Artemisia arborescens L Essential Oil–Loaded Solid Lipid Nanoparticles for Potential Agricultural Application: Preparation and Characterization

Submitted: May 31, 2005; Accepted: October 31, 2005; Published: January 3, 2006

Francesco Lai,[1,2] Sylvia A. Wissing,[2] Rainer H. Müller,[2] and Anna M. Fadda[1]

[1]Dipartimento Farmaco Chimico Tecnologico, Universitá degli Studi di Cagliari, Via Ospedale 72 09124 Cagliari, Italy
[2]Department of Pharmaceutical Technology, Biotechnology and Quality Management, The Free University of Berlin, Kelchstr, 31, D-12169 Berlin, Germany

ABSTRACT

The aim of this study was to formulate a new delivery system for ecological pesticides by the incorporation of *Artemisia arborescens* L essential oil into solid lipid nanoparticles (SLN). Two different SLN formulations were prepared following the high-pressure homogenization technique using Compritol 888 ATO as lipid and Poloxamer 188 or Miranol Ultra C32 as surfactants. The SLN formulation particle size was determined using Photon correlation spectroscopy (PCS) and laser diffraction analysis (LD). The change of particle charge was studied by zeta potential (ZP) measurements, while the melting and recrystallization behavior was studied using differential scanning calorimetry (DSC). In vitro release studies of the essential oil were performed at 35°C. Data showed a high physical stability for both formulations at various storage temperatures during 2 months of investigation. In particular, average diameter of *Artemisia arborescens* L essential oil–loaded SLN did not vary during storage and increased slightly after spraying the SLN dispersions. In vitro release experiments showed that SLN were able to reduce the rapid evaporation of essential oil if compared with the reference emulsions. Therefore, obtained results showed that the studied SLN formulations are suitable carriers in agriculture.

KEYWORDS: solid lipid nanoparticles, SLN, natural pesticide, in vitro release, agriculture.

INTRODUCTION

Solid lipid nanoparticles (SLN) are particles with a mean photon correlation spectroscopy (PCS) diameter of ~50 to 1000 nm. Lipids used for their production are solid at room temperature[1-4] and most of them have an approved status, such as the GRAS status, due to their low toxicity. Introduced at the beginning of the 1990s, SLN are an alternative carrier system for pharmaceutical and cosmetic ingredients. They combine the advantages of emulsions and liposomes with those of polymer nanoparticles, while simultaneously avoiding some of their disadvantages (eg, stability problems, toxicological problems).

SLN have been intensively investigated for dermal application,[4] parenteral[5,6] and peroral[7,8] administration, and ocular delivery.[9] However, only one article has been published to date for the use of SLN in agriculture.[10]

Pests are problematic for humankind for a myriad of reasons, such as decreased crop yield, reduced crop quality, and increased harvesting costs. The application of synthetic chemical pesticides to soil or plants produces toxic effects both in the environment, in plants, in humans, and in animals. Essential oils are good candidates for the substitution of conventional pesticides and many articles and patents for their use have been published in recent years.[11-16] The most attractive aspects of using essential oils as crop protectors are their very low mammalian and fish toxicity compared with synthetic pesticides and their nonpersistence in fresh water and soil.[15]

Artemisia arborescens L is an aromatic plant that is endemic in Mediterranean regions. It is an evergreen shrub from the *Asteraceae* family. Components of *Artemisia* genus have been used for centuries in folk medicine.[16,17] Recently, its antiviral properties have been demonstrated against Herpes simplex virus 1 in vitro.[18] *Artemisia arborescens* L essential oil also demonstrated pesticidal activity against *Aphis gossipy* (a pest of citrus fruits), adult and young *Bemisia tabaci*, and *Lymantria dispar* L (pest of *Quercus suber*) and was efficiently encapsulated in cross-linked alginate beads for a controlled release into the soil.[19] Obtained data showed the possibility of using this natural product as an ecological pesticide in both conventional and organic agriculture and in domestic and public use (eg, city garden).

However, the major inconvenience of the use of this oil and, in general, of essential oils is their chemical instability in the presence of air, light, moisture, and high temperatures that can determine the rapid evaporation and degradation of some active components.[11] Incorporation of essential oils in controlled-release formulations could solve these problems and offer several advantages. An ideal delivery

Corresponding Author: Anna M. Fadda, Dipartimento Farmaco Chimico Tecnologico, Universitá degli Studi di Cagliari, Via Ospedale 72 09124 Cagliari, Italy. Tel: +39 070 6758744; Fax: +39 070 6758553; E-mail: mfadda@unica.it

system should protect the essential oil from the environmental degradation process and prevent removal of these natural pesticides from their target before they can take effect. The ideal formulation should also maintain both a minimum effective and continuous controlled release of the essential oil allowing the use of much less natural pesticides for the same period of activity. Particular attention should also focus on the costs of the materials employed as well as of processing the formulation.

SLN have demonstrated their capacity to protect labile compounds such as tocopherol acetate, retinoids, and vitamin E from degradation.[20,21] Several studies also showed that the incorporation of volatile compounds into SLN prevents their rapid evaporation.[22,23]

SLN are produced using low-cost materials and the possibility for the scaling up of production by the high-pressure homogenization technique was demonstrated.[24,25] The use of a submicron particle system can also promote the adhesion on the leaf and fruits.

The purpose of this work was to study the incorporation of *Artemisia arborescens* L essential oil into SLN for agricultural application. Two different SLN formulations were prepared by high-pressure homogenization using Compritol 888 ATO as lipid, Poloxamer 188 or Miranol Ultra C32 (sodium cocoamphoacetate) as surfactants, and *Artemisia arborescens* L essential oil as a model drug. Encapsulation efficiencies (E%) were determined after purification of SLN dispersions from nonincorporated *Artemisia arborescens* L essential oil by gel chromatography. The physical stability of different formulations was studied for a period of 2 months at various temperatures (4ºC, room temperature [RT], 40ºC). The in vitro evaporation was studied at 35°C and was compared with the reference emulsions prepared using the essential oil and Poloxamer 188 or Miranol Ultra C32 at the same concentrations of the SLN formulations. Placebo SLN were also produced using the same method and surfactants and maintaining a 10% wt/wt concentration of lipid phase.

Materials and Methods

Materials

Artemisia arborescens L leaves were collected in the countryside around Usellus, Sardinia, Italy, during full blossom (May-June 2003). The leaves were identified and a voucher specimen was deposited in the Herbarium of the Department of Botany and Botanical Gardens, University of Cagliari.

Compritol 888 ATO, which was obtained from Gattefossé (Weilam Rhein, Germany), is declared as glycerol behenate with a melting point of 72ºC. It is a mixture of 12% to 18% mono-, 52% to 54% di-, and 28% to 32% triglycerides. The fatty acid fraction consists of >87% behenic acid (docosan acid). The surfactant Pluronic F68 (Poloxamer 188) was a gift from BASF AG (Ludwigshafen, Germany), Miranol Ultra C32 (sodium cocoamphoacetate) was from Rhodia (Frankfurt, Germany).

Methods

Essential Oil Extraction and Characterization

The fresh aerial parts of the plant (5000 g) were distilled in a steam apparatus with an aqueous phase recycling system for 3 hours. The obtained blue essential oil was separated from the aqueous phase solution and then dried over anhydrous sodium sulfate. The oil was stored at 4ºC until used.

The quali-quantitative analysis of the essential oil was performed by gas chromatography/ion trap mass spectrometry (GC/ITMS).

Gas Chromatography/Ion Trap Mass Spectrometry Analysis

A Varian CP 3800 gas chromatograph (Varian Inc, Palo Alto, CA) coupled with a Saturn 2000 ion trap mass spectrometer (ITMS) detector, a Varian CP 7800 autosampler, a split-splitless injector, and an MS ChemStation, were used. The column was a fused silica capillary DB-5MS (5% phenylmethylpolysyloxane, 30 m × 0.25 mm; film thickness 0.25 μm) (J&W Scientific Fisons, Folsom, CA). The injector and interface were at 150°C and 280°C, respectively. The oven temperature was programmed as follows: from 60°C to 180°C (3°C/min) and isothermally held for 15 minutes. Helium was the carrier gas at 1 mL/min; the sample (1 μL) was injected in the split mode (1:20). MS conditions were as follows: ionization mode electron impact (EI) from 50 to 450 amotic mass units (amu). The oil compounds were identified by comparison of their relative retention times with those of authentic samples or by comparison of their retention index (RI) relative to the series of n-hydrocarbons, and computer matching against commercial library[26,27] and homemade library mass spectra made up from pure substances and components of known oils and MS literature data. In Table 1 the composition of the most abundant molecules of the essential oil is given.

Preparation of SLN and Emulsions

For the preparation of SLN the *Artemisia arborescens* L essential oil was dissolved in the melted Compritol 888 ATO at 85ºC and the essential oil–loaded lipid dispersed in a hot aqueous surfactant solution. The mixtures were stirred with a T 25 Ultra Turrax (Janke und Kunkel GmbH and

Table 1. Main Components of *Artemisia arborescens* L Essential Oil as Determined by GC and GC-ITMS*

Component	Retention Time R_t	Area %
α-pinene	4.15	3.17
β-thujone	13.32	23.97
Camphor	15.30	35.73
beta-Carophyllene	19.67	3.32
Chamazulene	41.19	7.66

*GC indicates gas chromatography and GC-ITMS, gas chromatography/ion trap mass spectrometry.

Co KG Staufen, Germany) for 1 minute at 8000 rpm. The obtained pre-emulsion was then homogenized at high pressure (3 cycles, 500 bar) using an APV Micron Lab 40 (APV Systems, Unna, Germany) thermostated at 90ºC.

The references placebo SLN were prepared using the same method and the same surfactant concentration of relative loaded SLN formulations. For the preparation of the emulsion formulations, the essential oil was emulsified in a cold aqueous surfactant solution and then homogenized at high pressure (1 cycle, 500 bar). Details of SLN and emulsion formulations are given in Table 2.

Characterization of Solid Lipid Nanoparticles

Encapsulation Efficiency

Encapsulation efficiencies (E%) are expressed as a percentage of the total amount of *Artemisia arborescens* L essential oil found in the studied formulations at the end of the preparation procedure. The SLN dispersions were purified from nonincorporated *Artemisia arborescens* L essential oil by gel chromatography on Sephadex G50. The encapsulation efficiency was calculated using the following equation: $[(T-S)/T] \times 100$, where T is the total quantity of incorporated and nonincorporated essential oil in the SLN dispersion and S is the nonincorporated oil quantity separated with gel chromatography. Quantitative determination was performed spectrophotometrically using an Uvikon 940 (Kontron Instruments, Eching/München, Germany) UV spectrophotometer at 284 nm, after extraction and dilution with methanol for 1 hour in an ultrasonic bath.

Recovery of incorporated and nonincorporated essential oil accounted for more than 95% of the used dose. As previously reported, the spectrophotometric method was validated by comparison of the obtained results with those from GC/ITMS (as described in *Methods, Gas Chromatography/Ion Trap Mass Spectrometry Analysis*).[26] The spectrophotometric method results were also compared with those obtained by high-performance liquid chromatography (HPLC), where the most important components of the oil (camphor, b-thujone and chamazulene) were used as a "lead." The oil content was assayed by HPLC at several wavelengths (209, 245, and 284 nm), using a Waters 2690 liquid chromatograph, equipped with a Photodiode Array detector 996 (Waters Corp, Milford, MA). The mobile phases were methanol (solvent A) and water (solvent B). Separations were performed by the following linear gradient: 45% to 30% B in 15 minutes, 30% to 10% B in 25 minutes, at a flow rate of 1.0 mL/min. The column was Spherisorb 5 mm ODS2 (4.6 × 280 mm, Waters). Appropriate standard solutions of *Artemisia arborescens* L essential oil and authentic samples of the lead compounds in methanol were prepared and tested. All experiments were performed in triplicate.

Particle Size Analysis

The average diameter (Z-AVE) and polydispersity index (PI) of SLN were determined by PCS using a Zetasizer 4 (Malvern Instruments, Malvern, UK) at a fixed angle of 90º and at 25ºC. The aqueous SLN dispersions were diluted with distilled water before analysis. Each value is the average of 10 measurements. The laser diffraction particle size analysis (LD) was performed by a Coulter LS 230 (Beckmann-Coulter, Krefeld, Germany). The LD data were evaluated using the volume distribution method to detect even few large particles. Characterization parameters were the diameters LD 50, LD 90, and LD 99 (ie, a diameter LD 90 of 1 μm means that 90% of all particles have a diameter of 1 μm or less).

Table 2. Composition of *Artemisia arborescens* L Essential Oil–loaded SLN, Emulsions, and Reference Placebo SLN Formulations*

Formulation	Components	% (wt/wt)
SLN 1	Compritol 888 ATO	9.0
(SLN)	*Artemisia arborescens* L essential oil	1.0
	Poloxamer 188	5.0
	Water	85.0
SLN 1p	Compritol 888 ATO	10.0
(placebo	Poloxamer 188	5.0
SLN)	Water	85.0
SLN 2	Compritol 888 ATO	9.0
(SLN)	*Artemisia arborescens* L essential oil	1.0
	Miranol Ultra C32	2.5
	Water	87.5
SLN 2p	Compritol 888 ATO	10.0
(placebo	Miranol Ultra C32	2.5
SLN)	Water	87.5
EMU 1	*Artemisia arborescens* L essential oil	1.0
(emulsion)	Poloxamer 188	5.0
	Water	94.0
EMU 2	*Artemisia arborescens* L essential oil	1.0
(emulsion)	Miranol Ultra C32	2.5
	Water	96.5

*SLN indicates solid lipid nanoparticles; EMU, emulsion.

Zeta Potential

The particle charge was quantified as zeta potential (ZP) using a Zetasizer 4 at 25ºC. Measurements were performed in bidistilled water adjusted with sodium chloride to a conductivity of 50 microSiemens (mS)/cm. The pH values of the samples were always between 6.2 ± 0.9. Zeta potential was calculated from the electrophoretic mobility following the Helmholtz-Smoluchowski equation.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) was performed with a Mettler DSC 821e (Mettler Toledo, Greifensee, Switzerland). Samples containing ~15 mg nanoparticle dispersions (identical to 1-2 mg of solid lipid) were weighed accurately into standard aluminum pans using an empty pan as a reference. DSC scans were recorded at a heating and cooling rate of 5ºC/min. The samples were heated from 25ºC to 85ºC and cooled from 85ºC to 20ºC under liquid nitrogen. Enthalpies were calculated using the Mettler Star software.

Spraying of SLN

Sixty days after production, the *Artemisia arborescens* L–loaded SLN were sprayed using a garden spraying apparatus (Goizper, Antzuola, Spain). The sprayed SLN dispersions were collected in a beaker with water, and after dilution, the Z-AVE and PI were determined by PCS and LD as previously described.

In Vitro Release

Samples of the SLN formulations (SLN 1, SLN 2) were transferred into open glass vials and stored at 35°C for 48 hours. The essential oil was extracted from the SLN with methanol for 1 hour in an ultrasonic bath. The essential oil/methanol solution was filtered and then evaluated at a wavelength of 284 nm using a Uvikon 940 spectrometer (Kontron Instruments). The evaporation release of SLN formulations was compared with that of 2 emulsions (EMU 1 and EMU 2) prepared using the essential oil and Poloxamer 188 (EMU 1) or Miranol Ultra C32 (EMU 2) at the same concentrations used for SLN formulations (Table 2).

As previously reported,[26] the spectrophotometric method was formerly validated by comparison of the obtained results with those from GC/ITMS and HPLC (as described in *Characterization of Solid Lipid Nanoparticles, Encapsulation Efficiency*).

Results and Discussion

Essential Oil Extraction and Characterization

Distillation of the aerial part of *Artemisia arborescens* L in a steam apparatus gave a blue essential oil in good yield (0.8%). In Table 1, the composition of the most abundant molecules of the essential oil is given. As can be seen, monoterpene ketones, β-thujone and camphor, represent more than 50% of the essential oil.[28] Chamazulene, which is responsible for the blue color of the volatile oil, is also one of the main components.

Particle Size and Zeta Potential Measurements

Using the hot high-pressure homogenization technique, we were able to produce physically stable SLN formulations, both empty and *Artemisia arborescens* L essential oil–loaded. Compositions of the formulations are listed in Table 2.

The PCS data showed that the incorporation of *Artemisia arborescens* L essential oil into SLN led to a distinct decrease in SLN mean particle size only when Poloxamer 188 was used as a surfactant. One day after production, the SLN 1 and the relative placebo formulation SLN 1p had a size of 199 nm (0.224 PI) and 294 nm (0.288 PI), respectively, while the particle size of SLN 2 and the relative placebo formulation SLN 2p prepared using Miranol Ultra C32 as surfactant were 207 nm (0.285 PI) and 207 nm (0.249 PI), respectively (Figure 1).

The mean particle size of the loaded formulations increased only slightly after 2 months of storage, indicating a high physical stability of both SLN 1 and SLN 2 formulations at all storage temperatures (Figure 1). In particular, 60 days after production, SLN 1 showed the smallest increase when stored at 4ºC (207 nm), while the mean particle size of SLN 2 did not change at all. The PI values were always smaller than 0.35 indicating a fairly narrow size distribution of the particles.

The absence of particles in the micrometer range and aggregation was confirmed by LD particle size analysis (Figure 2). For both *Artemisia arborescens* L essential oil–loaded formulations SLN 1 and SLN 2, the obtained data showed an LD 99 smaller than 600 nm 60 days after production irrespective of storage temperature.

Generally it is accepted that ZP values of −30 mV and above characterize a stable formulation.[29] The SLN 2 formulation possessed a high ZP at day 1 (−36.2 ± 0.5 mV), which did not change during the 2 investigational months for all storage temperatures (Table 3) indicating a high long-term stability of this formulation. A reason for this is the negatively charged surfactant Miranol Ultra C32.

At day 1, the SLN 1 formulation prepared using the steric nonionic surfactant Poloxamer 188 showed a ZP value of −15.6 ± 0.5 mV, which decreased slightly during the investigational 2 months for RT and 4ºC storage temperature. However the SLN 1 formulation stored at 40ºC showed the lowest ZP value (−6.2 ± 2.8 mV) 60 days after production,

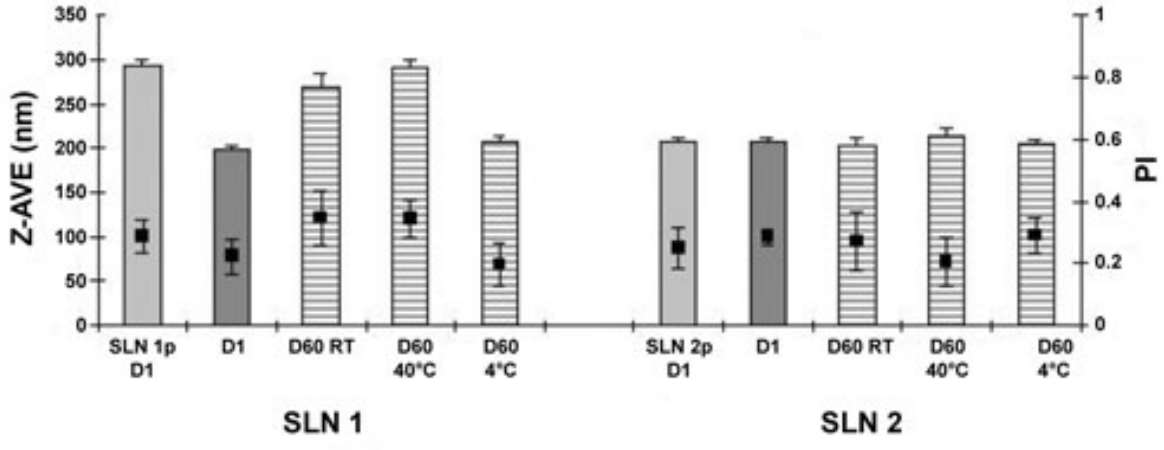

Figure 1. PCS Z-AVE and PI of *Artemisia arborescens* L essential oil–loaded SLN formulations (SLN 1, SLN 2) stored at RT, 4ºC, and 40ºC for 1 day (D1) and 60 days (D60) after production and placebo formulations (SLN 1p and SLN 2p) 1 day after production.

which explains the greater change of particle size after storage measured by PCS at this temperature. This finding is because the tails, trains, and loops formed by Poloxamer on the particle surface do not act so efficiently when temperature increases.[29] Too much kinetic energy enters the system, which can lead to destabilization.[30]

Encapsulation Efficiency

Compritol 888 ATO was chosen as the main component of the studied SLN formulations because a preliminary lipid screening had shown that its SLN formulations were the most stable. In fact, no separation of essential oil was detected at the light microscope during 48 hours of testing. Both SLN formulations showed a high capability of entrapping the essential oil. In particular the E% of SLN 1 and SLN 2 were 87% and 92%, respectively. The high incorporation capability of Compritol 888 ATO SLN is achieved because of a high lipophilicity of the essential oil. Figure 3 shows a GC/ITMS of pure and SLN-encapsulated *Artemisia arborescens* L essential oil. The GC/ITMS chromatogram, as confirmed by the HPLC analysis, shows that no change in the composition of the most abundant components of the oil occurred during SLN preparation. A more detailed study of essential oil composition and stability during storage will be discussed in a further paper.

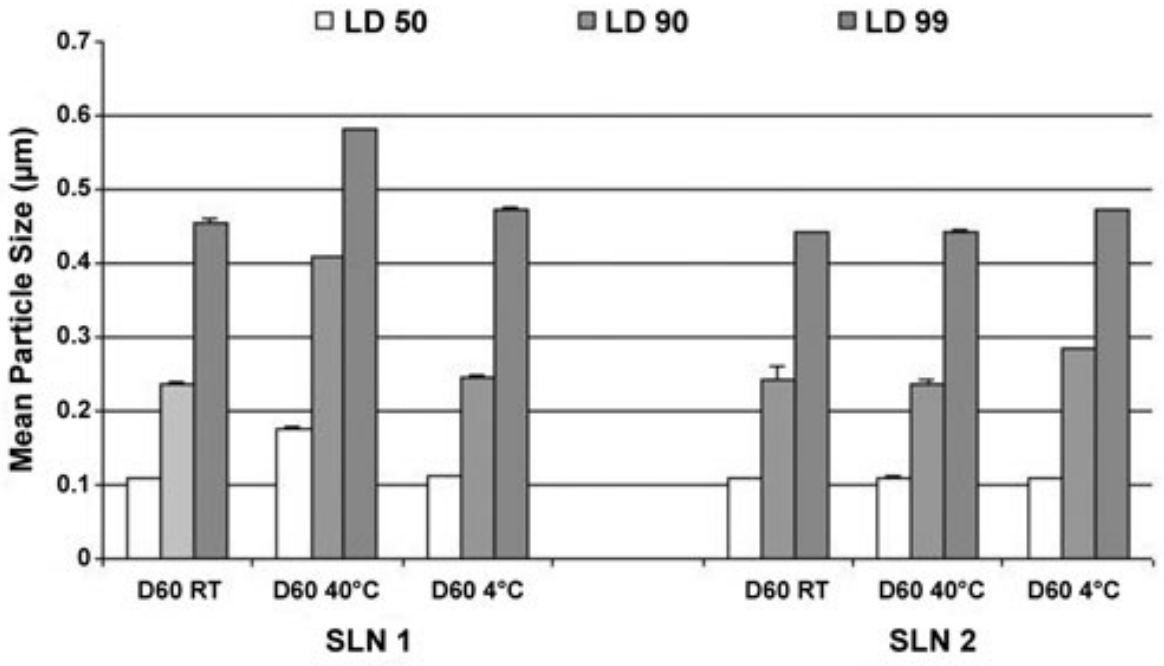

Figure 2. LD 50, LD 90, LD 99 values of *Artemisia arborescens* L essential oil–loaded SLN formulations (SLN 1, SLN 2) stored at RT, 4ºC. and 40ºC for 60 days (D60) after production LD data: volume distribution.

Table 3. Zeta Potential Measurements (in mV) of *Artemisia arborescens* L–loaded SLN Formulations in Bidistilled Water (50 μS/cm) Stored at Room Temperature (RT), 4ºC, and 40ºC, 1 Day (D1) and 60 Days (D60) After Production*

Formulations		Zeta Potential (mV)
SLN 1	**D1**	−15.6 ± 0.5
	D60 RT	−12.1 ± 0.7
	D60 40°C	−6.2 ± 2.8
	D60 4°C	−12.1 ± 0.7
SLN 2	**D1**	−36.2 ± 0.5
	D60 RT	−37.3 ± 0.3
	D60 40°C	−34.7 ± 0.7
	D60 4°C	−39.0 ± 0.9

*SLN indicates solid lipid nanoparticles.

Crystallinity of SLN

The bulk lipid melts between 61.5ºC and 72.5ºC with a melting point at 71.2ºC (Figure 4 and Table 4). The SLN 1 and SLN 2 heating curves differ distinctly from bulk Compritol showing a broadening of the heating curve (peak) and a reduction of the melting point to 65.7ºC and 66.7ºC, respectively, and thus indicating an increased number of lattice defects.

It has been reported that the lipids can recrystallize in the alpha, beta, or beta′ modifications. Using DSC analysis, cooling scans are the most sensitive method to detect polymorphic forms. zur Mühlen et al[30] and Freitas and

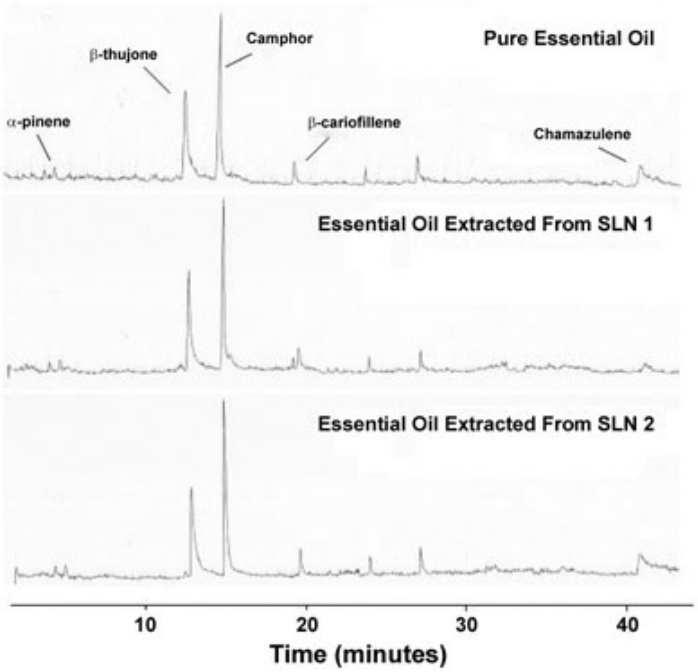

Figure 3. GC chromatograms of pure and SLN encapsulated *Artemisia arborescens* L essential oil.

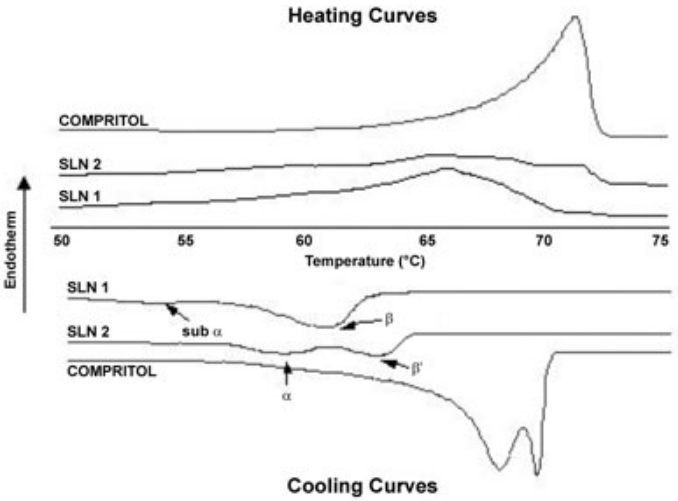

Figure 4. DSC heating and cooling curves of bulk lipid (Compritol) and SLN formulations (SLN 1, SLN 2) 1 day after production. The curves were not standardized.

Muller[31] used this method to investigate the different crystallization phase of Compritol SLN. The cooling curves obtained 1 day after production showed that the 2 formulations recrystallized in different polymorphic forms. The SLN 1 cooling curve shows a main peak at 60ºC, which can be attributed to the beta modification and a shoulder at 53ºC, which indicates a presence of a subalpha modification. The SLN 2 cooling curve on the contrary shows 2 main peaks at 62ºC and 59ºC that suggest the presence of beta′ and alpha modifications.

The melting peaks and enthalpies of *Artemisia arborescens* L essential oil–loaded SLN (SLN 1, SLN 2) 1 day and 60 days after production stored at RT, 4ºC, and 40ºC are reported in Table 4. The melting enthalpy of pure Compritol (lipid) is used as a reference (100%) to calculate a theoretical percentage of the crystallinity of SLN formulations. The data confirmed that Compritol SLN have a high degree of crystallinity as found for other SLN formulations prepared using the same lipid.[29,31]

When comparing pure lipid with SLN 1 and SLN 2 at day 1, the melting enthalpy of pure lipid was 110.1 J/g, while those of SLN 1 and SLN 2 were 10.7 J/g and 8.6 J/g, respectively. Considering that the lipid content of both formulations was 9%, we can suggest that these data represent a crystallinity index of 107.7% and 86.2%. These values demonstrated that lipid crystallization occurred at least partly and that no supercooled melts were present in the *Artemisia arborescens* L essential oil–loaded SLN.[1] However, as seen in Figure 4, the lipid is present partly in the alpha-modification after 1 day and is thus likely to undergo polymorphic transition during storage.

In general during storage the crystallinity index of both formulations increased as demonstrated by the day 60 data.

Table 4. Melting Peaks and Enthalpies of *Artemisia arborescens* L Essential Oil–loaded SLN Stored at Room Temperature (RT), 4ºC, and 40ºC at Day 1 (D1) and Day 60 (D60)*

Formulations		Melting Peak (°C)	Enthalpy (J/g)	Crystallinity Index (%)
Compritol		71.2	110.1	100.0
SLN 1	D1	65.7	10.7	107.7
	D60 RT	68.5	11.6	117.5
	D60 40°C	68.8	13.1	133.0
	D60 4°C	69.0	11.0	111.5
SLN 2	D1	66.7	8.6	86.2
	D60 RT	68.1	9.4	94.0
	D60 40°C	69.7	11.3	114.5
	D60 4°C	66.3	9.3	93.8

*SLN indicates solid lipid nanoparticles. The melting enthalpy of pure Compritol (lipid) is used as a reference (100%) to calculate the theoretical percentage of crystallinity of SLN formulations. The enthalpy values were not standardized.

This data suggested the repair of lattice defects during storage at all storage temperatures. However, for both formulations, storage at 4ºC determined a very slight increase in the crystallinity index, suggesting the possibility of maintaining the formulations in their present polymorphic forms for a longer period when stored at this temperature.

Spraying of SLN

It has been reported that in some suboptimal stabilized SLN formulations, shear forces (like pressing through the needle of a syringe or the force during spray drying) might potentially promote an increase in the particle size distribution, which can cause particle aggregation and a gelation of the formulation.[30,32] For the application in agriculture, this new delivery system for ecological pesticide must be sprayed over the plants or trees. Figure 5 shows the variation of the average diameter and PI of SLN 1 and SLN 2 formulations before and after spraying (sprayed formulations).

The PCS data showed that the sprayed SLN dispersions of both SLN 1 and SLN 2 formulations had average diameters below 300 nm.

The spraying process of the SLN 1 formulation led to a small increase in average size. However, the formulation SLN 1 stored at 40ºC was not sprayable: the pressure exerted by the spraying apparatus led to a gelation of the SLN dispersion, and no liquid was collected over the orifice of the apparatus. As described in the paragraph above, this phenomenon is due to a low ZP of this formulation 60 days after production. The ZP value was not high enough to avoid the collision of particles during spraying and a gel network formed.

The formulation SLN 2 showed no change in average diameter after spraying for all storage temperatures confirming high stability. The LD data showed that all particles of all sprayed formulations were in the submicron range.

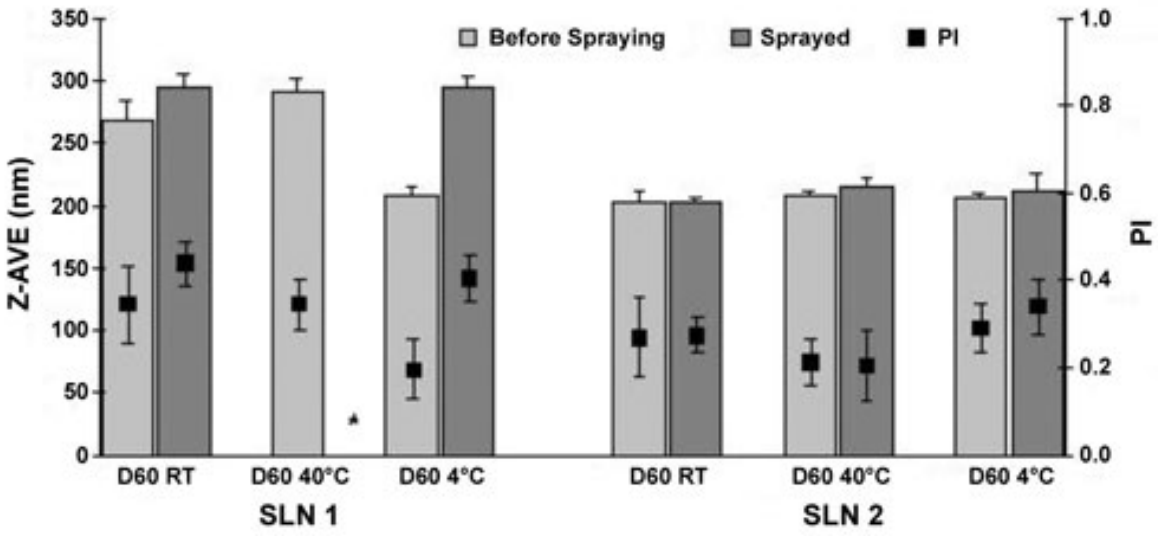

Figure 5. Z-AVE and PI of SLN 1 and SLN 2 *Artemisia arborescens* L essential oil–loaded SLN 60 days after production stored at RT, 4ºC, and 40ºC before and after spraying. *The SLN 1 dispersion stored at 40ºC for 60 days was not sprayable.

In Vitro Release

The amount of essential oil needed to achieve pesticidal activity strongly depends on the type of pest and the cycle of the insect life. For example, *Artemisia arborescens* L essential oil demonstrated a LC_{100} = 0.1 mg/cm^2 (lethal concentration expressed as mg of essential oil/cm^2 of foliar surface) when tested against adult *Bemisia tabaci* insects and LC_{100} = 0.18 mg/cm^2 when tested against young *Bemisia tabaci*. The concentration of the different SLN formulations (1%) is in the same order of value (0.5%-1.5%) as the marketed ready to use essential oil-based emulsion pesticide formulations.

An important point to consider when studying design formulations for agricultural applications is the capability to release the active substance in a controlled manner. Different studies have shown that the rapid evaporation, leaching, and degradation of some active substances can lead to a dramatic decrease of formulation performance.[33,34] For this reason pesticides have been encapsulated into different microcapsules or microspheres of different polymers.[33,34] Several studies have shown that the incorporation of volatile compounds into SLN prevents their rapid evaporation.[22,23] To investigate the capability of SLN to prevent the rapid evaporation of the incorporated *Artemisia arborescens* L essential oil, samples of the SLN 1 and SLN 2 formulations and EMU 1 and EMU 2 emulsion formulations were transferred into open glass vials and stored at 35°C for 48 hours. Figure 6 shows the comparison of the in vitro cumulative evaporation release of *Artemisia arborescens* L essential oil from SLN and emulsion formulations. The figure clearly shows that the incorporation of the essential oil into both SLN formulations (SLN 1, SLN 2) determined a decrease in its evaporation when compared with the emulsion formulations (EMU 1, EMU 2). After 1 hour, the EMU 1 and EMU 2 showed a burst release with a loss respectively of 21.6% and 13.52% of the total essential oil in the formulations. This burst effect was not present in the case of the SLN formulations (SLN 1, SLN 2), which had a loss of only 2.98% and 5.94%, respectively, after 1 hour. Moreover the emulsion formulations showed a higher cumulative release rate (slope of the curves) than that of the SLN formulations. In general during the first 24 hours all formulations showed a higher release rate than in the subsequent hours. In the case of SLN formulations, this higher release rate in the first 24 hours is probably due to the evaporation of the not incorporated essential oil, adsorbed on the lipid nanoparticle surface.

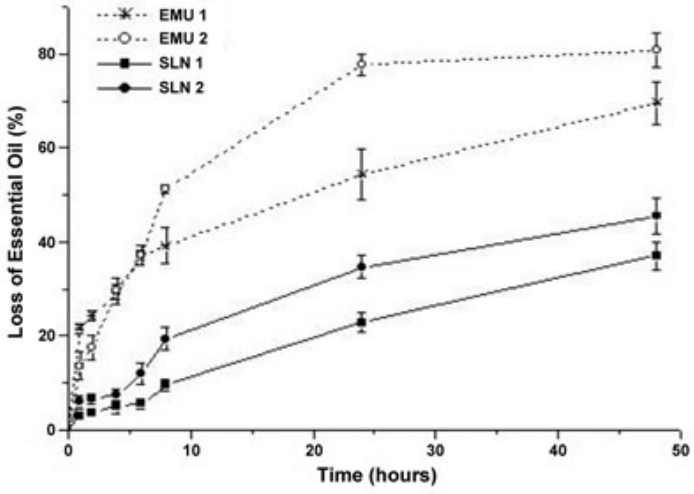

Figure 6. In vitro evaporation release of *Artemisia arborescens* L essential oil from SLN formulations (SLN 1, SLN 2) and related emulsions EMU 1 and EMU 2 stored at 35°C.

After 48 hours, the cumulative release of the EMU 1 and EMU 2 formulations was 69.56% and 80.77%, respectively, while for SLN 1 and SLN 2 it was 37.07% and 45.51%, respectively.

Data also showed that the use of different surfactants affects the cumulative release for both SLN and emulsion formulations. In particular, the use of Poloxamer 188 (EMU 1, SLN 1) instead of Miranol Ultra C32 (EMU 2, SLN 2) decreased the evaporation of the *Artemisia arborescens* L essential oil.

Comparison of release data obtained by the spectrometry method with those from GC/ITMS also showed that there was not any selective loss of the main oil components. Reciprocal area ratios of these compounds did not change throughout the study.

CONCLUSION

Results obtained during this study showed that SLN are good potential carriers for ecological pesticides in agriculture. All studied formulations demonstrated a high physical stability and a good capability to reduce the essential oil evaporation. The best results were obtained with SLN 2 formulation (Miranol Ultra C32 surfactant), which did not vary in size even after the spraying procedure.

ACKNOWLEDGMENTS

This study was partially supported by a grant from Assessorato all'Igiene e Sanità, Regione Autonoma della Sardegna, Progetti di ricerca e di educazione sanitaria.

REFERENCES

1. Mehnert W, Mader K. Solid lipid nanoparticles: production, characterization, and applications. *Adv Drug Deliv Rev.* 2001; 47:165–196.

2. Müller RH, Lucks JS, inventors. Arzneistofftrager aus festen Lipid-teilchen, Feste Lipidnanospharen (SLN). European patent 0605497. March 1996.

3. Gasco MR, inventor. Method for producing solid lipid microspheres having a narrow size distribution. US patent 5250236. October 5, 1993.

4. Wissing SA, Müller RH. Solid lipid nanoparticles as carrier for sunscreens: in vitro release and in vivo skin penetration. *J Control Release.* 2002;81:225–233.

5. Zara GP, Cavalli R, Bargoni A, Fundaro A, Vighetto D, Gasco MR. Intravenous administration to rabbits of non-stealth and stealth doxorubicin-loaded solid lipid nanoparticles at increasing concentrations of stealth agent: pharmacokinetics and distribution of doxorubicin in brain and other tissues. *J Drug Target.* 2002;10:327–335.

6. Chen DB, Yang TZ, Lu WL, Zhang Q. In vitro and in vivo study of 2 types of long-circulating solid lipid nanoparticles containing paclitaxel. *Chem Pharm Bull (Tokyo).* 2001;49:1444–1447.

7. Zhang Q, Yie G, Li Y, Yang Q, Nagai T. Studies on the cyclosporin A loaded stearic acid nanoparticles. *Int J Pharm.* 2000;200:153–159.

8. Lai F, Wissing SA. Peroral administration of SLN. *Acta Techn Leg Med.* 2003;XIV:2–3.

9. Cavalli R, Gasco MR, Chetoni P, Burgalassi S, Saettone MF. Solid lipid nanoparticles (SLN) as ocular delivery system for tobramycin. *Int J Pharm.* 2002;238:241–245.

10. Frederiksen HK, Kristensen HG, Pedersen M. Solid-lipid nanoparticle formulations (SLN) of the pyrethroid gamma-cylalothrin (GCH): incompatibility of the lipid and the pyrethroid. *J Control Release.* 2003;86:243–253.

11. Pillmoor JB, Wright K, Terry AS. Natural products as a source of agrochemicals and leads for chemical synthesis. *Pestic Sci.* 1993; 39:131–140.

12. Chiasson H, Belanger A, Bostanian N, Vincent C, Poliquin A. Acaricidal properties of *Artemisia absinthium* and *Tanacetum vulgare* (Asteraceae) essential oils obtained by 3 methods of extraction. *J Econ Entomol.* 2001;94:167–171.

13. Bessette SM, Enan EE, inventors. Insecticidal compositions for household pests containing rosemary oil. World patent 0100032. January 2001.

14. Moretti MDL, Sanna-Passino G, Demontis S, Bazzoni E. Essential oil formulations useful as a new tool for insect pest control. *AAPS PharmSciTech.* 2002;3:E13.

15. Isman MB. Plant essential oils for pest and disease management. *Crop Prot.* 2000;19:603–608.

16. Sherif A, Hall RG, el-Amamy M. Drugs, insecticides and other agents from *Artemisia. Med Hypotheses.* 1987;23:187–193.

17. Ballero M, Poli F, Sacchetti G, Loi MC. Ethnobotanical research in the territory of Fluminimaggiore (south-western Sardinia). *Fitoterapia.* 2001;72:788–801.

18. Sinico C, De Logu A, Lai F, et al. Liposomal incorporation of *Artemisia arborescens* L essential oil and *in vitro* antiviral activity. *Eur J Pharm Biopharm.* 2005;59:161–168.

19. Lai F, Sinico C, Valenti D, Casu L, Loy G, Fadda AM. *Artemisia arborescens* L essential oil-loaded beads: preparation and characterization. Proceedings of the 30th Annual Meeting & Exposition of the Controlled Release Society (CRS); July 19-23, 2003; Glasgow, Scotland, Minneapolis: Controlled Release Society; 2003:45.

20. Wissing SA, Müller RH. A novel sunscreen system based on tocopherol acetate incorporated into solid lipid nanoparticles. *J Cosmet Sci.* 2001;23:233–243.

21. Jenning V, Gohla SH. Encapsulation of retinoids in solid lipid nanoparticles (SLN). *J Microencapsul.* 2001;18:149–158.

22. Wissing SA, Mäder K, Müller RH. Prolonged efficacy of the insect repellent lemon oil by incorporation into solid lipid nanoparticles (SLN™). Third World Meeting APGI/APV; April 3-6, 2000; Berlin, Germany, Mainz, Germany: APV; 2000:439–440.

23. Wissing SA, Mäder K, Müller RH. Solid Lipid Nanoparticles (SLN) as a novel carrier system offering prolonged release of the perfume allure (Chanel). *Proc Intern Symp Control Rel Bioact Mater.* July 9-13, 2000:311–312.

24. Hildebrand GE, Dingler A, Runge SA, Müller RH. Medium scale production of solid lipid nanoparticles (SLN). *Proc Int Symp Control Rel Bioact Mater.* 1998:968–969.

25. Müller RH, Dingler A, Schneppe T, Gohla S. Large scale production of solid lipid nanoparticles (SLN) and nanosuspensions (DissoCube). In: Wise D, ed. *Handbook of Pharmaceutical Controlled Release Technology.* New York, NY: Marcel Dekker Inc; 2000:359–376.

26. Adams RP. *Identification of the Essential Oil Components by Gas-Chromatography/Mass Spectroscopy.* Carol Stream, IL: Allured Publishing Corp; 1995.

27. National Institute of Standards and Technology. NIST Scientific and Technical Databases [database online]. The NIST Mass Spectral Search Program for the NIST/EPA/NIM Mass Spectral Library Version 1.7, 1999.

28. Sacco T, Frattini C, Bicchi C. Constituents of essential oil of *Artemisia arborescens. Planta Med.* 1983;47:49–51.

29. Freitas C, Müller RH. Effect of light and temperature on zeta potential and physical stability in Solid Lipid Nanoparticles (SLN) dispersions. *Int J Pharm.* 1998;168:221–229.

30. Freitas C, Müller RH. Correlation between long-term stability of solid lipid nanoparticles (SLN) and crystallinity of the lipid phase. *Eur J Pharm Biopharm.* 1999;47:125–132.

31. zur Mühlen A, zur Mühlen E, Niehus H, Mehnert W. Atomic force microscopy studies of solid lipid nanoparticles. *Pharm Res.* 1996; 13:1411–1416.

32. Freitas C, Muller RH. Spray-drying of solid lipid nanoparticles (SLN). *Eur J Pharm Biopharm.* 1998;46:145–151.

33. Dailey OD, Dowler CC. Polymeric microcapsules of cyanazine*:* preparation and evaluation of efficacy. *J Agric Food Chem.* 1998; 46:3823–3827.

34. Mogul MG, Akin H, Hasirci N, Trantolo DJ, Gressen JD, Wise DL. Controlled release of biologically active agents for purposes of agricultural crop management. *Resource Conservation Recycling.* 1996;16:289–320.