Potential of antioxidant-loaded solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) for the management of neurodegenerative diseases

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Introduction

Neurodegenerative diseases *(NDs), such as* ***Alzheimer's disease*** *and* ***Parkinson's disease****, constitute a major health problem as they affect millions of people worldwide (1).*

Extensive evidence highlights a common pathophysiological mechanism in these diseases, the ***oxidative stress*** (2)*. Therefore,* ***the use of antioxidants arises as a promising approach in NDs therapy, potentially limiting the neuronal loss and slowing the disease progression*** (3).

Aim

- **To develop and characterize solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) for brain delivery loaded with 1000 µg/mL of an antioxidant extract (AE) and 1000 µg/mL of pure antioxidant (PA).**
- **To evaluate the biocompatibility of the developed formulations in human neuronal SH-SY5Y cells differentiated into a dopaminergic phenotype.**

Methods

- ***Particle size*** *was evaluated by* ***laser diffractometry*** *(****LD****), using a Mastersizer 3000, and by* ***dynamic light scattering*** *(****DLS****), using a Malvern nanozetasizer.*
- ***Polydispersity index (PDI) and zeta potential*** *(****ZP****) were also evaluated using a Malvern nanozetasizer. Before the experiments, the samples were diluted with ultrapure water (1:100). All measurements were performed using a backscatter angle of 173°. For particle size measurements, the particle refractive index and the absorption index were set to 1,6 and 0,001, respectively.*
- ***Encapsulation efficiency:*** *evaluated, indirectly, by measuring the amount of non-encapsulated compound by high performance liquid chromatography conected to an UV detector (HPLC-UV).*
- ***Biocompatibility****: exposure of differentiated SH-SY5Y to different concentrations of formulations (0 - 500 µg/mL), for 24 h, followed by cytotoxicity evaluation by the MTT reduction and neutral red (NR) uptake assays.*

Results

Particle size, polydispersity index and zeta potential

	Time (days)	D10 (nm)	D50 (nm)	D90 (nm)	Z-Ave (nm)	PDI	ZP (mV)	EE (%)
Empty SLN	0	43,20 ± 0,00	69,80 ± 0,00	105,00 ± 0,00	98,04 ± 1,20	0,21 ± 0,01	-20,03 ± 0,64	-
	180*	18,00 ± 0,00	41,30 ± 0,00	109,00 ± 0,00	98,11 ± 0,64	0,23 ± 0,01	-22,70 ± 0,36	-
Empty NLC	0	14,30 ± 0,00	30,80 ± 0,00	91,90 ± 0,01	109,03 ± 0,40	0,23 ± 0,01	-23,47 ± 1,08	-
	180*	20,40 ± 0,00	52,20 ± 0,00	129,00 ± 0,00	116,03 ± 1,80	0,22 ± 0,00	-23,83 ± 0,46	-
AE - loaded SLN	0	17,80 ± 0,00	45,30 ± 0,00	122,00 ± 0,01	106,97 ± 2,52	0,22 ± 0,02	-24,13 ± 0,38	99,99 ± 0,00
	180*	18,30 ± 0,00	48,20 ± 0,01	128,00 ± 0,02	104,37 ± 0,40	0,22 ± 0,01	-23,60 ± 0,52	99,99 ± 0,00
AE – loaded NLC	0	16,20 ± 0,00	38,90 ± 0,00	106,00 ± 0,00	117,30 ± 2,16	0,22 ± 0,02	-23,27 ± 0,45	99,61 ± 0,04
	180*	17,50 ± 0,00	45,40 ± 0,00	124,00 ± 0,00	174,17 ± 1,21	0,31 ± 0,01	-23,33 ± 0,38	99,99 ± 0,00
PA – loaded SLN	0	20,20 ± 0,00	63,40 ± 0,00	3520,00 ± 0,62	110,20 ± 6,48	0,35 ± 0,06	-24,33 ± 1,23	98,75 ± 0,88
	30*	20,40 ± 0,00	65,10 ± 0,00	3450,00 ± 0,04	106,55 ± 9,36	0,46 ± 0,07	-29,80 ± 1,06	-
PA – loaded NLC	0	19,90 ± 0,00	59,20 ± 0,00	244,00 ± 0,00	97,61 ± 0,39	0,29 ± 0,03	-23,30 ± 1,06	98,55 ± 0,39
	30*	20,00 ± 0,00	60,30 ± 0,00	265,00 ± 0,00	109,00 ± 1,00	0,33 ± 0,02	-28,40 ± 0,95	-

*** Stored at 20,0 ± 0,5 °C; AE: antioxidant extract; PA: pure antioxidant.**

Results

Biocompatibility

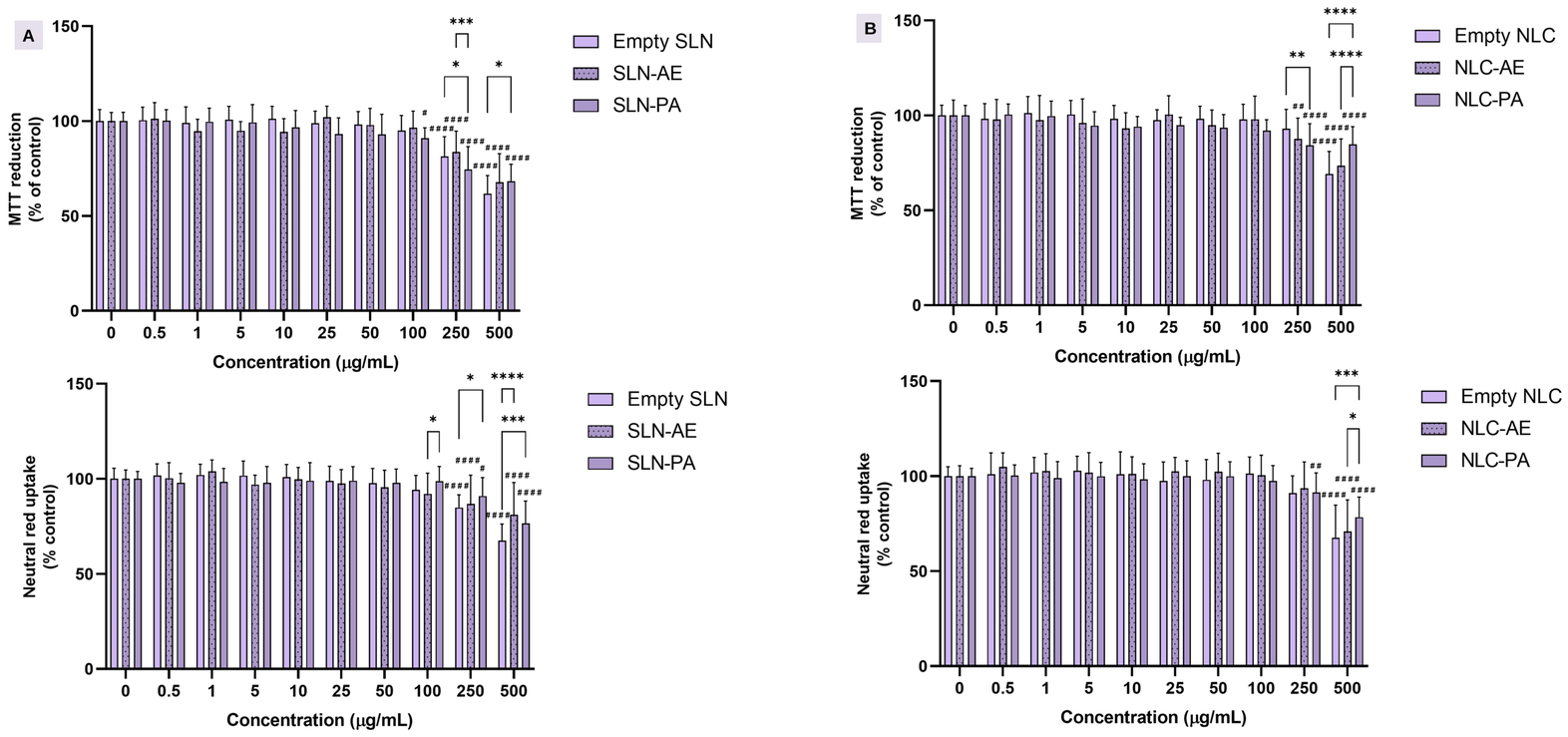

Figure 1. Cytotoxicity of the developed SLN (A) and NLC (B) formulations (0 – 500 µg/mL) evaluated in differentiated SH-SY5Y cells by the neutral red uptake and MTT reduction assays, 24 h after exposure. AE: antioxidant extract; PA: pure antioxidant.
Results are expressed as Mean + SD from 4 independent experiences, performed in triplicate. Statistical comparisons were made using two-way ANOVA to compare means of different groups, followed by the Tukey's multiple comparisons test ($^{\#}p < 0.05$; $^{\#\#}p < 0.01$; $^{\#\#\#\#}p < 0.0001$ for each formulation *vs*. 0 µg/mL; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; $^{****}p < 0.0001$ for comparisons between formulations, at each concentration). In all cases, *p* values < 0.05 were considered significant.

Biocompatible for concentrations up to 100 µg/mL

Conclusion

Six months after storage at 20.0 ± 0.5 ◦C, in the case of SLN and NLC with antioxidant extract, and one month after storage, in the case of SLN and NLC with pure antioxidant, ***the characterization parameters underwent only slight changes. Biocompatibility studies*** *in human neuronal cells, SH-SY5Y cells differentiated into a dopaminergic phenotipe, showed that the developed* ***formulations are safe at concentrations up to 100 µg/mL.*** *The results of this study highlighted the potential of using lipid nanoparticles loaded with natural antioxidants in the management of neurodegenerative diseases, although more experiments are needed to confirm this evidence.*

References

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