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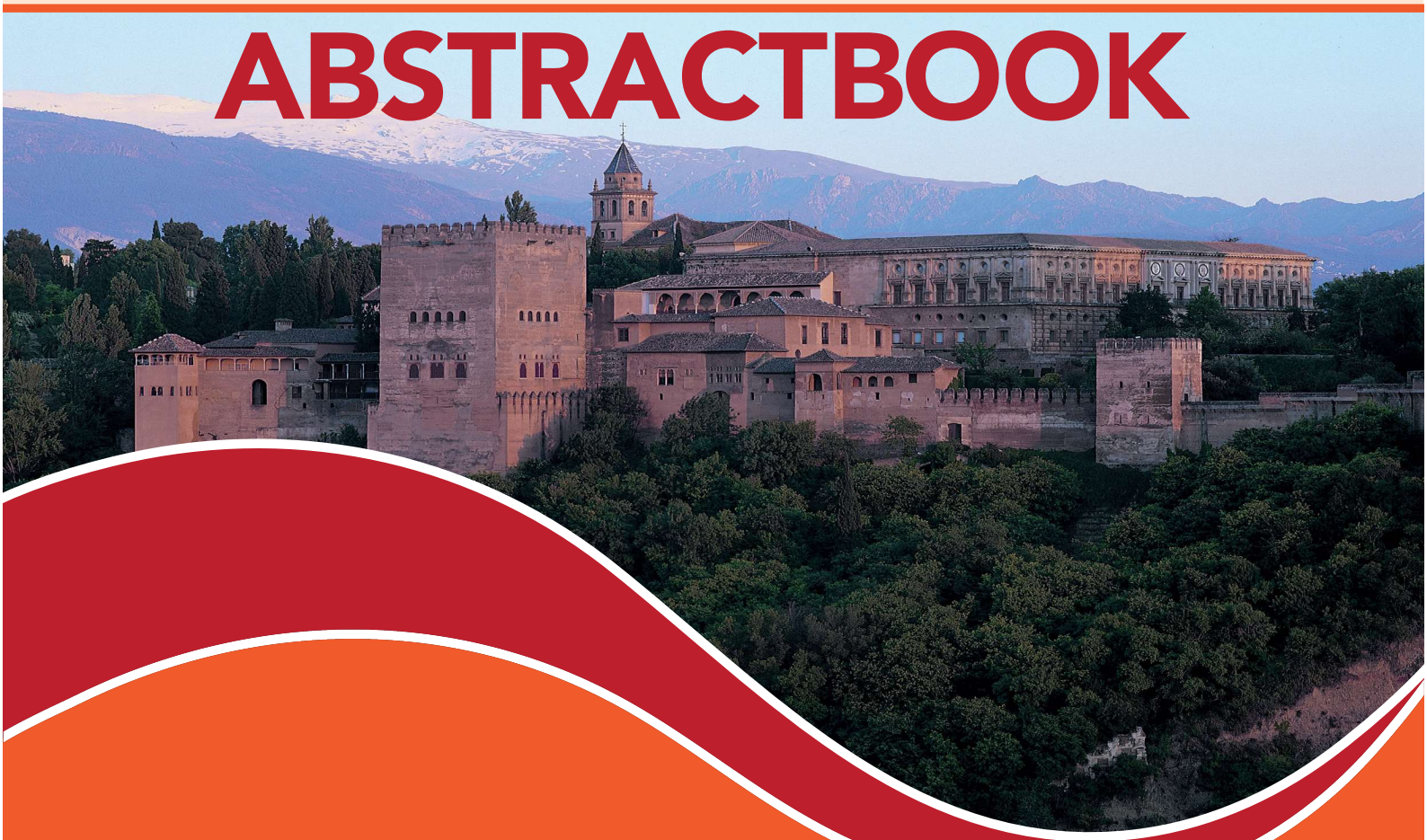
ABSTRACTBOOK



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Nanostructured lipid carriers loaded with *Salvia off.* extract for intranasal delivery

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INTRODUCTION

Considering that Alzheimer's pathophysiology involves more than one aspects and that single target drugs are not always efficacious in the desired extent, promoting multi-targeted drug therapy would be better pathway to achieve the desired treatment. Most of the natural products are multi-target as they are rich reservoir for drug discovery because of their diversity and complexity of structures. It has been suggested, on the basis of traditional medicine, its in vitro cholinergic binding properties and modulation of mood and cognitive performance in humans, that *Salvia officinalis* L. might potentially provide a novel natural treatment for Alzheimer's disease [Akhondzadeh et al. 2003]. However, having in mind that the blood brain barrier (BBB) is a significant barrier for efficient drug delivery to the brain, alternative ways of administration are becoming increasingly popular. Research has shown that avoiding circulation and direct delivery to the brain is possible following intranasal administration where most likely drugs reach the brain using axonal transport through the olfactory and trigeminal nerve [Pardeshi and Belgamwar, 2013]. Taking into account the previous, the aim of this study was formulation of nanostructured lipid carriers (NLC) loaded with freeze-dried methanolic extract of *Salvia officinalis* (FSE) for intranasal application and hence evaluation of the influence of total lipid to organic phase ratio upon NLC-FSEs` properties (particle size, encapsulation efficiency (EE), in vitro release as well as antioxidant activity).

MATERIALS AND METHODS

Materials

Phospholipon 90H was kindly donated from Phospholipid, Germany. Oleic acid (OA) was purchased from Sigma-Aldrich, Germany, Tween 80 from Merck, Germany. Poloxamer 407 from BASF, Germany and Ethanol 96% from Alkaloid, Macedonia. All other reagents were of analytical grade and used without any modification.

Preparation of NLC-FSE

NLCs were prepared by solvent evaporation method. Briefly, the lipid phase consisted of phospholipon as solid lipid (0.150 g) and OA (0.065 g) as liquid lipid was dissolved in ethanol on magnetic stirrer (70 °C, 250 rpm, Jenway, UK). Afterwards FSE (25 mg) was added. Ratio of total lipid and organic solvent was varied: 1:10, 1:20, 1:30, 1:40 and prepared formulations were coded as NLC-FSE1, NLC-FSE2, NLC-FSE3 and NLC-FSE4, accordingly. Lipid phase was added dropwise to aqueous phase (9.03 g water solution of 2% Tween 80 and 0.5% Poloxamer 407) and stirred continuously at previously mentioned conditions until complete evaporation of ethanol. The obtained hot lipid emulsions were cooled to room temperature by magnetic stirring (25 °C, 250 rpm, Variomag, Multipoint HP 15, Germany) and kept at 4 ºC overnight to allow the recrystallisation of the lipid for NLCs formation.

Characterization of NLC-FSE

Particle size determination

Particle size and particle size distribution were determined by laser diffractometry (Mastersizer 2000, Hydro 2000S, UK) using previously validated method. The conditions were as follows: 2520 rpm/stirrer rate and obscuration 1 %. Before measurement, samples were dispersed in 0.9 % NaCl and stirred in the cell for 5 minutes at the same conditions. Particle size was expressed as D_{50} (nm), while particle size distribution as span factor.

Transmission electron microscopy

Morphological properties were examined using transmission electron microscopy (TEM) (JEM-1400, Jeol, Japan) attached to a digital camera (Veleta TEM Camera, Olympus, Germany) and controlled by the iTem software v.5.2.

Encapsulation efficiency

Separation of nonencapsulated FSE from NLC was performed by centrifugal ultrafiltration using Vivaspin 20 ultrafiltration spin columns, 1000 KDa (Sartorius Stedim Biotech GmbH, Germany). FSE content was determined by previously validated HPLC method [Cvetkovikj et al. 2013]. EE (%) was calculated from linear regression of the external standard of FSE.

In vitro release studies

In vitro release study of NLC-FSEs was carried out by membrane diffusion method (MEMBRA-CEL® dialysis tubing, regenerated cellulose, MWCO 7000 RC, Serva,

Germany). The dissolution tests were performed in phosphate buffer pH 7.4 (Eur. Ph. 9.0) (37 °C, 100 rpm) under sink conditions at appropriate time intervals (0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8 and 24 h). Samples were analyzed by previously mentioned HPLC method. NLC-FSE dissolution data modeling was performed with DDSolver 1.0 program (menu-driven add-in program for Microsoft Excel).

Antioxidant activity

Antioxidant activity of FSE incorporated in NLC-FSEs compared to FSE alone was determined with β-carotene bleaching assay carried out at 37 °C according to the method developed by Wettasinghe and Shahidi [1999]. Antioxidant activity was expressed as $IC_{50\%}$ (the concentration of FSE encapsulated into NLC as well as FSE alone (mg/ml) inhibiting 50% of b-carotene oxidation).

All analyses were performed in triplicates.

RESULTS AND DISCUSION

Characterization of NLC-FSE

Using solvent evaporation method four different formulations of NLC-FSEs were prepared and subjected to further characterization.

Surface morphology of prepared NLC-FSEs was examined and generally, a spherical morphology with smooth surface appearance was observed.

NLC-FSEs` particle size, EE and $IC_{50\%}$ are presented in Table 1. Particle size of NLC-FSE2 was significantly smaller compared to other formulations (one way ANOVA, Fischer LSD, $p<0.05$). Span values ranged from 1.418 ± 0.928 to 3.825 ± 0.198.

Formulation	D_{50} (nm)±SD	EE (%)±SD	$IC_{50\%}$ (mg/ml)±SD
NLC-FSE1	145±2.64	80.15±2.2	$0.037\pm0.8*10^{-3}$
NLC-FSE2	132±0.97	77.71±1.8	$0.04\pm1.3*10^{-3}$
NLC-FSE3	143±1.14	76.61±2.1	$0.043\pm0.4*10^{-3}$
NLC-FSE4	140±1.13	78.45±1.9	$0.045\pm1.9*10^{-3}$
FSE	/	/	$0.07\pm2*10^{-3}$

Table 1. Particle size, encapsulation efficiency and antioxidant activity of prepared formulations

Considering that diameter of olfactory axons of humans are between 100 and 700 nm [Morrison and Constanzo, 1992], it might be anticipated that particle size of prepared formulations is suitable for intracellular transport to the brain *via* the neural pathway.

Although statistically insignificant, it can be noticed that NLC-FSE1 (ratio of total lipid to organic solvent (1:10) has the highest EE.

The release profiles of prepared NLC-FSEs, are shown in Figure 1. It could be concluded that prolonged release was obtained as only ~40% of FSE was released over 24h. Comparing the values of correlation coefficient (R^2) of different kinetics models, the best fit was established for the Peppas and Sahlin model, where values of *k1* and *k2* indicated dominance of Fickian diffusion and insignificant effect of case II transport, accordingly.

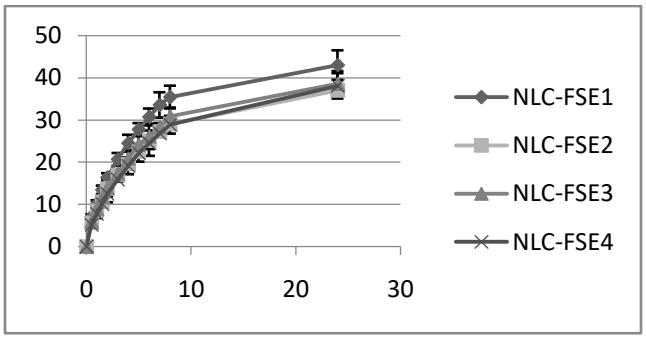

Figure 1. In vitro release profiles of prepared NLC-FSE

Lowest $IC_{50\%}$ value was observed for NLC-FSE1 (Table 1) thus indicating higher antioxidant activity compared to NLC-FSE 2-4 as well as FSE alone most likely due to the faster FSE release (Figure 1).

CONCLUSION

Influence of total lipid to organic phase ratio on NLC-FSEs` properties was investigated. Obtained results were in favor of their potential for intranasal administration and efficient treatment of Alzheimer's disease.

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