Quantification of peptide adsorption on lipid nanocapsules without prior purification or separation process

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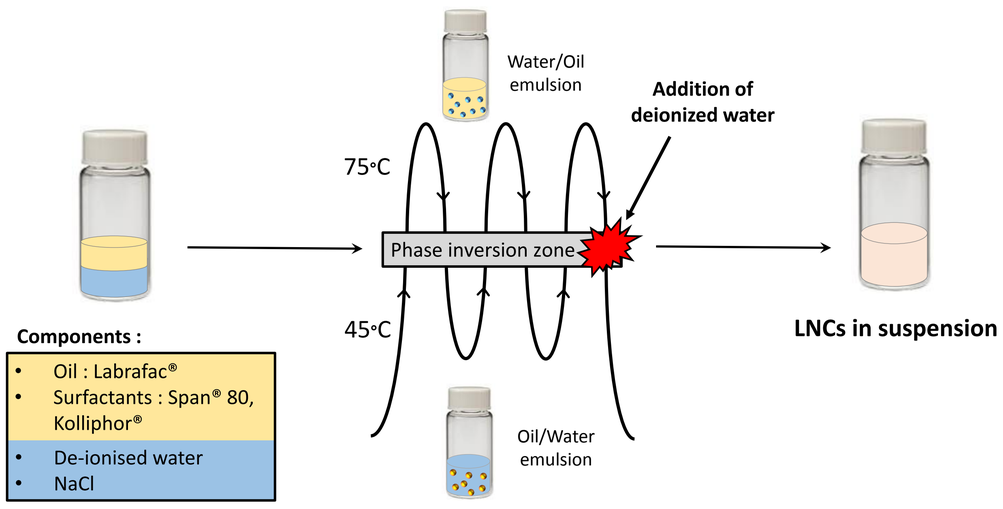
To quantify the encapsulation rates of active agents (drug, protein, etc.) in nanoparticles, the separation of non-loaded agents from the loaded ones is often reported in literature, using dialysis or centrifugal filters.[1,2] However, these techniques are more and more questioned, and the low quality of the separation could lead to over or underestimated data.[3,4] To avoid the biases, a method was developed to **quantify free peptide proportions without prior separation step from peptide adsorbed at the nanoparticle surface**, using NFL-TBS.40-63 (NFL) and lipid nanocapsules (LNCs) as peptide and nanoparticle models, respectively.

1. LNC formulation[5,6]



2. NFL adsorption on LNCs

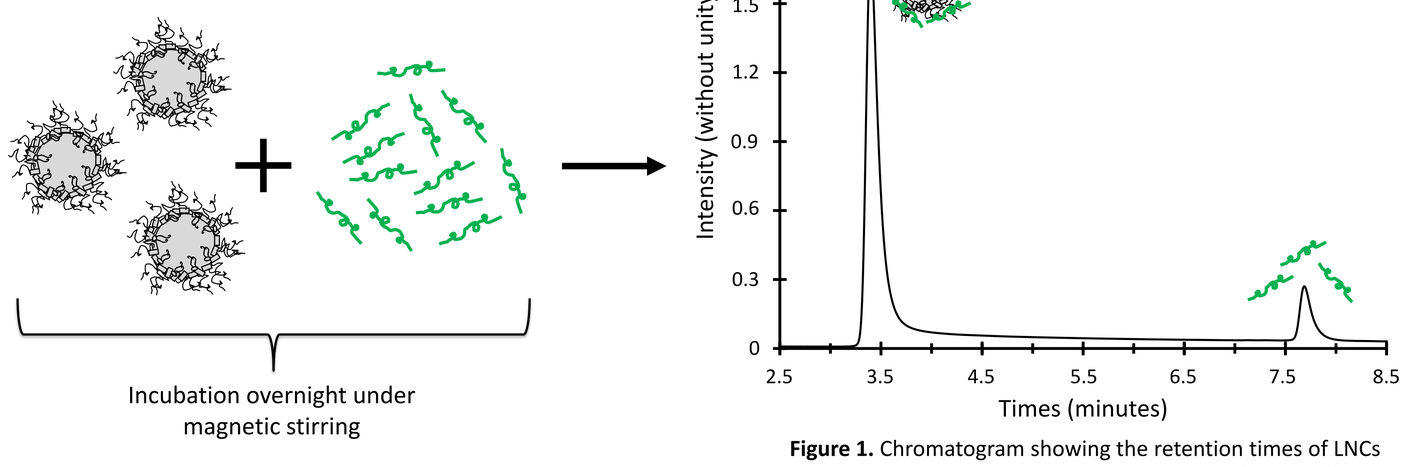
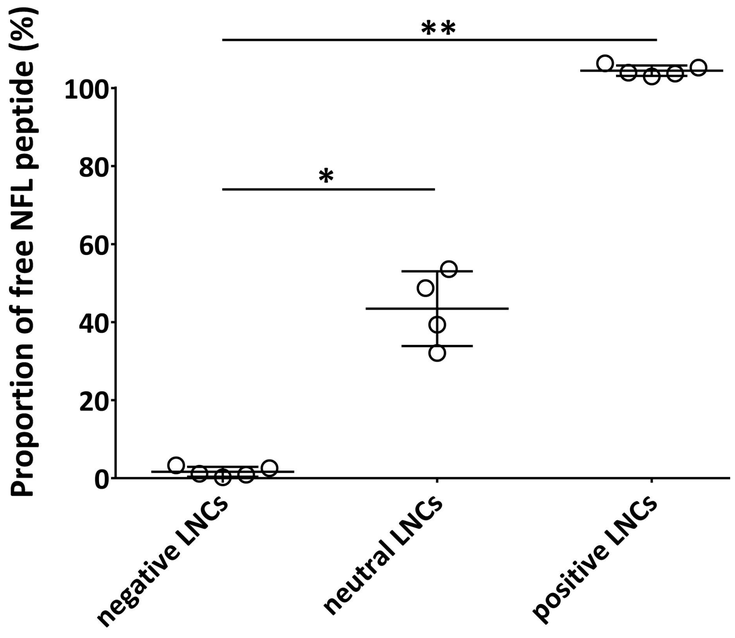


Figure 1. Chromatogram showing the retention times of LNCs (3.5 minutes) and NFL (7.7 minutes) on size exclusion column.

3. Influence of LNC charge on NFL adsorption



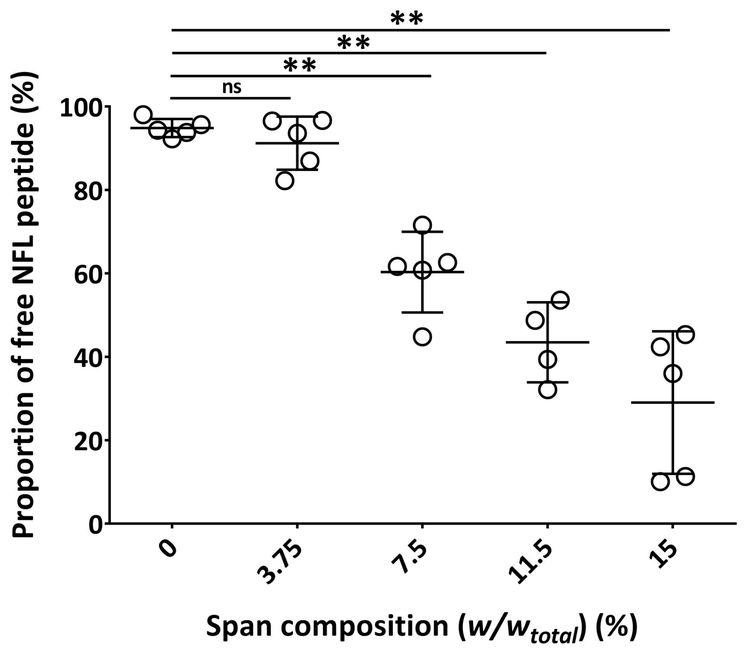
Better NFL adsorption with **negatively charged LNCs**.

Results consistent with the positive charge of NFL.[7]

Hypothesis : **electrostatic interactions** between NFL and LNCs.

Figure 2. Influence of LNC charge on NFL adsorption. LNCs were used at 0.975 mg/mL and NFL at 0.1 mg/mL. Negative and positive LNCs are respectively with 2.5 % of S-Chol and 5 % of DDAB (w/w_{Lab}). The LNC size corresponds to 50 nm. (Mann-Whitney test ; * $p < 0.05$; ** $p < 0.01$; n = 4-5 ; mean ± SD)

4. Influence of LNC composition on NFL adsorption



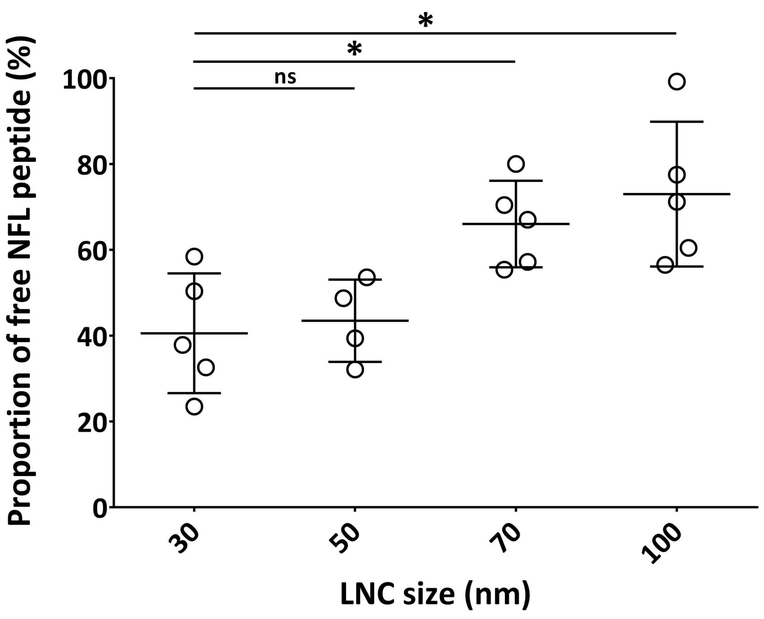
Better NFL adsorption with **high Span® 80 composition** at LNC surface.

When Span® 80 composition increases, NFL adsorption is enhanced.

Hypothesis : **hydrogen bonds** between the NFL and Span® 80 at LNC surface.

Figure 3. Influence of Span® 80 composition of LNCs on NFL adsorption. LNCs were used at 0.975 mg/mL and NFL at 0.1 mg/mL. The LNC size corresponds to 50 nm. (Mann-Whitney test ; ** $p < 0.01$; ns $p > 0.05$; n = 4-5 ; mean ± SD).

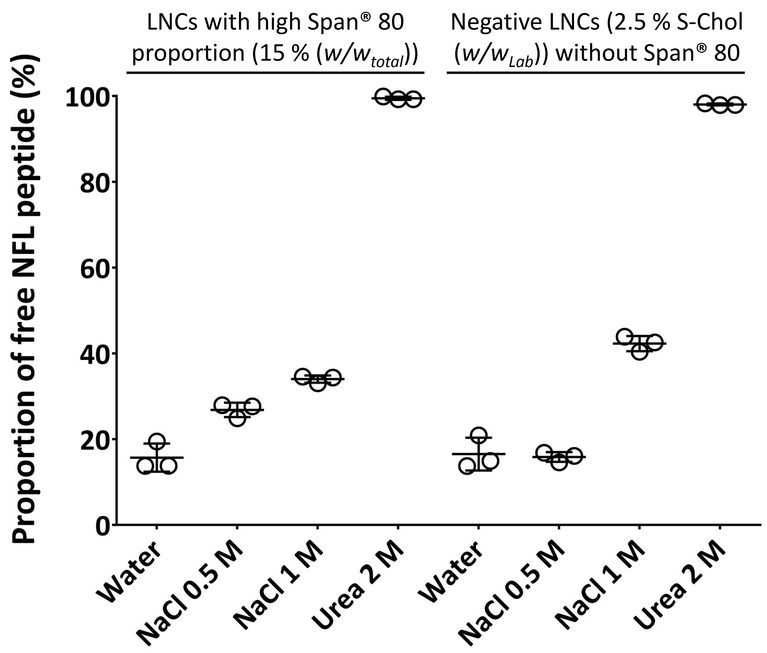
5. Influence of LNC size on NFL adsorption



When the LNC surface increases, NFL adsorption is enhanced.

Figure 4. Influence of LNC size on NFL adsorption. LNCs were used at 0.975 mg/mL and NFL at 0.1 mg/mL. (Mann-Whitney test ; * $p < 0.05$; ns $p > 0.05$; n = 4-5 ; mean ± SD)

6. Interaction inhibition between NFL and LNCs



Inhibition of NFL adsorption at LNC surface when NaCl and urea are present.

Proof of electrostatic interactions and hydrogen bonds between LNCs and NFL.

Figure 5. Inhibition of interactions between NFL and LNCs in presence of NaCl and urea. LNCs were used at 9.75 mg/mL and NFL at 0.1 mg/mL. The LNC size corresponds to 50 nm. (n = 3 ; mean ± SD)

Thanks to a size exclusion method, the **separation between nanoparticles and active agent and their quantification are performed simultaneously**. This single step is a real benefit for analysis accuracy and robustness, minimizing the biases compared to sequential steps for separation and quantification. In this particular case, the best combinations of concentrations/types of LNCs and NFL were determined to achieve a **total NFL adsorption at LNC surface**. This technique could be a **promising tool to characterize other nanoparticle/active agent interactions**.

References

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Acknowledgments

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