Molecular Crystals and Liquid Crystals
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl20

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Version of record first published: 28 May 2010

To cite this article: I. Lacatusu, N. Badea, A. Murariu, C. Nichita, D. Bojin & A. Meghea (2010): Antioxidant Capacity of Lipid Nanoparticles Loaded with Rosemary Extract, Molecular Crystals and Liquid Crystals, 523:1, 260/[832]-272/[844]

To link to this article: http://dx.doi.org/10.1080/15421401003719886

Mol. Cryst. Liq. Cryst., Vol. 523: pp. 260/[832]–272/[844], 2010

ISSN: 1542-1406 print/1563-5287 online
DOI: 10.1080/15421401003719886

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This work presents a simple, available and effective method for preparation of lipid nanoparticles loaded with a natural extract containing active principles. The purpose of the research is to synthesize new lipid nanoparticles with enhanced antioxidant activity, by using two types of lipid carriers. The lipid nanoparticles were produced by emulsification with high shear homogenisation method using biocompatible emulsifiers with excellent skin compatibility as surfactants. The developed nanoparticles were characterized for particle size, polydispersity index, zeta potential, morphology, crystallinity of the lipid matrices and antioxidant activity. Both types of synthesized lipid nanoparticles exhibit an improved antioxidant activity.

Keywords Antioxidant activity; lipid nanoparticles; olive oil; rosemary extract

1. Introduction

Solid lipid nanoparticles (SLNs) were developed by Műller and Gasco at the beginning of the 1990s as alternative carrier systems to emulsions, liposomes and polymeric nanoparticles [1,2] for poorly water-soluble compounds. Upon introduction to aqueous biological environments, lipophilic molecules exhibit instability, food interactions, reduced bioavailability, non-specific targeting and other toxic effects that produce undesired immunogenic responses and reduced efficacy [3,4]. By encapsulation of lypophilic compounds in biocompatible physiological lipids which decreases the danger of acute and chronic toxicity, all these problems are avoided. SLN are produced by replacing the liquid lipid (oil) of a traditional o/w emulsion by a solid lipid or a blend of solid lipids [1]. These lipid nanoparticles have gained an increased attention in the pharmaceutical industries because of their ability to overcome deficiencies of nanoscalar colloidal systems; actualy, they combine

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the advantages of lipid emulsion systems and polymeric nanoparticle systems with physical and chemical stability of the encapsulated compound [4].

After SLN development, nanostructured lipid carriers (NLC) were discovered since 1999 as the latest generation of nanoscalar encapsulation systems. The nanostructured lipid carriers (NLC) are distinguishable from solid lipid nanoparticles (SLN) carriers by the composition of the solid particle matrix [5,6]. In the second generation of the lipid nanoparticle technology, the particles are produced using blends of solid lipids and liquid lipids (oils), preferably in a ratio of 70:30 up to a ratio of 99.9:0.1 [3]. In a preferred scenario, the liquid lipids form droplets within the solid lipid particles matrix. According to this model, the NLC nanoparticles are more advantageous carrier systems than SLN because they present a less ordered lipid matrix (also solid at body temperature) which may provide a high loading capacity (e.g., more space for the accommodation of guest molecules due to the liquid lipid) and to a more controlled drug release (due to the encapsulating solid lipid) [3,6]. Some authors defined this imperfectness of crystalline structure as ''the perfectness'' of the NLC system [7]. The structures of lipid nanoparticles depend on the production parameters and the chemical nature of lipid and active ingredients. Besides these, surfactant blend used in the formulation and also viscosity of lipid and aqueous phase have also significant influence on the outcome of the procedure. Therefore, in this study some lipid blends formed by cetyl palmitate (CP), glycerol stearate (GS) and a natural antioxidant oil, unexplored for NLC preparation – olive oil (OO), were chosen as the solid and solid/liquid cores, respectively. In addition, a variety of different emulsifiers have been used to prepare SLN and NLC, including polyoxyethylenesorbitan monooleate (Tween 80), a mixture of alkylpolyglycoside and cetylstearyl alcohol (Emulgade PL 68/50) and phosphatidyl coline (phospholipid from soybean), in combination with a co-surfactant (Synperonic F68). The selection of emulsifiers was based on a few considerents. Firstly, Tween 80 is one of the most employed surfactant in pharmaceutical industry and it has interesting characteristics to be used in drug formulations, because of the presence of poly (ethyleneglycol) (PEG) chains in its structure [8]. Liu *et al.* showed that Tween 80 was the most effective nonionic surfactant to avoid the formation of aggregates [9]. Secondly, Emulgade PL 68/50 is a wax mixture of alkylpolyglycoside and cetylstearyl alcohol used as a non-ionic self emulsifier base in cosmetic oil/water emulsions. It is a biocompatible emulsifier with excellent skin compatibility. Thirdly, by using an emulsifier based on lecithin, the phospholipids bilayer structure which is formed around the lipid core may increase the drug loading capacity and surface modification also enables stabilization of colloidal particles especially when generation of the nanoparticles is carried out in an aqueous medium [10]. Finally, a member of Poloxamer family – Synperonic PE/F 68 was used as co-surfactant in this study. The Poloxamer compounds consist in triblock copolymers that have generated a much interest in the field of drug controlled release due to their ability to form gels in response to changes in temperature. It consists of ethylene oxide (EO) and propylene oxide (PO) monomers in an arrangement that, in aqueous media, allows the formation of self-assembled micelle structures, based on the relative difference in hydrophobicity between PO and EO (the cores of PO and water are surrounded by coronas consisting of EO and water) [11].

The nanoparticles can be divided into two main families: nanospheres, which have a homogeneous structure in the whole particle, and nanocapsules, which exhibit a typical core-shell structure [12]. A main challenge of the formulation of nanoparticles

is adapting the choice of its structure to the final aims of drug delivery: biocompatibility of the lipid matrix, physicochemical properties of the lypophil drug and therapeutic goals. Hence, the objective of this investigation was to develop lipid nanoparticles by SLNs and NLCs loaded with a lypophil antioxidant mixture – Rosemary extract (RE) as a model drug, by facile and simple high shear homogenization technique and to evaluate the viability of SLN/NLC in preserving and improving antioxidant activity of RE as compared to native RE in methanol.

The Rosemary extract belonging to *Rosmarinus officinalis L.* (*family Lamiaceae*) is a vegetal extract rich in flavonoid compounds, that possess a variety of bioactivities, being appreciated for its antioxidant, antimicrobial or antitumoral properties [13,14]. The market for antioxidants supplied from vegetal sources is expected to experience a huge growth and the requirement for natural products is growing rapidly as well. The antioxidant effects of natural extracts are more effective than other many individual antioxidants [15,16]. The antioxidants contained in Rosemary extract protect the body cells against damage caused by free radicals. They include flavonoids, monoterpenes and phenolic diterpenes which are well known for their ability to slow down the production of free radicals [17]. Recent researches revealing even many benefits associated to this vegetal extract, includ its ability to prevent cancer and age-related skin damage. Two of the most important ingredients in Rosemary, which are considered to be largely responsible for many of these therapeutic actions, are caffeic acid and rosemarinic acid – both being potent antioxidant and anti-inflammatory agents [18]. These two natural acids are effective in reducing inflammation which may contribute to asthma, liver disease and heart disease [19]. According to other researchers, Rosemary extract protects the individual components of skin cells, which may prevent age-related skin damage such as wrinkles [20]. Another benefit of Rosemary extract has been shown to be the ability to inactivate toxins and then eliminate them from the liver, before they can inflict any serious damage [21], thus stimulating the liver to work more efficiently.

Therefore, the primary goal of this work was to characterize the processing factors affecting the characteristics of the Rosemary extract loaded into SLNs and NLCs, including the optimal conditions for their preparation, the morphology and thermal behavior of them, as well as the effect of the olive oil on antioxidant capacity of NLCs formulations. The developed RE-SLN and RE-NLC systems may provide a beneficial alternative to conventional antioxidant formulations, which could become attractive colloidal carriers suitable for the local formulations based on active substances (lypophile natural antioxidants) used for cosmetic or pharmaceutic purpose.

2. Experimental Part

2.1. *Materials*

Rosemary extract (RE), a white greenish-yellow amorphous powder, was extracted from *Rosmarinus officinalis L.* and chemically identified using UV, IR, NMR and MS [22]. RE was supplied from National Institute for Chemical-Pharmaceutical Research and Development (Romania). Polyethylene glycol sorbitan monooleate (Tween 80) and Rutin were obtained from Merck, Germany; Synperonic PE/F68 (block copolymer of polyethylene and polypropylene glycol) and L-α-Phosphatidylcholine were purchased from Fluka, Sigma Aldrich Chemie GmbH; n-Hexadecyl

Palmitate (CP), 95% was obtained from Acros Organics, USA; Olive oil, Emulgade PL68/50 (wax mixture of alkylpolyglycoside and cetylstearyl alcohol) and Glycerol stearate (GS) were offered by Elmiplant S.A., Romania.

2.2. *Rosemary Extract – SLNs and – NLCs Preparation*

The Rosemary extract loaded SLNs and NLCs were prepared by the high shear homogenization technique. The composition of each formulation is illustrated in Table 1. Briefly, a lipid phase (10%, w/w) which contain the blends of solid lipids or liquid and solid lipids (in case of NLC) was melted at about 80°C and certain amount of Rosemary extract was added to obtain a clear molten solution. In a hot water phase consisting of 3% or 5% surfactant mixtures (w/w, in a molar ratio of Tween 80 or Emulgade PL 68/50:Synperonic F68:Lechitin = 1:0.25:0.25) and deionized water to 100% (w/w), the melted lipid phase was gradually dispersed and the resulted emulsion was kept under stirring for 2 h at 80°C. The aqueous surfactant solution was complied with a high speed stirring (15 000 rpm) for 3 minutes, before the mixing the two phases. Then, the hot pre-emulsion was further processed by a high shear homogenization at 25 000 rpm for 15 minutes (with a Lab High-Shear Homogenizer SAII-20 type; 0 ~ 28.000 rpm; power of 300 W, Shanghai Sower Mechanical & Electrical Equipment Co., Ltd., China). The lipid dispersions loaded with Rosemary extract (RE-SLNs/RE-NLCs) were solidified by adding of an appropriate deionized water volume (the volume ratio of pre-emulsion to water was 1:2) and cooled at room temperature. The physical lipid mixture was produced by melting of pure lipids at 80°C and unloaded-SLN was obtained in the same manner as loaded-SLNs, by replacing the natural extract with deionized water. The excess water is removed by lyophilization in order to increase the SLN particle concentration (by using a Christ Delta 2–24 KD lyophilizer, Germany). The SLNs/NLCs dispersions with various content of Rosemary extract were freeze-dried in the following conditions: 1. cooling to −40°C during 4 h, heating to −20°C within 4 h at 0.12 mbar and heating to 20°C within 20 h at the same pressure; 2. heating within 4 h to 30°C at 0.01 mbar and hold for 8 h. The lyophilized SLNs/NLCs have contained an amount of natural extract between 1.3÷2.5% (as comparing with Rutin-SLN that contains 7% active ingredient).

2.3. *Characterization Methods for RE-SLNs and RE-NLCs*

The information regarding the size distribution and the mean particle sizes of RE-SLNs and RE-NLCs suspensions were evaluated by photon correlation spectroscopy (PCS) on a Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, UK) at a scattering angle of 90° at 25°C, by using a He-Ne laser of 633 nm. The particle size analysis data were evaluated using hydrodynamic diameter (by intensity distribution). Zeta potential measurements were performed on the same equipments. The experimental measurements were repeated 3 times for each sample, for $z_{average}$ and zeta potential determinations. The SLNs and NLCs dispersions were diluted with deionized water (initial SLN/NLC dispersions:water = 1:50) to ensure that the light scattering intensity fulfill the sensitivity range conditions of instrument. Size measurements were made on the day of production and 3 months after their production.

The morphology of loaded SLNs and NLCs was examined by transmission electronic microscopy, by using a Philips 208 S (Netherlands) after dropping three

Table 1. Composition and physico-chemical characterization of RE loaded SLN and NLC formulations, % (w/w)

	Composition (w/w)			Average size (nm)			
Formulation	RE (%)	Lipid (%)	Surfactant (%)	Mean (nm)	Width (nm)	Pdl index	Zeta potential (mV)
Rutin-SLN-1*	1	10 (CP:GS = 1:1)	3 (Tween 80:Synperonic F68:Lechitin)	91.5	55.0	0.40	−39.6
RE-SLN-2	0.33	10 (CP:GS = 1:1)	3 (Tween 80:Synperonic F68:Lechitin)	68.4	35.7	0.26	−63.4
RE-SLN-3	0.17	10 (CP:GS = 1:1)	3 (Tween 80:Synperonic F68:Lechitin)	57.4	41.0	0.26	−77.8
RE-SLN-4	0.17	10 (CP:GS = 1:1)	3 (Emulgade:Synperonic F68:Lechitin)	209.4	125.6	0.21	−22.5
RE-NLC-1	0.17	10 (CP:GS:OO = 1:1:0.22)	3 (Tween 80:Synperonic F68:Lechitin)	68.2	33	0.25	−87.4
RE-NLC-2	0.17	10 (CP:GS:OO = 1:1:0.50)	3 (Tween 80:Synperonic F68:Lechitin)	68.3	43.4	0.23	−87.5
RE-NLC-3	0.17	10 (CP:GS:OO = 1:1:0.84)	3 (Tween 80:Synperonic F68:Lechitin)	85.9	63.5	0.26	−88.7
RE-NLC-4	0.17	10 (CP:GS:OO = 1:1:0.50)	3 (Emulgade:Synperonic F68:Lechitin)	162.9	140.5	0.42	−62.9
RE-NLC-5	0.17	10 (CP:GS:OO = 1:1:0.50)	5 (Emulgade:Synperonic F68:Lechitin)	157.33	123.9	0.44	−64.1

*SLN-1 was prepared for comparative purpose and contains a pure flavonoid (Rutin).
**Each value represents the average for $n = 3$.

times the SLN/NLC dispersion sample directly to the copper grid and dried in room temperature for 5 min. The TEM images were recorded after three months of preparation.

Thermograms were recorded with a differential scanning calorimeter Jupiter, STA 449C (Netzsch). Samples were heated at the scanning rate of 3°C/min over a temperature range between 30 and 100°C. For DSC measurements, a standard creuzet of Al_2O_3 was used.

The antioxidant activity (AA) of SLNs and NLCs loaded with Rosemary extract has been determined and compared with that of pure Rosemary extract by chemiluminescence method (CL), using luminol $+H_2O_2$ as generator system, in tampon TRIS-HCl, pH = 8.6 by using a Chemiluminometer Turner Design TD 20/20, USA. The antioxidant activity of methanol solutions of Rosemary extract/Rutin, lyophilized Rutin-SLN, lyophilised RE-SLNs and RE-NLCs (with the same concentration of active compound: Rutin or Rosemary extract) was calculated by using the relation [23]:

$$\%AA = \frac{I_0 - I_s}{I_0} \cdot 100$$

where: I_0 = the maximum CL for standard at t = 5 s; I_s = the maximum CL for sample at t = 5 s.

3. Results and Discussion

3.1. *Characterization of the Developed Rosemary Extract – SLNs and NLCs*

Physically stable SLNs and NLCs loaded with Rosemary extract (RE-SLNs/RE-NLCs) were produced with promising antioxidant properties. In Figure 1 it is shown the size distribution of optimized nanoparticle dispersions measured by dynamic light scattering. The RE-SLN and RE-NLC dispersions are distributed completely in the nanometer range with a relatively narrow size distribution. The mean particle sizes and surface charges of the developed RE-SLNs, RE-NLCs and Rutin-SLN (for comparative purpose) are shown in Table 1. It can be seen that the size of the lipid nanoparticle dispersions was strongly dependent on the type

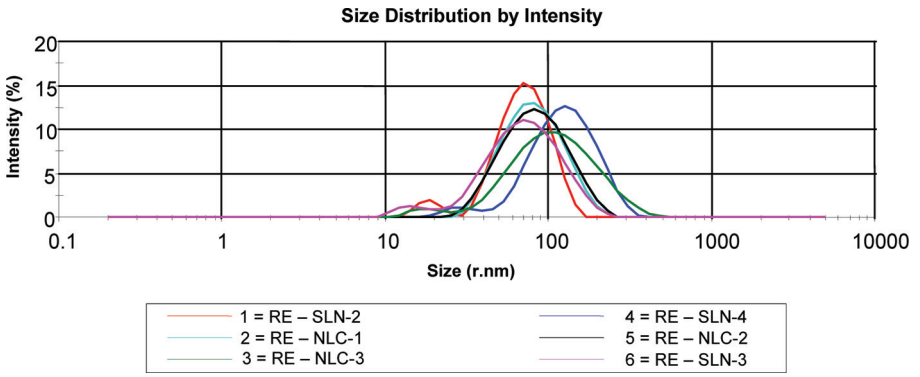


Figure 1. Size distribution of RE-SLNs and RE-NLCs dispersions, by DLS technique.

of surfactant and slightly dependent on the solid/liquid lipid composition and the type of loaded compound. When an individual flavonoid was loaded into SLN, the mean size was 91.5 nm, but with a relatively high polydispersity index (Pld = 0.40), while by loading the natural extract in lipid matrices, the mean sizes were decreased for both SLN and NLC systems prepared with Tween 80 as main emulsifier. Regarding the type of surfactants, SLNs and NLCs prepared with Emulgade PL68 as main surfactant were significantly larger (the average size >157 nm, with a good polydispersity for SLN-4 −0.21, while for the NLC-4 and NLC-5 the polydispersity values were increased up to 0.42 for 3% Emulgade and 0.44 for 5% Emulgade, respectively) than those prepared with a mixture of Tween 80, Lecithin and Synperonic surfactants. These results demonstrate that the mixture of alkylpolyglycoside and cetylstearyl alcohol does not manifest an efficient self-assembling tendency as comparing with a conventional non-ionic surfactant. For an evaluation of behavior of Emulgade emulsifier, its content was increased in a NLC formulation up to 5%. The average size was slowly decreased in this case. This result suggests that, in case of Emulgade non-ionic emulsifier, the steric hindrance is an additional effect which increases the size, as may be observed by larger values of the curve width for SLN-4, NLC-4, and NLC-5, as compared to the lipid dispersions prepared with Tween 80 as the main emulsifier. By comparing the SLNs formulations with variable amounts of RE, the size decreases when the content of vegetal extract was reduced at half. Among the formulations tested, the sample RE-SLN-3 with 0.17% RE (wt) and mixture of Tween 80, Lecithin and Synperonic F68 manifests the smallest size (57.4 nm) with a good polydispersity index (Pld = 0.26).

In order to elucidate the influence of liquid lipid component on the performance of the particulate systems, a modification of solid lipid matrix was realized by incorporating of a liquid lipid – olive oil. Thus, some lipid emulsions that contain variable amounts of OO (between 10÷30% olive oil from total lipid content of 10%, w/w) as the liquid lipid core were prepared. It was found that the variation in the lipid composition had also a significant effect on the mean particle size, by using 0.17% RE (wt). As can be shown in Table 1, the nanoparticles prepared only with solid lipid mixture (GS:CP = 1:1) are smaller than those prepared with a combination of solid and liquid lipids. This result reveals that the SLN particles after encapsulation of liquid lipid up to 30% (from entire lipid content), the size of particles increases due to the swollen core of the particles loaded with liquid oil. The obtained results shown that by increasing the lipid content from 10 to 30% (w/w), the size distribution of the lipid emulsions prepared with 10 and 20% olive oil is similar and they exhibited the same size (∼68 nm), while the average size of NLC was increased up to 86 nm when the liquid lipid proportion reaches 30 wt%. Also, its size distribution is broader (Fig. 1). For all these three NLCs formulations, the polydispersity indices were in a range between 0.23÷0.26, suggesting that the nanoparticles are monodispersed.

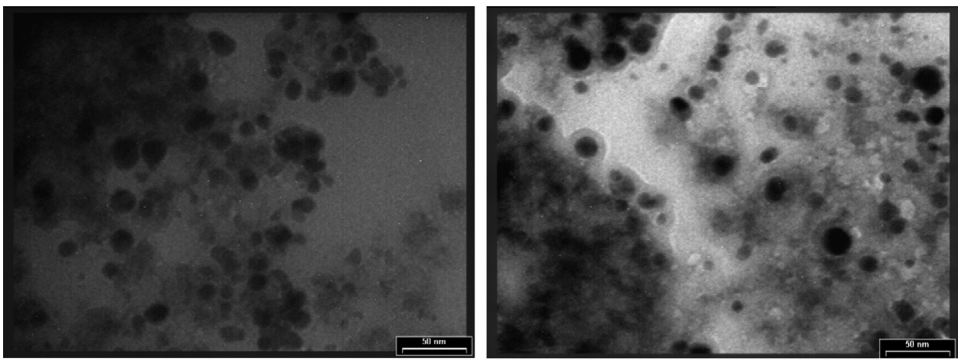
A deeper understanding of the stability conditions in SLN and NLC suspensions requires the knowledge of zeta potential values. The analysis of the zeta potential, which is the electrostatic potential at the plane of shear, allows predictions about the physical storage stability of colloidal dispersions. Zeta potential values higher than −30 mV (absolute value) show good physical stability, being optimized when they reach approximately −60 mV. Thus, the particle aggregation is not likely to occur for charged particles with high zeta potential due to electrostatic repulsion among the particles [24]. In this study, the zeta potential value was revealed to be dependent on the type of surfactants, lipids, and also on the amount of RE incorporated. The

mean zeta potentials (average for $n = 3$) of RE-SLNs and RE-NLCs were indicated in Table 1. The zeta potential values of almost all loaded-SLN and -NLC dispersions (except some prepared with Emulgade emulsifier) were in the range $-39 \div -88$ mV (Table 1), showing that both SLNs and NLCs should possess a good physical stability. The NLC samples with olive oil, present the most electronegative zeta potentials ($\xi = -87 \div -89$ mV), while the RE-SLN prepared by using Emulgade did not fulfill the stability conditions ($\xi = -22$ mV).

There were three drug incorporation models for lipid nanoparticles: solid solution model, core-shell model with drug-enriched shell and core-shell model with drug-enriched core [3]. The lypophile distribution inside the lipid matrix of SLNs/NLCs is determined by the preparation method. Based on the preliminary tests investigating the effects of different factors (amount of extract, surfactant type and lipid composition) on RE-SLNs/RE-NLCs, the TEM analysis was applied for two optimized formulations. From Figure 2 it can be seen that SLN and NLC exibit quite similar morphology. Both images show that the SLN and NLC particles are spherical or near-spherical in shape, but their structural arrangement is rather different. The experimental results reveal that NLC particles are different in their inner microstructures. The microstructure of the NLC particles is more ordered, with a visible shell of surfactants that surrounded the lypophile core, while the inner part of the SLN particles shows a rather amorphous structure. According to TEM images, we consider that the incorporation of RE into NLC should fit to the core-shell model. The size of SLN and NLC loaded with RE are about tenth of nanometer, as can be estimated from Figures 2A and B.

By combining results on particle size and zeta potentials, evaluated from DLS and TEM, it is clear that good physical stability of nanoparticles could be obtained and particle aggregation during storage is not likely to occur, due to electrostatic or steric repulsion among the particles. Therefore, this method provided a high stability and relative good dispersion quality of the particles.

DSC measurements offer an overview on the melting and crystallization behavior of crystalline materials like lipid nanoparticles. In order to investigate the different effects on the crystalization behavior of RE-SLNs and RE-NLCs prepared by HSH technique, by detecting whether the crystallinity was different



(A) (B)

Figure 2. TEM images of SLN-3 (A) and NLC-2 (B) formulations (with 0.17% RE); (bar scale 50 nm).

or not in the lipid matrices due to their compositions, the lyophilized SLNs and NLCs loaded with Rosemary extract, the unloaded SLN and also the physical lipid mixture (CP:GS = 1:1 for SLN and CP:GS:OO = 1:1:0.5, for NLC, respectively w/w), were studied by DSC measurements. From Figures 3 and 4 it is evident that when the pure lipid mixture was turned into SLN or NLC, the melting point was changed for both types of lipid carriers. The pure physical lipid mixtures exhibits broader melting peaks (between 38–55°C), while, for example, the unloaded-SLN exhibits a maximum peak located at 50.2°C. This result may be intrpreted by the presence of surfactant which conffers a more ordered arrangement of lipids, as can be seen by the narrow melting range, as compared to the pure physical mixture of lipids. Moreover, by comparing the unloaded- with loaded-SLNs, the incorporation of Rosemary extract and Rutin leads to a decrease peak intensity for loaded-SLNs, that underline a disturbance of crystallin arrangement towards a more dezordered network.

Regarding the NLCs formulations (Fig. 4), due to the olive oil presence within the lipid mixtures, two DSC shoulders appear compared to SLNs formulations. According to the DSC thermograms, the melting peak range of the solid lipid which converts towards two shoulders after incorporating of olive oil into the solid lipid cores (of NLC) suggests the presence of two polymorph states and imperfections in the crystalline lattice of the lipid cores. Thus, the peak location of GS/CP/OO nanospheres prepared with Emulgade as main surfactant, are slightly shifted towards higher temperatures (50.2°C and 55°C, for RE-NLC-4, and 50.3°C and 57°C, for RE-NLC-5, respectively) compared to that of pure physical lipid mixture (46°C and 54°C). In case of RE-NLC prepared with Tween/Lecithin and Synperonic surfactants, the disordered network (as comparing to loaded-SLNs) is observed together with the disappearance of the shoulder at the right side. These results confirm the difference between SLN and NLC that underlines the less ordered lipid matrix for NLC systems.

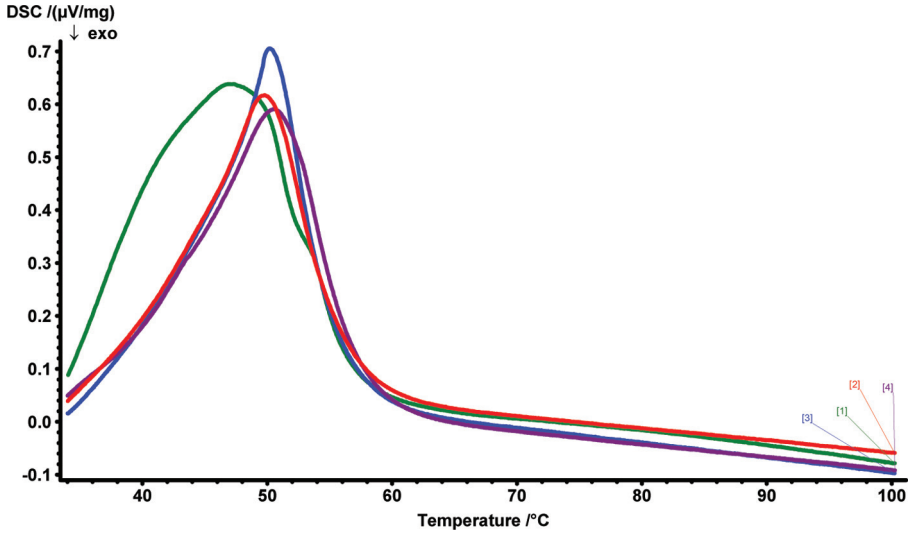


Figure 3. DSC curves of: 1 = physical lipid mixture; 3 = unloaded-SLN; 2 = Rutin-SLN; 4. RE-SLN.

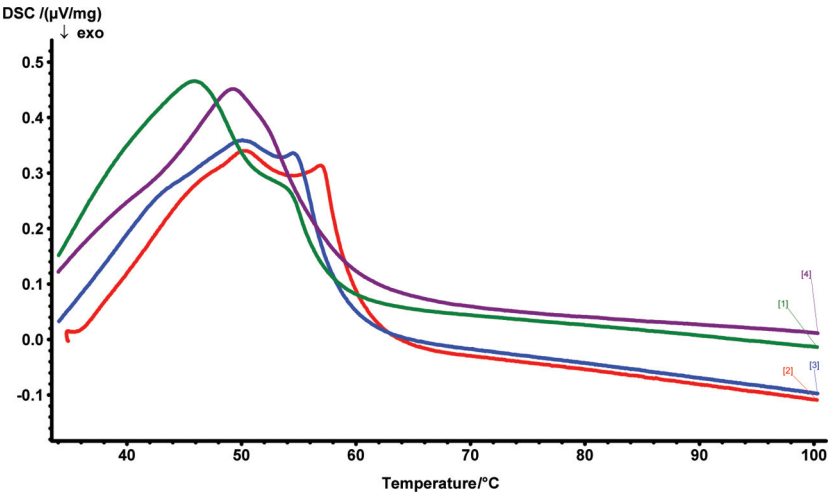


Figure 4. DSC curves of: 1 = physical lipid mixture of GS:CP:OO, 2 = RE-NLC-5; 3 = RE-NLC-4; 4 = RE-NLC-2.

3.2. *Antioxidant Activity of Developed Lipid Nanoparticles. Effect of Natural Lipid Oil (Olive Oil) on the Antioxidant Activity of NLC*

Based on the above results, the influence of the type and amount of loaded compound, lipid phase and surfactant type on the antioxidant properties of developed SLNs/NLCs was also experimentally investigated. The experiments were carried out on methanol solutions of lyophilized Rutin-SLN, RE-SLNs and RE-NLCs. The antioxidant activity of RE entrapped in SLNs and NLCs was evaluated in comparison to alcoholic solution of pure RE. The mixture of wax, oil and glyceride-based SLNs and NLCs comprising similar composition of natural extract mixture (0.1 mg/L active compound for sample prepared with 0.17% RE) were found to enhance the antioxidant activity of RE. Firstly, the incorporation of an amount of 1% Rutin into a solid lipid mixture (GS:CP = 1:1) lead to an antioxidant activity of 86.4% compared to 74.2% for Rutin alcohol solution. This Rutin-SLN formulation was prepared in order to underline the effect of a complex mixture of a natural extract, referring to an individual antioxidant compound. Therefore, the next experiments were realized by decreasing the loaded extract content to 0.33% and 0.17% RE, respectively. As can be seen from Figure 5, the AA of the nanoparticles loaded with Rosemary extract was enhanced by 24% for RE-SLN-2 (prepared with 0.33% RE, AA = 86.25%) and 84%, for RE-SLN-3 (prepared with 0.17%, AA = 83.4%), respectively, in comparison to alcohol solutions of active compounds (AA = 69.6% for RE 0.33% and AA = 32% for RE 0.17%). These results reveal that the increasing of AA was due to a synergistic effect of natural extract completed by the presence of the complex structural lipid mixture.

In case of NLCs formulations, the total amount of lipid was kept constant in the experiments (10%), and only the proportion of the liquid oil in the total lipid was changed. The effect of OO concentration on the AA properties was evaluated by varying the concentration from 10% to 30% (w/w, from the total lipid content), while maintaining the amount of surfactant mixture (3% mixture of Tween 80, Lecithin and Synperonic F68). The results presented in Figure 6 showed that when

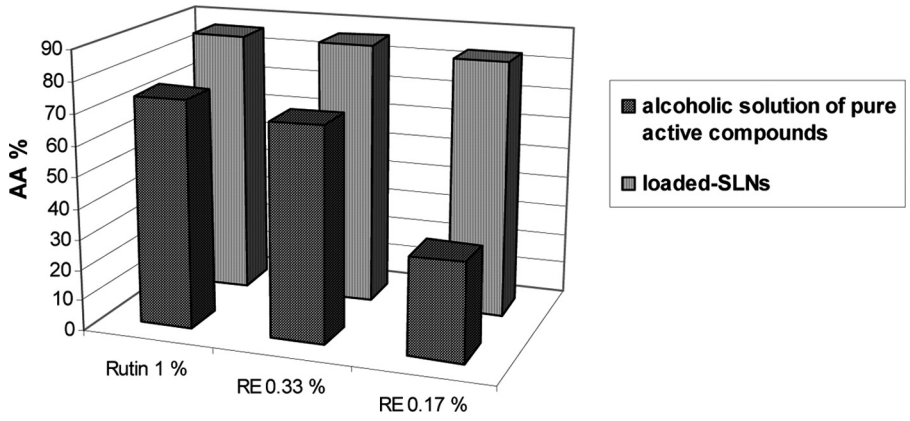


Figure 5. Antioxidant activity of Rutin-SLN and RE-SLNs formulations.

increasing the amount of liquid lipid concentration from 10% to 20% (w/w), the AA of lipid nanoparticles firstly increased from approximatively 74% to 83.1%, respectively, and then decreased up to 77.7% when the liquid oil proportion reaches 30 wt%. Therefore, 20% OO was found to be an optimum composition of liquid lipid. By using a higher content of natural liquid lipid, as was shown by DLS measurements (that resulted in larger particle size and broader size distribution), the structural complexity of lipids was also increased and probably it does not allow a good loading of RE inside lipid matrix.

Moreover, having in view the synergistic effect of complex lipid mixture, the AA of unloaded NLC (prepared with 20% OO) was also determined and it presents a value of AA = 70.2%. By comparing the AA of unloaded SLN (66.8%) with that of unloaded-NLC (70.2%), it may be supposed that the presence of OO in the lipid matrix has a quite reduced effect on the AA, probably due to the less amount of OO that could not influence the AA of final NLC.

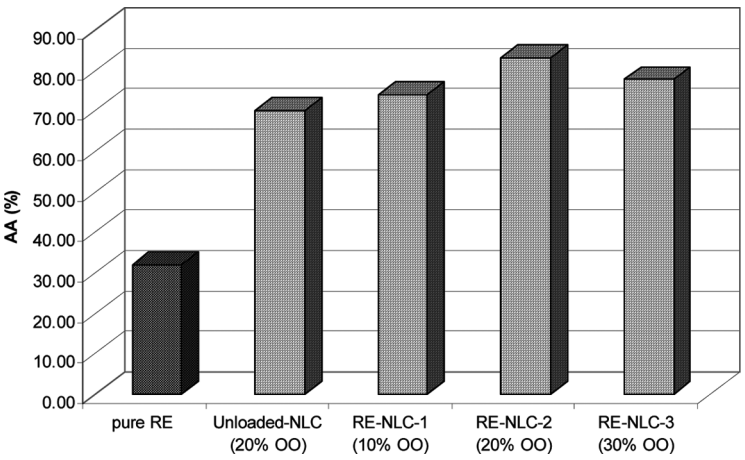


Figure 6. Antioxidant activity of RE-NLCs formulations, prepared with Tween 80/Lechitin/Synperonic surfactants mixture.

The antioxidant activity of RE-NLCs formulations was also evaluated for 3% and 5% surfactant mixtures of Emulgade PL68, Lecithin and Synperonic F68 (results not shown in Fig. 6). In this case, as expected, the AA registered an enhancing effect, but not as significant as in case of using Tween as main surfactant (also demonstrated by DLS measurements). The AA values for these two formulations were 74.6% and 77.0% (for RE-NLC prepared with 3% surfactant mixture and 5% surfactant mixture, respectively).

Furthermore, the synergistic effect of lipids was greatly dependent on the type of lipid and surfactant mixture. As a result, the antioxidant activity of RE could be effectively enhanced by loading in SLNs and NLCs together with incorporation of a liquid lipid.

4. Conclusion

In this study, the antioxidant activity of a natural antioxidant extract (Rosemary extract) was improved by loading Rosemary extract into two model lipid carriers (SLN and NLC), using a HSH technique. The antioxidant activity of RE-SLN and RE-NLC can be also enhanced by selecting suitable types of surfactant, lipid and proper preparation conditions. The applied synthesis technique was demonstrated to be a simple, available and effective method to prepare SLN and NLC loaded with Rosemary extract, which are homogeneously distributed into lipid matrices. The results indicated that non-ionic surfactants combined with an ionic surfactant (Tween 80, Emulgade PL68, Lecithin and Synperonic F68) showed obviously a good emulsification efficiency in the preparation. They increased the zeta potential of nanoparticles, thus leading to improvement in the physical stability of the systems. The RE-SLNs and RE-NLCs produced had good particle size stability with low average size ($57 \div 85$ nm) and polydispersity (≤ 0.26), and high negative potential at lower surfactant content ($\xi = -39 \div -88$ mV).

Inclusion of RE into the lipid nanoparticles were shown to enhance the antioxidant activity that increased up to 1.6 times in both SLN and NLC formulations, as comparing to RE methanolic solution. In addition, the antioxidant capacity was evaluated in RE-NLC system with the increase of liquid lipid and Emulgade concentration. The results of the characterization and evaluation of loaded-SLN/NLC systems have been proven the suitability and compatibility of an indigenous natural lipid – olive oil as a novel liquid lipid, unexplored in the drug delivery. Finaly, this study has demonstrated that olive oil could be very useful in the preparation of stable lipid nanoparticles when it is combined with two kinds of solid lipid matrices.

Acknowledgments

The authors gratefully acknowledge the Romanian National CNCSIS-UEFISCSU program ''IDEI'' (ID_1050/2007) for its financial support and to S.C. Elmiplant, Romania for materials offered.

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