

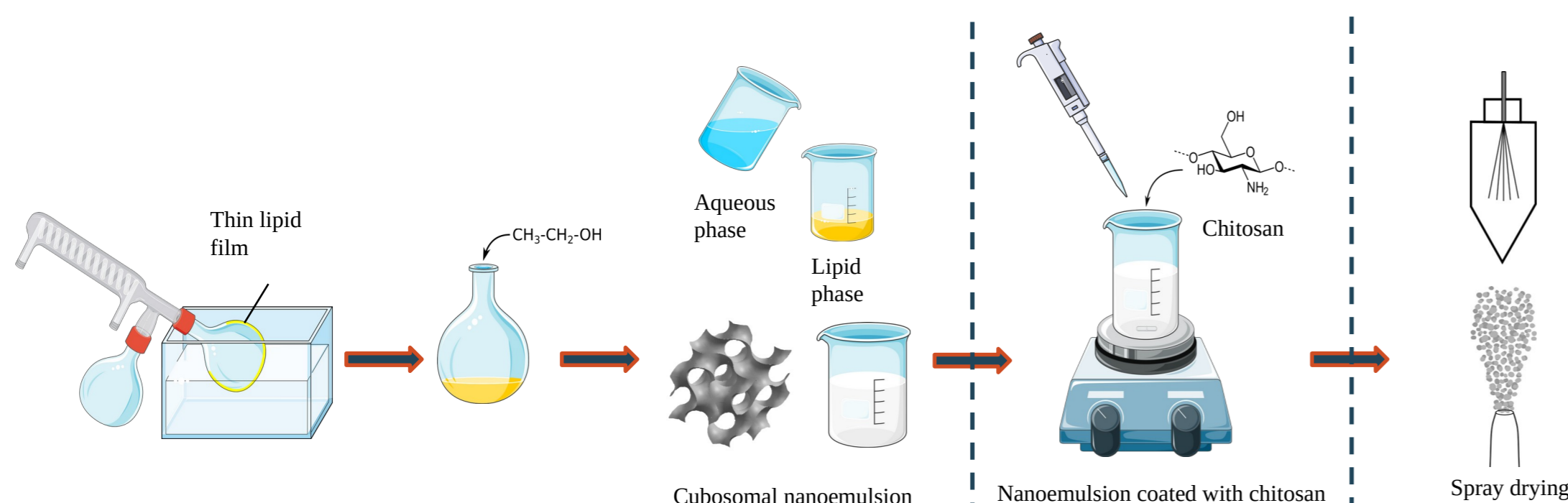
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## Introduction

This work aims to formulate, characterize, and evaluate two types of cubosomal nanoparticles (CNPs) designed for nose-to-brain (N2B) delivery. Paliperidone palmitate (PP), a second-generation antipsychotic drug with poor patient compliance due to its systemic side effects, was used as a model. Its direct administration to the brain may reduce these side effects by decreasing the administered dose. Cationic CNPs (CCNPs) and chitosan based CNPs (ChCNPs) were developed to evaluate the influence of the formulation on the nanoparticle properties (i.e., particles size (PS), polydispersity index (PDI), zeta potential (ZP), loading capacity (LC) and encapsulation efficiency (EE)), the mucoaffinity, the cellular uptake, and the nasal deposition (using a 3D printing nasal cast).

## Production method



## Characterization

NPs	Nanoparticle characteristics					Powder characteristics					
	LC (%)	PS (nm)	PDI	EE (%)	ZP (mV)	Dv10 (μm)	Dv50 (m)	Dv90 (m)	Span	Yield %	% Moisture
ChCNPs	70	305.7 ± 22.54	0.166 ± 0.022	99.7 ± 0.1	+42.4 ± 0.2	8.19 ± 0.33	12.65 ± 0.37	23.33 ± 10.47	1.66 ± 1.27	75.86 ± 2.86	1.710 ± 0.180
		5	116.5 ± 2.5	0.253 ± 0.027	98.0 ± 0.4	+66.4 ± 1.2	11.91 ± 0.28	14.34 ± 0.82	38.55 ± 10.03	1.84 ± 0.59	77.98 ± 3.78

## Mucoaffinity test

The strong mucin electrostatic interaction with the CCNPs could easily be explained by the high positively charged surface due to the incorporation of the cationic lipid. The molar concentration of DOTAP was higher than the one with the chitosan (6.2E-2 mole/g and 7.0E-4 mole/g of formulation for CCNPs and ChCNPs, respectively).

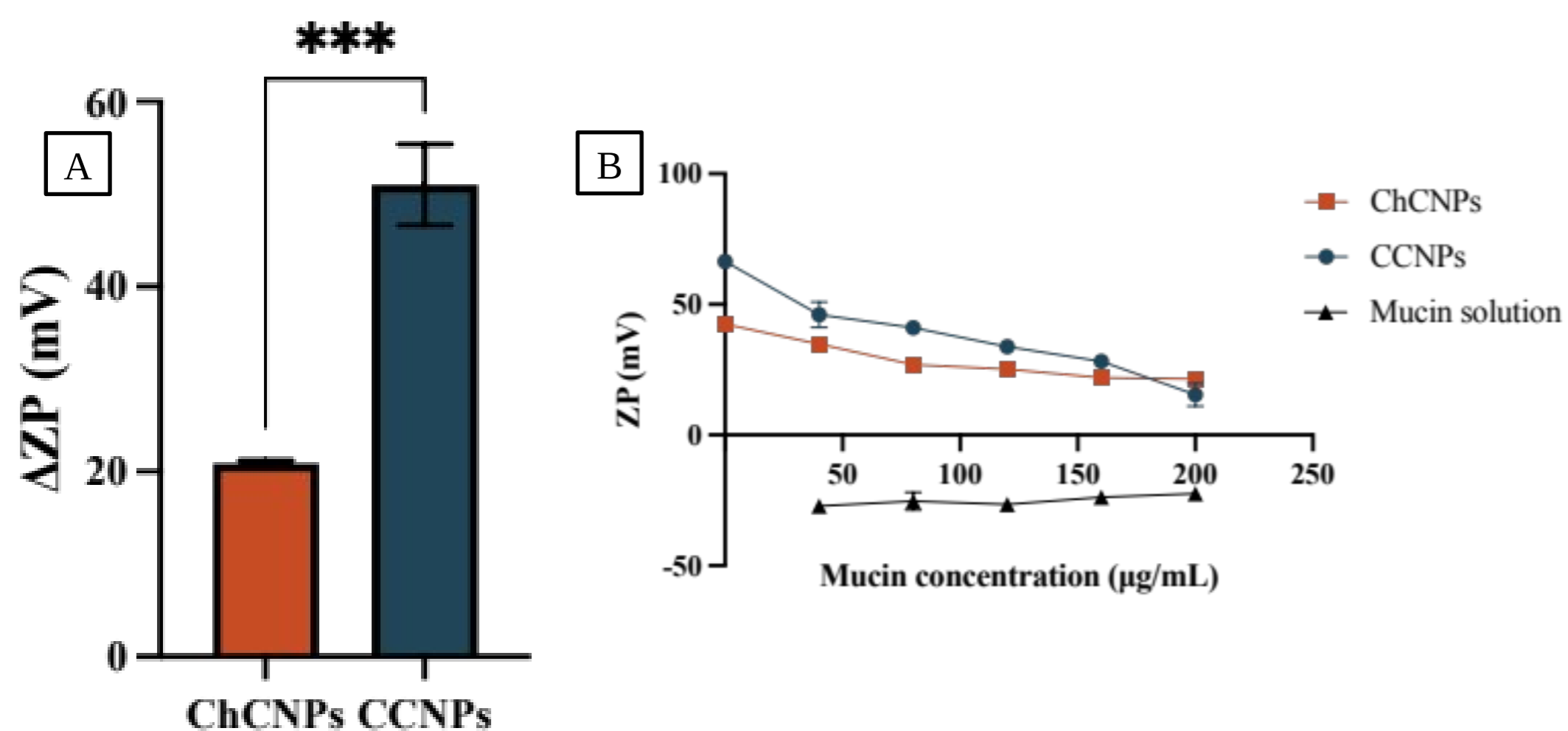


Figure 1: (A) Comparison of the  $\zeta$ ZP of the CCNPs and ChCNPs. The results are expressed by mean  $\pm$  SD. A statistical student t-test concluded a significant difference between the  $\zeta$ ZP of the two formulations (\*\*\*) p-value < 0.001. (B) Influence of the mucin concentration on the ZP of each CNPs formulation.

## Cell culture and permeation study

Figure 2 illustrates the Alcian blue assay which was used to characterize mucin production by RPMI 2650 cells during the growing when we go from liquid-covered cultivation to air-liquid interface cultivation (ALI). Indeed, the ALI conditions increased the aerobic exposition, which correlates with epithelial barrier formation.

Both formulations were characterized by a significant higher  $P_{app}$  than pure PP. Such data confirmed the interest in improving the mucoaffinity of the cubosomes with a cationic lipid (CCNPs) or adding a permeation enhancer such as chitosan to open the TJs (ChCNPs). The  $P_{app}$  coefficient was increased from  $2.57E-08 \pm 3.025E-08$  cm/s for the pure PP, to  $1.72E-05 \pm 0.21E-05$  cm/s for the CCNPs and finally to  $3.00E-05 \pm 0.24E-05$  cm/s for the ChCNPs due to their mucoaffinity and ability to open the thigh junctions.

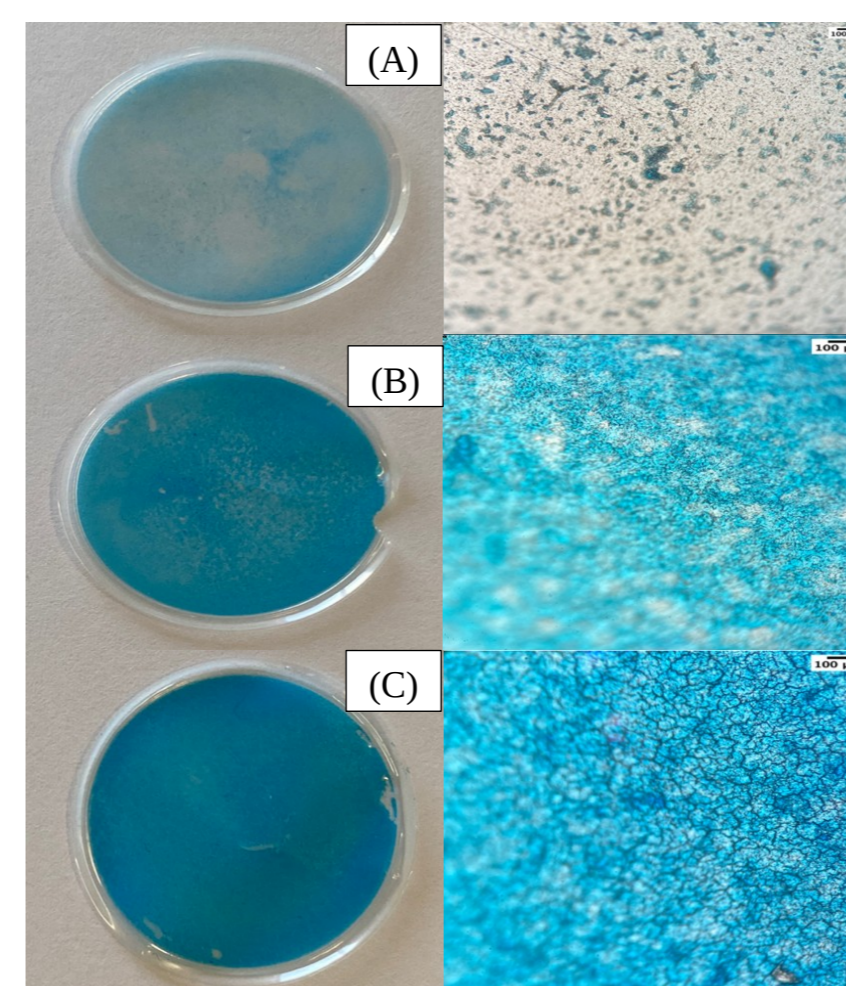


Figure 2: Staining of mucus present in RPMI 2650 cells at (A) day 1, (B) day 11 and (C) day 22. (A', B' and C' represent RGBb<sup>†</sup> histogram for A, B and C images). <sup>†</sup> RGB<sub>b</sub> (Red-Green-Blue) ratio corresponding to the average value over the whole picture of the blue component was then divided by the sum of the three channels.

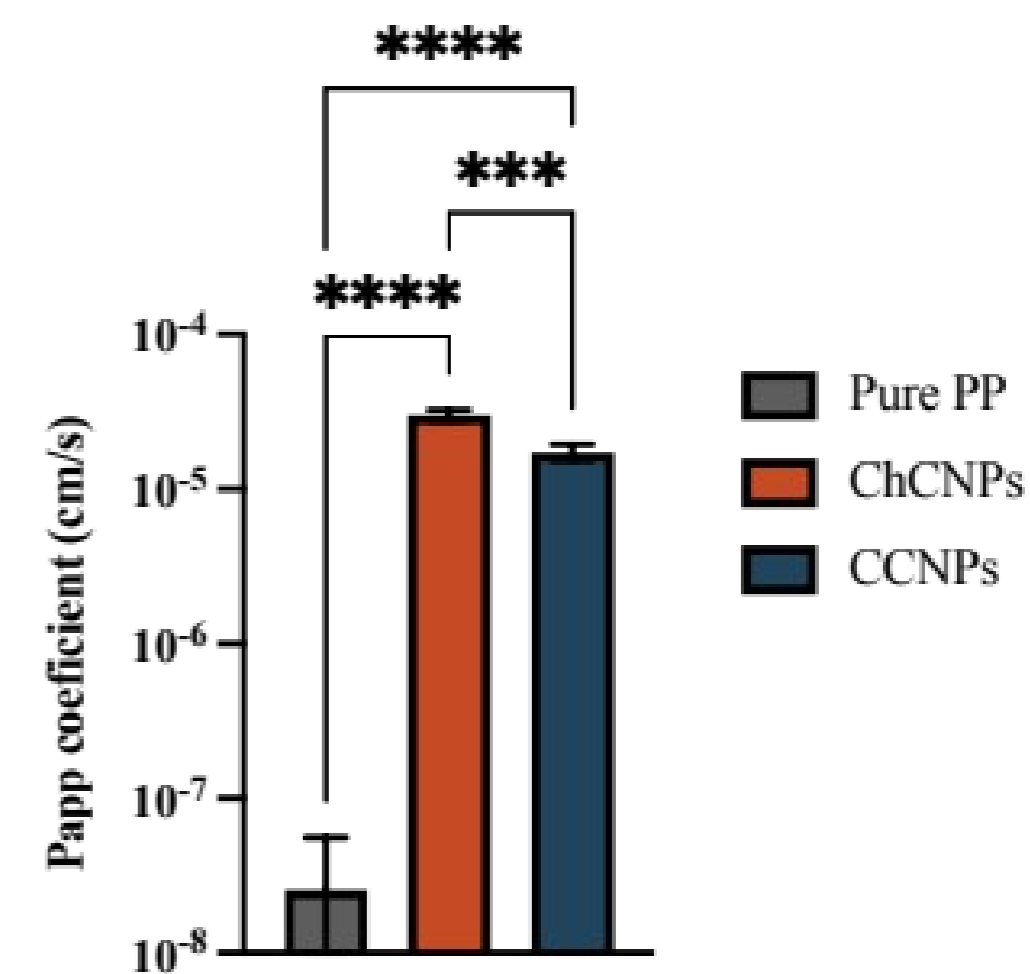
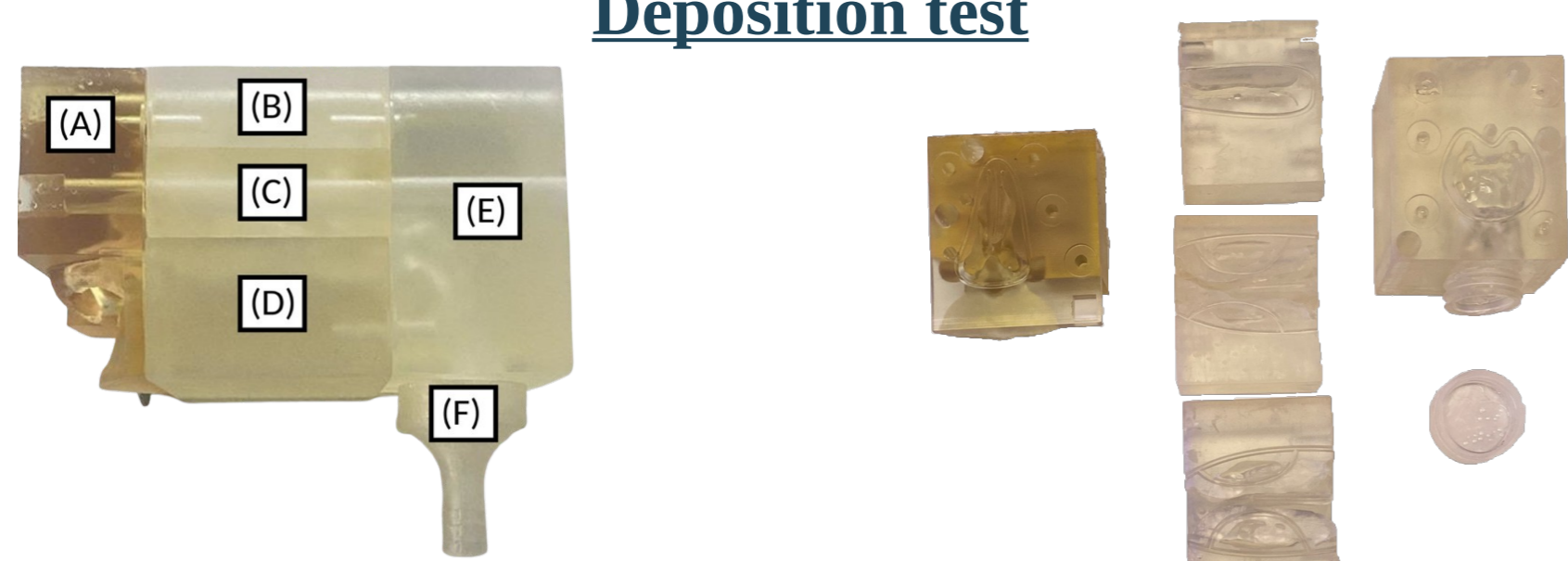


Figure 3: Apparent permeability coefficient of the pure PP, ChCNPs, and CCNPs formulation. The y axis is expressed in log 10, (\*\*\*) p-value < 0.001 and (\*\*\*\*) p-value < 0.0001. n=3, mean  $\pm$  SD.

## Deposition test



### Percentage of powder deposition

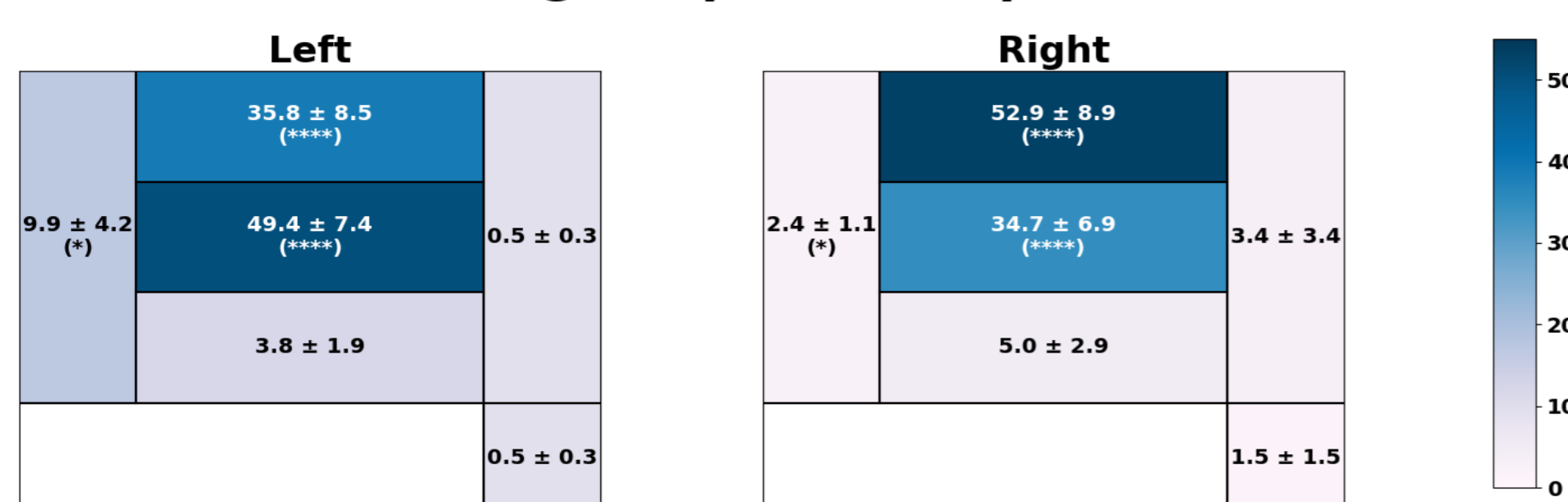


Figure 4: Percentage of powder deposition in (A) nostril, (B) olfactory, (C) middle turbinates, (D) lower turbinates, (E) nasopharynx, and (F) post-nasal fraction in left and right side for combined formulations. Results are expressed by mean  $\pm$  SD. A one-way ANOVA statistical test was realized to compare the powder deposition in right and in left side in each part of the nasal cast. (\*) indicate a p-value < 0.05 and (\*\*\*\*) indicate a p-value < 0.0001.

## Conclusion

ChCNPs was selected:

- ✓ High mucoaffinity => positive surface charged
- ✓ Higher  $P_{app}$  than CCNPs => appropriate particles size and open thigh junctions
- ✓ Reaches well the olfactory region => appropriate powder properties