Anle 138b and Vitamin C Solid Lipid Nanoparticles: *in vitro* studies for intranasal administration in Parkinson disease

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Introduction

Anle 138b is an a-synuclein inhibitor, proposed in anti-Parkinson therapy since 2020 (Clinical trials at 300 mg/daily). However, being poorly water soluble, some enabling strategies have been investigated to administer it in aqueous medium. Herein, redispersable Solid Lipid Nanoparticles (SLNs) have been studied as a modified drug delivery systems suitable for the intranasal route and, for the purpose of Parkinson disease application, two active agents have been combined, namely Anle 138b and Vitamin C (Vit C).

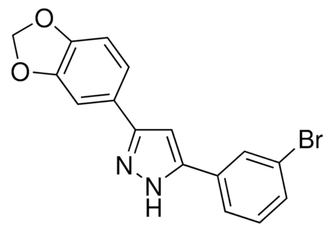

Figure 1. Anle 138b

Vitamin C Selection was selected for the following reasons:

- To reduce the damage due to oxidative stress
- To slow down neurodegeneration progression

SLNs containing Anle and Vitamin C

For Anle 138b and Vit C Encapsulation Efficiency percentages in the SLNs[1] were provided equal to 73 ± 2% and 76 ± 12%, respectively. Mean particle size of the resulting SLNs was 96 ± 10 nm with a zeta potential value of -4.0 ± 0.3 mV. Via the spectrophotometric assay of DPPH test[2] on the freeze dried SLNs, the 99±1% of antioxidant activity was obtained for Anle 138b-Vit C-SLNs, evidencing the protective role of Vit C, although it was encapsulated in the SLNs rather than being as free substance in solution. From TEM microphotograph of Anle 138b-Vit C-SLNs spherical shape was deduced (Figure 2).

Table 1. Pysicochemical properties of SLNs containing Anle 138b and/or Vit C.
**p≤0,001

	Size (nm)	IP	Zeta Potential (mV)	Encapsulation Efficency (%)
Vit C SLNs	120 ± 8	0.4 ± 0.008	-13.4 ± 1.6 **	49.7% ± 7
Anle 138b + Vit C SLNs	96 ± 10 **	0.29 ± 0.004	-4.0 ± 0.3**	Anle 138b: 73% ± 2 Vit C: 76% ± 12
Anle 138b SLNs[a]	99 ± 3**	0.38 ± 0.004	-5.0 ± 0.2**	65% ± 2
Plain SLNs[1]	141 ± 11	0.35 ± 0.004	-9.7 ± 0.8	-

DPPH assay. For evaluation of the *in vitro* antioxidant activity, the spectrophotometric assay of DPPH test[2,3] on the freeze dried SLNs was performed, resulting in 99±1% of antioxidant activity for Anle 138b-Vit C-SLNs, evidencing the protective role of Vit C, although it was encapsulated in the SLNs rather than being as free substance in solution (Table 2) .

Table 2. DPPH tests carried out on the investigated SLNs

Sample	Antioxidant Activity %
Free Vit C	≥100
Anle + Vit C SLNs	99.9±0.1
Vit C SLNs	94.8±2.7
Anle SLNs	79.1±0.0
Plain SLNs[2,4]	72.8 ± 5.3
Free Anle	NOT DETERMINED

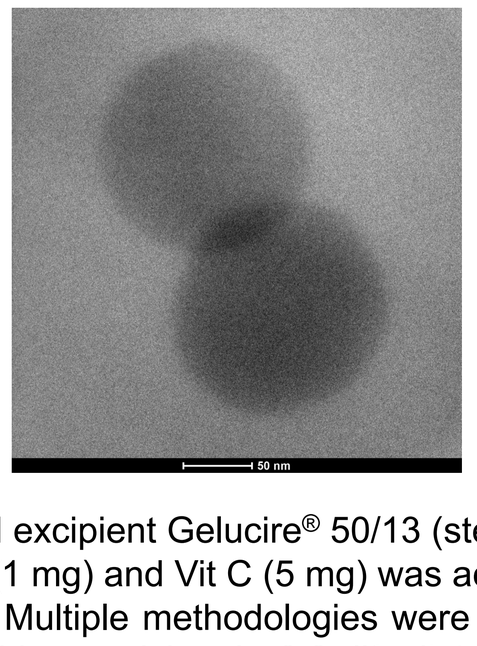

Figure 2. TEM visualization of Anle 138b-Vit C SLNs

SLNs containing Anle and Vitamin C-*In vitro* release test

To corroborate the assessment of the efficacy of Vit C in the SLNs, via DPPH test, Anle 138b-SLN and plain SLNs were found to provide 79 ± 1% and 73 ± 5%, respectively of antioxidant activity (Figure 3A). In SNF release medium, less than 10% of Anle 138b and Vit C was delivered from SLNs. On the other hand, from release studies in CSF, Anle 138b reached 40% of delivery maybe due to the ionization equilibrium involving the pyrazole ring of the drug (Figure 3B).

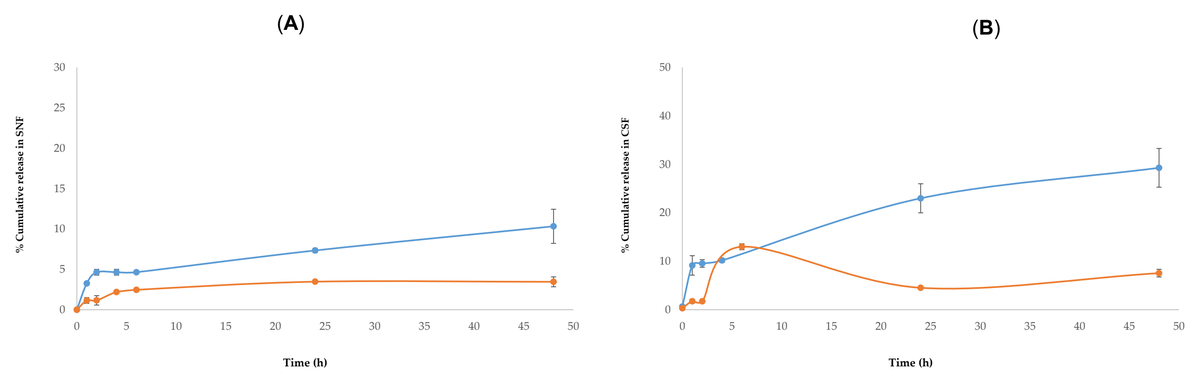

Figure 3. *In vitro* release studies concerning Anle 138b-Vit C SLNs. Blue: Anle 138b; Orange: Vit C. Simulated Nasal Fluid (A, pH 5-6) Simulated Cerebrospinal Fluid (B, pH 7)

Cell viability evaluation. When resazurin viability assay was carried out on SHSY-5Y cells, up to 300 mM of Vit C contained in the SLNs for both 24 and 48h no toxicity was detected at 24h (Figure 4A, B). However, as reported in Figure 4B, at the longest incubation time and at the highest Vit C concentration, a reduction in cell viability was observed.

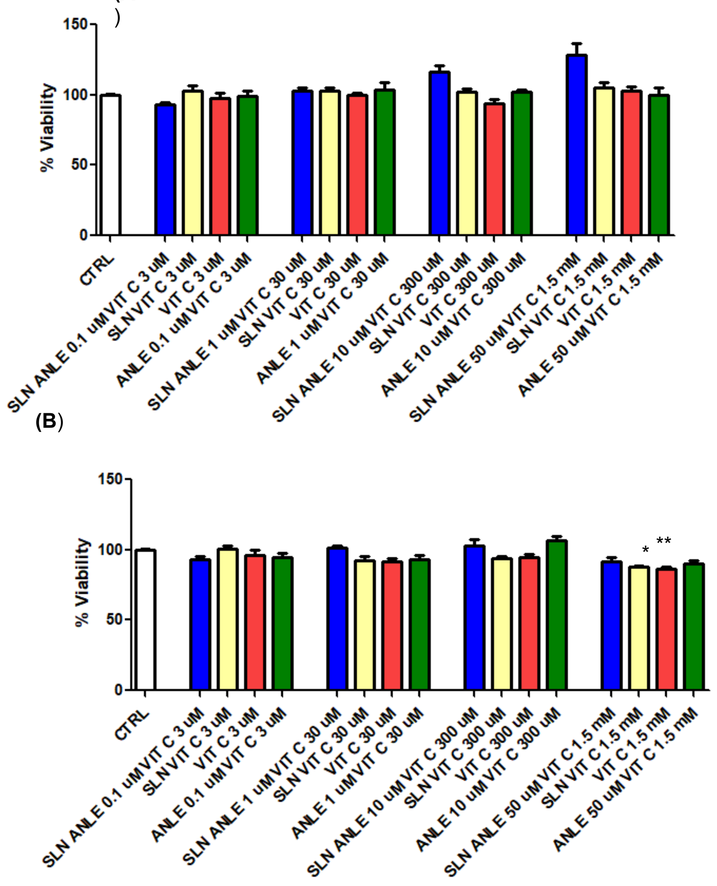

Figure 4. Resazurin test- for assessment of cytobiocompatibility with SHSY5Y cell model line for 24 h (A) and 48h (B) # p<0.05. # # p<0.01.

Materials and Methods

Solid lipid nanoparticles (SLNs) made of the lipid excipient Gelucire® 50/13 (stearoyl polyoxyl-32 glycerides NF) were produced according to the melt emulsification method. Co-loading of Anle 138b (1 mg) and Vit C (5 mg) was achieved by dissolving the former in the melt lipid and the latter in the diluted acetic phase of the pre-emulsion. Multiple methodologies were carried out for SLNs characterization such as size and zeta potential measurements, the in vitro evaluation of antioxidant activity via 2,2-difenil-1-picrylhydrazyl (DPPH) assay[2] and in vitro release tests in Simulated Nasal Fluid (SNF) and Cerebrospinal Simulated Fluid (CSF).
Furthermore, for cytocompatibility assessment, neuronal SHSY-65Y cells were treated with the SLNs at different doses and times of exposure.

Conclusions

Co-loading of Anle 138b and Vit C in the SLNs was seen to provide high encapsulation efficiency of both agents and preliminary in vitro results are promising in view of more cell experiments needed to verify the feasibility of nose-to-brain-delivery

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