Formulation and characterization of loaded rosmarinic acid solid lipid nanoparticles

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NanoFormulation2012
28 May - 1 June 2012 Barcelona, Spain www.nanoformulation2012.com

Introduction

During the last decade has been a growing interest in the formulation of new food and nutraceutical products containing compounds with antioxidant activity. Unfortunately, certain compounds such as polyphenols are not stable when incorporated in certain food matrices. The use of solid lipid nanoparticles (SLNs) has been extensively reported and represents an alternative carrier system to traditional colloidal carriers. Furthermore, SLNs combine advantages such as biocompatibility and biodegradability, physical stability, protection of incorporated compounds, controlled release and specific targeting (Parhi and Suresh, 2010). The increasing demand for functional foods with beneficial effects for human health, has opened the doors to the use of SLN in the food industry, with the incorporation of natural compounds with beneficial purposes.

With this aim, SLNs were prepared by a hot melt ultrasonication method using Witepsol E85 as lipid and Tween 80 as surfactant. The effects of lipid proportion in the lipid mixture and surfactant concentration were evaluated. Also, the stability of the nanoparticles during 28 days was tested in aqueous solution stored at 4 ºC, tracking the mean particle size, polydispersity index (PI) and zeta potential (ZP). Thermal analyses of SLNs were performed using DSC (Differential Scanning Calorimetry) and crystallinity index (CI%) was obtained through enthalpy values of SLN formulations and raw materials. The loading efficiencies as well surface properties and morphology were also evaluated.

Materials & Methods

Preparation of SLNs

The SLNs were prepared by hot melt ultrasonication method using rosmarinic acid (RA) (0.15 mg/mL) and Witepsol E85 as lipid according a 3^2 factorial design. As surfactant, tween 80 at different percentages (viz. 1, 2 and 3%, v/v) and Witepsol E85 (0.5, 1 and 1.5%, w/v) were used to optimize the formulation. Witepsol E85 was warmed up to 5 ºC above the melting point of the lipid (i.e. 44 ºC). The surfactant solution was added to the lipid and polyphenol solution and then homogenized during 1 min at 70% of intensity in sonicator. The resulting solution was left to cool at room temperature. SLNs were stored at 4 ºC during 28 days.

Particle size and zeta potential analyses

The average particle size (PS), polydispersity (PI) and zeta potential (ZP) were analyzed by phase analysis light scattering using ZetaPALS, Zeta Potential Analyzer (Holtsville, New York, USA); samples were diluted with MilliQ-water to suitable concentration and were analyzed using an angle of 90 degrees at 25 ºC.

Thermal properties determination

DSC thermograms of the materials used and final SLNs were obtained using differential scanning calorimetry (DSC-60, Shimadzu, Columbia, USA). The measurements were performed on freeze-dried SLN, and 3 mg of SLN were placed on an aluminum pan and the thermal behavior determined in the range of 20-100 ºC at a heating rate of 10 ºC/min. Enthalpies were calculated by equipment software. The crystallinity indexes (CI%) of SLNs were calculated using the following equation:

$$CI\% = \frac{\text{Melting enthalpy}_{\text{SLN dispersion}}\ (J/g)}{\text{Melting enthalpy}_{\text{bulk material without rosmarinic acid}}\ (J/g).\text{Concentration}} \times 100$$

Morphology properties of SLNs

The morphology of nanoparticles was investigated by SEM (Scanning Electron Microscopy). Briefly, an amount of freeze-dried SLNs were placed in proper supports and coated with gold using a Sputter Coater (Polaron).

Loading efficiency

The loading efficiency (LE) was calculated by measuring the concentration of rosmarinic acid in supernatants by HPLC according the method described by Fonte, *et al.*, (2011). The LE was determined according to the following formula:

$$LE\% = \frac{\text{Total amount of Rosmarinic Acid} - \text{Amount of Rosmarinic Acid on Supernatant}}{\text{Total amount of Rosmarinic Acid}} \times 100$$

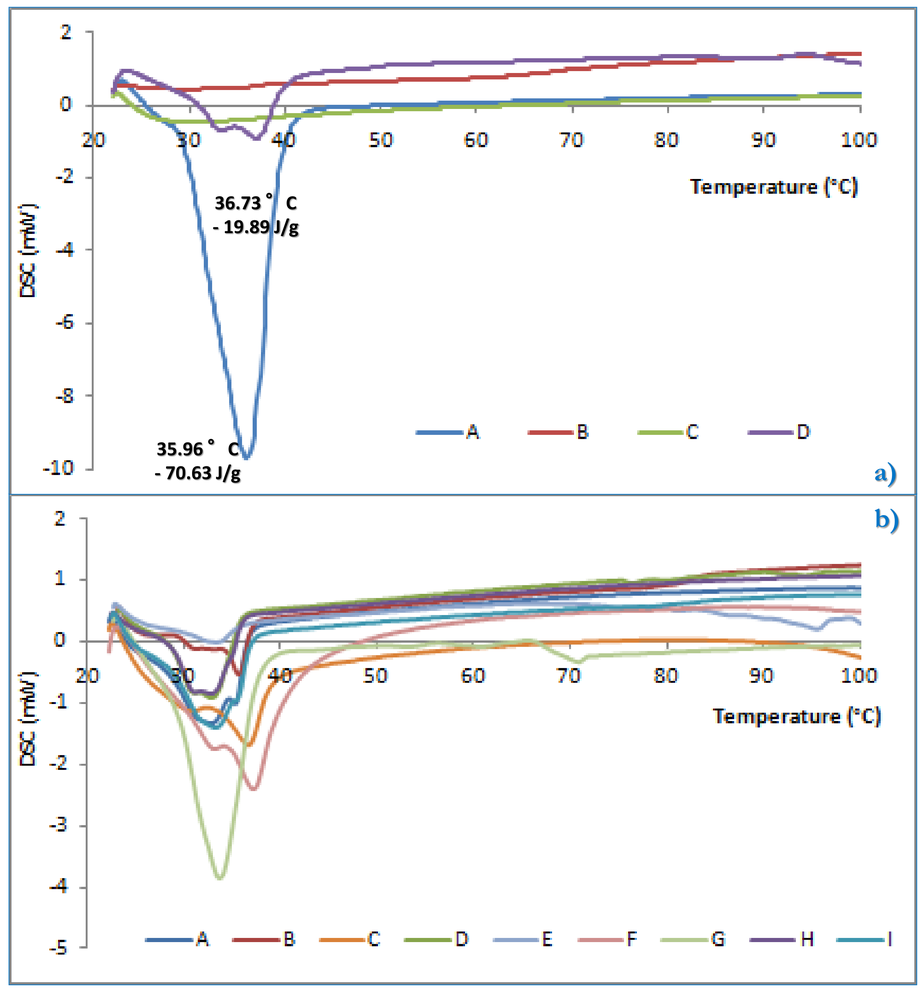

Fig. 1: Differential scanning calorimetry thermograms of the ingredients **(a)** and the SLNs **(b)**. **(a)** Raw materials: **(A)** Witepsol E85, **(B)** Rosmarinic acid, **(C)** Tween 80 **(D)** 1.0% Witepsol E85: 2% Tween 80 without RA. **(b)** Percentage of Witepsol E85 (w/v) and percentage of Tween 80 (v/v), **(A)** 0.5% : 1%, **(B)** 0.5% : 2%, **(C)** 0.5% : 3%, **(D)** 1.0% : 1%, **(E)** 1.0% : 2%, **(F)** 1.0% : 3%, **(G)** 1.5% : 1%, **(H)** 1.5% : 2%, **(I)** 1.5% : 3%.

Table 1: Physical properties of the SLNs throughout storage time, formulated with the different % of Witepsol E85 (w/v) and percentage of Tween 80 (v/v), **(A)** 0.5% : 1%, **(B)** 0.5% : 2%, **(C)** 0.5% : 3%, **(D)** 1.0% : 1%, **(E)** 1.0% : 2%, **(F)** 1.0% : 3%, **(G)** 1.5% : 1%, **(H)** 1.5% : 2%, **(I)** 1.5% : 3%.

SLN Witepsol	Days	A	B	C	D	E	F	G	H	I
Particle size (PS) (nm)	0	411 ± 1	1149 ± 117	659 ± 289	646 ± 291	1303 ± 147	1400 ± 31	650 ± 328	372 ± 80	423 ± 109
	28	821 ± 48	956 ± 225	872 ± 132	663 ± 130	648 ± 5	694 ± 194	588 ± 223	583 ± 97	769 ± 102
Polydispersity index (PI)	0	0.23 ± 0.03	0.28 ± 0.11	0.23±0.03	0.29±0.09	0.30±0.10	0.29±0.09	0.32±0.05	0.25±0.09	0.28±0.04
	28	0.30 ± 0.05	0.30 ± 0.01	0.25 ± 0.03	0.28 ± 0.09	0.20 ± 0.09	0.29 ± 0.03	0.30 ± 0.03	0.24 ± 0.10	0.29 ± 0.06
Zeta potential (ZP)	0	-38.4 ± 3.00	-39.4±3.11	-39.1 ± 3.15	-38.2 ± 2.89	-38.7 ± 3.01	-38.2 ± 2.92	-38.0 ± 2.89	-38.7 ± 3.03	-38.6 ± 3.02
	28	-38.9 ± 3.00	-38.2±2.94	-38.2 ± 2.91	-39.5 ± 3.12	-37.7 ± 2.87	-38.3 ± 2.94	-38.0 ± 2.89	-38.8 ± 3.01	-37.8 ± 2.86
Loading efficiency (%)	0	99.88	99.89	99.87	99.84	99.80	99.83	99.78	99.80	99.76
	28	99.75	99.82	99.81	99.89	99.89	99.89	99.93	99.93	99.85
Thermal properties of lyophilized SLN	Enthalpy (J/g)	-31.88 ± 5.9	-17.47 ± 0.0	-19.55 ± 8.4	-24.10 ± 8.32	-21.19 ± 0.00	-40.31 ± 0.00	-35.11 ±0.01	-24.03 ±2.01	-31.56 ± 11.71
	Melting T (ºC)	35.74	38.50	36.55	35.53	37.03	37.12	38.11	35.52	36.04
	CI%	90.27	49.47	55.36	34.13	30.85	57.07	33.14	22.68	29.79

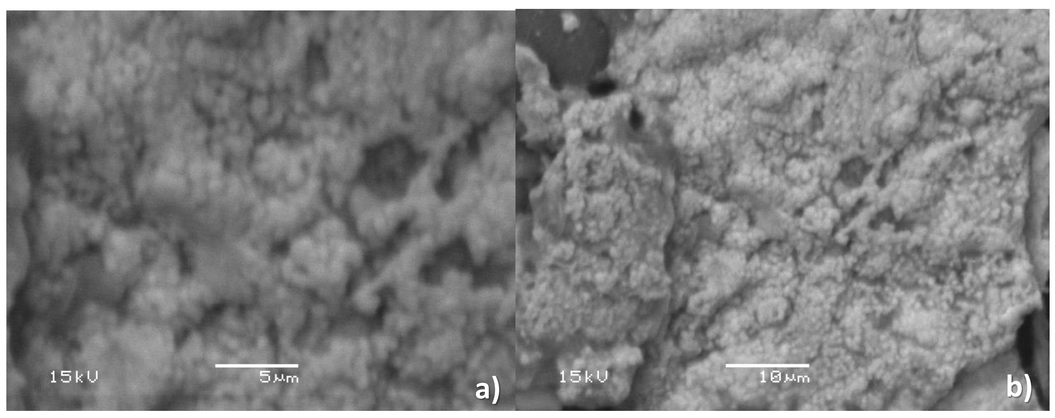

Fig. 2: Micrographs of SLNs by scanning electron microscope (SEM). Percentage of Witepsol E85 (w/v) and percentage of Tween 80 (v/v), **(a)** 1.0% : 1%, 5000x and **(b)** 1.5% : 2%, 2000x.

Discussion

- In general, after 28 days, increasing the lipid content decreases the mean PS, but the average of PS increased when using higher contents of Tween 80; with exception of the formulations B and E;
- There were no significant differences in the PI ($P>0.05$). Nevertheless, low PI indicates better stability of SLN over time, as example the formulations A, C and H;
- In general, all formulations resulted in a moderate high negative value of ZP, with values between -37.0 and -40.0, which indicates moderate to good stability of SLN throughout time (Muller, 1996);
- The percentage of loading efficiency is high for all formulations (≈ 99.8%), which means that the polyphenol entrapment does not change with the different formulations tested and throughout storage;
- The melting temperature is similar for all the formulations, rang of temperature between 35.5 - 38.5 ºC, including the formulation without RA (36.7 ºC; Fig 1aD), and are in the range of the value of Witepsol E85 (36.0 ºC; Fig 1aA), which means that the formulations does not change the chemical stability of the lipid used;
- All enthalpy values are negative; formulation B showed a lower enthalpy value, which suggests that the SLNs have lower particle arrangement, in contrast with F, which showed a highest value;
- High values of CI% normally leads to a bigger drug release, as example the formulation A, but with the increase of lipid percentage in the formulation the CI% decreases;
- Figure 2 shows micrographs with rounded shape SLNs of Witepsol E85.

Conclusion

- **The smallest, physical and thermally stable SLNs were those for H formulation (1.5% lipid and 2 % Tween 80). With high negative ZP the SLNs presented good stability. The formulations do not affect the polyphenol entrapment.**

References

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Acknowledgements

FCT Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA EDUCAÇÃO E CIÊNCIA

Authors acknowledge FCT- Fundação para a Ciência e Tecnologia for funding research work., through project (PTDC/AGR-ALI/117808/2010 and project PEst-OE/EQB/LA0016/2011