a mixture of precirol and almond oil, was investigated, and their application as reservoir systems of preservatives and their antimicrobial activity were tested. As known, the partitioning between water and an oily phase increases with the length of the aliphatic chain in position 4 of the aromatic ring of parabens; therefore, the intrinsic chemical properties of the chosen molecules may influence loading into SLN and NLC as well as the release rate from nanoparticles, so making possible modulation of the properties of the designed systems.

Preparation and Characterization of SLN and NLC

SLN and NLC were prepared according to the hot homogenization technique. The composition of all the investigated formulations is reported in Table I.

Even if parabens are allowed in cosmetic formulations in concentrations up to 0.4% for a single paraben and 0.8% for their mixtures (4), a concentration of 0.6%, for both single paraben and their mixtures, was used throughout the study in order to make a direct comparison between the different formulations.

It is well known that the loading capacity of SLN depends on both the solubility of the guest molecule in the lipid and the structure of the lipid matrix. When a highly purified lipid (i.e. tristearin or tripalmitin) is used for the preparation of SLN, an ordered matrix with few imperfections is formed, which cannot accommodate large amounts of guest molecule. Therefore, Precirol ATO5, a mixture of tri-, di- and monoglycerides of palmitic and stearic acids, was chosen as lipid phase. Moreover, it is reported in the literature (13) that blending a solid lipid with oil leads to less ordered lipid matrices, providing the possibility for higher encapsulation efficiencies. For this reason, a mixture of precirol and almond

Table I. Composition of the SLN and NLC Formulations Tested

Formulation	Precirol % (*w*/*w*)	Almond oil % (*w*/*w*)	Lutrol F68 % (*w*/*w*)	Sodium cholate % (*w*/*w*)	MP % (*w*/*w*)	PP % (*w*/*w*)	BP % (w/w)
Blank SLN	10.0	–	3.1	1.2	–	–	–
Blank NLC	8.5	1.5	3.1	1.2	–	–	–
SLN-MP	10.0	–	3.1	1.2	0.6	–	–
SLN-PP	10.0	–	3.1	1.2	–	0.6	–
SLN-BP	10.0	–	3.1	1.2	–	–	0.6
NLC-MP	8.5	1.5	3.1	1.2	0.6	–	–
NLC-PP	8.5	1.5	3.1	1.2	–	0.6	–
NLC-BP	8.5	1.5	3.1	1.2	–	–	0.6
SLN-PP:BP_1:2	10.0	–	3.1	1.2	–	0.21	0.39
SLN-PP:BP_1:1	10.0	–	3.1	1.2	–	0.3	0.3
SLN-PP:BP_2:1	10.0	–	3.1	1.2	–	0.39	0.21

SLN solid lipid nanoparticles, *NLC* nanostructured lipid carriers, *MP* methyl paraben, *PP* propyl paraben, *BP* butyl paraben

Table II. Particles Size, Zeta Potential and Entrapment Efficiency of the SLN and NLC Formulations

Formulation	Mean diameter (nm)	PDI	*Z* potential (mV)	Entrapment efficiency (%)		
				MP	PP	BP
Blank SLN	136.1±2.1	0.265±0.013	−33.2±1.2	–	–	–
Blank NLC	161.3±3.4	0.242±0.031	−32.7±0.6	–	–	–
SLN-MP	145.8±6.5	0.212±0.026	−29.3±1.4	76.2±0.1	–	–
SLN-PP	131.9±5.8	0.250±0.012	−28.9±0.5	–	82.3±12.6	–
SLN-BP	126.9±14.2	0.241±0.022	−27.4±2.2	–	–	93.7±2.7
NLC-MP	129.8±0.2	0.276±0.004	−30.4±3.5	61.4±6.4	–	–
NLC-PP	134.0±6.0	0.236±0.003	−31.0±2.1	–	70.4±10.0	–
NLC-BP	143.6±3.7	0.235±0.019	−28.2±1.2	–	–	97.0±3.5
SLN-PP:BP_1:2	122.1±1.5	0.230±0.012	−27.7±0.8	–	62.3±7.7	84.3±15.7
SLN-PP:BP_1:1	136.6±5.9	0.240±0.013	−26.9±1.2	–	90.7±9.3	91.9±4.2
SLN-PP:BP_2:1	124.7±4.7	0.245±0.007	−28.6±1.2	–	80.9±0.1	99.0±12.6

oil 85:15 (*w*/*w*) was also used for the preparation of lipid nanoparticles. After cooling, all the samples were analysed with PCS for mean diameter and polydispersion index of the particles. The results are reported in Table II.

The presence of almond oil does not deeply affect the characteristics of the particles. It is only possible to evidence small variations of the mean diameter probably due to a lower level of organization of the lipid matrix. The values of zeta potential are always negative as a consequence of the presence of the used anionic surfactant, sodium cholate, which inserts its lipophilic portion inside the particles and exposes the hydrophilic charged one toward the aqueous phase. SLN were loaded with the three parabens alone or in mixture, whereas NLC were loaded only with the three preservatives alone (Table I). The characteristics reported in Table II show that the presence of the preservatives produces only slight modifications in the mean diameter of the particles, which maintain a good homogeneity and are always included in a narrow range from 122 to 146 nm. Zeta potential values are always negative and able to assure the stability of the dispersed system.

Encapsulation Efficiency

In order to determine the amount of parabens loaded inside the nanoparticles, aliquots of each nanosuspension were eluted through a column of Sephadex G-75. After freeze-drying, the residues were extracted with methanol and analysed by UV spectrophotometry for formulations with single paraben and by HPLC analysis for formulations containing mixtures of parabens. The results of these analyses are reported in Table II.

The entrapment efficiency is, as expected, deeply influenced by the lipophilic characteristics of the preservative: the highest the logP value of the paraben, the biggest the encapsulation efficacy. No significant differences in the entrapment efficiency are observed when almond oil was added to precirol for the preparation of nanoparticles. However, it can be evidenced that almond oil increased the entrapment efficiency of butyl paraben from 93 to 97% (Table II: see NLC-BP *vs.* SLN-BP), while it decreased the encapsulation of methyl and propyl paraben by 12–15% (Table II: see NLC-MP *vs.* SLN-MP and NLC-PP *vs.* SLN-PP)

Release Studies

Because spoilage microorganisms must have water for metabolic processes, they will thrive only in the aqueous phase of a formulation. Taking into consideration the lipophilic properties of the encapsulated preservatives, it is necessary to prove their release from the nanoparticles to the water phase of the formulation, where they are needed. Therefore, release studies from

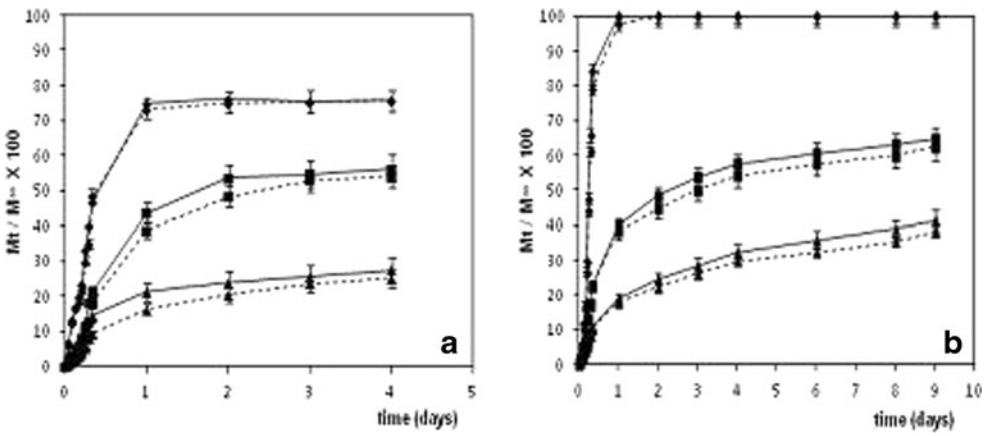

Fig. 1. Release profiles [$(M_t/M_\infty)\times100$] of MP (*black diamond*), PP (*black square*) and BP (*black triangle*) from SLN and NLC, in steady (**a**) and sink (**b**) conditions, respectively. *Solid lines* refer to SLN (SLN-MP, SLN-PP and SLN-BP), *dotted lines* refer to NLC (NLC-MP, NLC-PP and NLC-BP). The release studies were performed in distilled water at r.t.

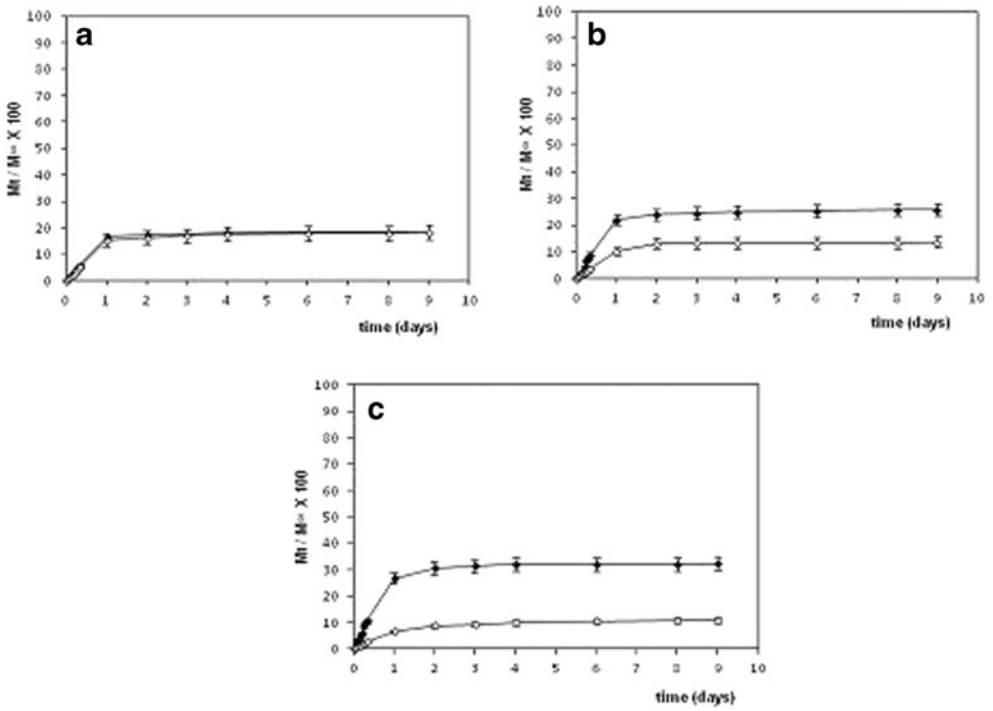

Fig. 2. Release profiles $[(M_t/M_\infty)\times 100]$ of PP (*black diamond*) and BP (*white diamond*) from SLN-PP:BP_1:2 (**a**), SLN-PP:BP_1:1 (**b**) and SLN-PP:BP_2:1 (**c**) in steady conditions. The release studies were performed in distilled water at r.t. for 9 days

formulations SLN-MP, SLN-PP, SLN-BP, NLC-MP, NLC-PP and NLC-BP were performed under two different experimental conditions: steady state and sink conditions. The release profiles are reported in Fig. 1.

The first type of experiments simulates the conditions of the formulation during storage (Fig. 1a, steady state). After an initial burst effect, much more evident for methyl than for propyl and butyl paraben, the concentration of each paraben gets a plateau which depends on its partition equilibrium between the two phases ($\log P_{MP}$, 1.88; $\log P_{PP}$, 2.90; $\log P_{BP}$, 3.41). The presence of almond oil influences only slightly the release profiles and, above all, within the first 24 h of the experiments, and it is more marked for butyl than propyl paraben, whereas it is almost insignificant for methyl paraben.

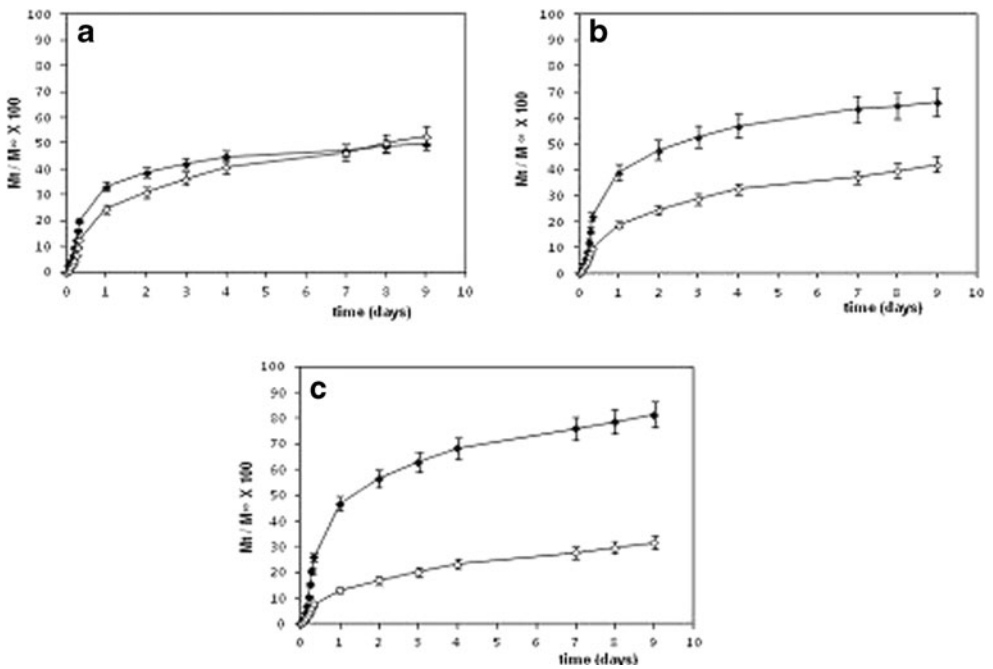

Fig. 3. Release profiles $[(M_t/M_\infty)\times 100]$ of PP (*black diamond*) and BP (*white diamond*) from SLN-PP:BP_1:2 (**a**), SLN-PP:BP_1:1 (**b**) and SLN-PP:BP_2:1 (**c**) in sink conditions. The release studies were performed in distilled water at r.t. for 9 days

The second set of release experiments (Fig. 1b, sink conditions) simulate the use conditions, when the formulation may turn contaminated by consumers. Any presence of microorganisms would consume the preservative and decrease its concentration in the aqueous phase. Therefore, the nanoparticles should act as reservoir systems, releasing the preservative and restoring a concentration sufficient to prevent the growth of contaminating microorganisms.

The SLN capacity of acting as reservoir system is shown both in the steady state (Fig. 1a) and sink conditions (Fig. 1b) and obviously depends on the lipophilic characteristics of the entrapped paraben. As shown by the sink experiments, MP, the most water soluble among the parabens used, is released very quickly, and it diffused out from the nanoparticles almost completely after the first medium replacement. On the contrary, a slower release was observed for both PP and BP and after 9 days, and many replacements of the water compartment, the lipid nanoparticles are depleted only by 60–65% of PP and 30–35% of BP with a slight difference of 5% between the two studied systems. For this reason, mixtures of PP and BP were tested in the next experiments.

The presence of almond oil slightly modifies the properties of the systems slowing down the release of the guest molecules, as already explained for Fig. 1a. Due to the slight effect of the oil on both the entrapment efficiency and the release rate of parabens, it was decided to further study the antimicrobial activity of nanoparticles made of only precirol and encapsulating mixtures of PP and BP.

Preparation and Characterization of SLN Containing Mixtures of PP and BP

A well-designed reservoir system for long-term preservation of multidose cosmetic products should assure the presence of the preservatives in the aqueous phase at concentration high enough to avoid any microbial proliferation. Anyway if the preservative is too much lipophilic, the release rate could be too slow and the preservation of the formulation not guaranteed during all its life; on the contrary, if it is too water soluble it could be released too quickly. Therefore, in order to have a well-preserved formulation, and on the basis of the just performed experiments, PP and BP were co-encapsulated within SLN, in different weight ratios. MP was not taken into consideration because it is immediately released from both SLN and NLC and therefore not suitable for the aim of the work. The composition of the formulations is reported in Table I as SLN-PP:BP_1:2, SLN-PP:BP_1:1, SLN-PP:BP_2:1. Also in this case, the samples were analysed with PCS in order to determine mean diameter, polydispersion index and zeta potential values of the particles (results reported in Table II).

Table III. Antifungal Activity of Parabens Against *C. albicans* ATCC 10231

	MIC (μg/ml)
PP	500
BP	125
PP:BP_1:2	125
PP:BP_1:1	125
PP:BP_2:1	125

Table IV. Results of Challenge Test on the Chosen SLN Formulations Against *C. albicans* ATCC 10231

	Log reduction			
	2 days	7 days	14 days	28 days
SLN-PP	3–4	4	5	5
SLN-BP	4	4	5	5
SLN-PP:BP_1:2	4	4	5	5
SLN-PP:BP_1:1	4	4	5	5
SLN-PP:BP_2:1	4	4	5	5

The characteristics of the nanosuspensions are very similar to those obtained with only one preservative. The particles show nanometric dimensions, good polydispersion index and high absolute value of zeta potential, able to assure a good stability of the disperse system. The release profiles of the parabens from the three systems performed under steady state and sink conditions are reported in Figs. 2 and 3, respectively.

The results of both the release studies show that the adopted ratios of PP and BP are able to give amounts of parabens sufficient to assure the system preservation. In particular, the formulation containing the mixture of PP:BP_2:1 seems to be particularly suitable to obtain a good long-term preservation. In fact, propyl paraben is quickly released from the nanoparticles, reaching a concentration high enough to hypothesize the preservation of the system from its first use, while the slow release of BP should be able to maintain the formulation free from microorganisms for a long time, also after repeated microbial insults. In any case, all the systems containing PP and BP, alone or as a mixture, were exposed to preliminary tests for their antimicrobial activity.

Antimicrobial Tests

Parabens encapsulated or not within lipid nanoparticles were tested against *C. albicans* ATCC 10231. First of all, the minimal inhibitory concentration values (MIC100), defined as the lowest preservative concentration that prevented any discernible growth with respect to the control, relative to single parabens and to mixtures of PP and BP are reported in Table III.

As already known from the literature (20), BP is more active than PP as a preservative. Furthermore, the associations of PP and

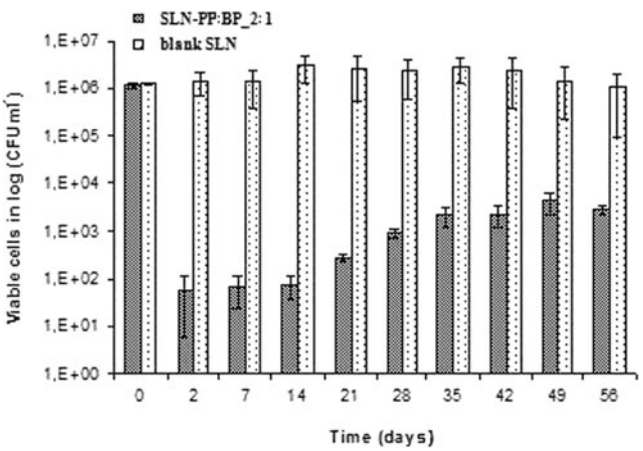

Fig. 4. Results of repeat insult microbial test performed on formulation SLN-PP:BP_2:1 against *C. albicans* ATCC 10231

BP show the same MIC value of BP alone. After that, challenge tests were carried out on all the systems containing PP and BP alone and as mixtures; the results are reported in Table IV.

Blank SLN, without preservatives, were also inoculated and included in each test as positive control. All the formulations were challenged at time zero and re-challenged every 7 days up to 28. They were able to produce more than two logarithmic reductions of viable cells with respect to the control without preservatives, in accordance with the values requested by challenge tests. Moreover, the efficacy of formulation SLN-PP:BP_2:1 goes on unaltered up to 56 days under extreme conditions of microbial contamination (Fig. 4).

CONCLUSIONS

SLN and NLC encapsulating methyl, propyl and butyl paraben with loading capacity up to 97%, depending on the lipophilic characteristic of the guest molecule, were prepared. The obtained systems were able to modulate the release rate of parabens and act as a reservoir system, both in steady state, simulating the storage conditions, and in sink conditions, simulating the use of the product by consumers. SLN systems, loaded with propyl and butyl paraben both alone and as a mixture, were tested against *C. albicans* ATCC 10231, showing a good antimicrobial activity. The best preservative system was obtained when a mixture of propyl paraben/butyl paraben 2:1 was used. In fact, the fast release of the less active propyl paraben toward the aqueous phase and the slow and constant release of the more efficient butyl paraben were able to preserve the formulation from microbial growth for a long time.

SLN technology, applied for sustained delivery systems of lipophilic preservative compounds, could allow lowering the level of preservatives in cosmetics so preventing the risk of their overpreservation and, above all, making possible to limit their level in the aqueous phase, to which the effect of skin irritation may be joined.

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