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New protocol to evaluate release in lipophilic medium: story of misinterpretation related to the use of fluorescent dyes

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ABSTRACT
The release from loaded-lipid nanocapsules (LNC) to dye-free oil medium (direct way) or from an oil phase containing dye to non-loaded LNC (back way) was performed in various conditions, by changing the nature of the dye and the oil, the volume and the LNC dilution. Results showed that the encapsulated dyes inside the LNC core (proved by surface tension), were able to be released, according to a partition coefficient behavior. In contrast, when the dyes were entrapped in the surfactant shell of LNCs, no release was observed. Dye-loaded LNC were put in cell culture and dye diffusion was clearly observed from LNC to cells, without LNC uptake.

INTRODUCTION
Since biological fluids are hydrophilic, in vitro release of drug or dye from nanoparticles against water or buffers is a classical experiment to test formulations. Nevertheless, with encapsulated hydrophobic compounds, to our knowledge, no precise protocol was described for in vitro release studies. When release was operated against water or buffer, only the stability of the vector was assessed, and the hydrophobic compounds did not leave the nanoparticles due to thermodynamic considerations. An original protocol was developed to study the release of amiodarone from nanoparticles to liposomes or non-loaded particles. The results were not easy to extrapolate, due to the presence of the dialysis membrane separating the two media. Here, we proposed a new and simple method to study the in vitro release of dyes from nanoparticles to lipophilic media, without any medium localization in nanoparticles were brought. Finally, the release properties observed in vitro were illustrated with cell culture, showing the possibility of misinterpretation if proper characterization was not previously performed.

RESULTS

Examples of results (LNC suspension, oily phase, and LNC formulation).

With Nile Red-loaded LNC, dye can be released from LNC to an oil phase. With Nile Red-loaded oily phase, dye can enter inside LNC from the oily phase. A partition coefficient was observed between LNC and the oily phase: after the separation protocol, about 65% of Nile Red was inside LNC and 35% in the oily phase, whatever the direct or back way. Nile Red proportion in each phase did not depend on the concentration of Nile Red but depended on R (the volume ratio of oil in LNC and oil in the outer phase, as for a partition coefficient). No effect of size was observed. Oily phase nature modified the partition coefficient values. LNC suspension dilution affected the partition coefficient (data not shown). A partition coefficient was also observed with 6-Coumarine.

For DiI, DiO and DiO, an opposite effect was observed, with no release from dye-loaded LNC and no entry in LNC from the oily phase containing the dye. These dyes remained inside the LNC whatever the concentration and R (volume ratio of oil in LNC and oil in the outer phase).

CONCLUSION
A new protocol was developed to evaluate the release of hydrophobic active ingredients from nanocapsules to lipophilic media. As proof of concept, hydrophobic dyes were used but it could be applied to hydrophobic drugs, to obtain information on their release pattern (immediate or sustained profile). It will allow improvement of interpretation about cellular uptake and in vivo cell distribution.

REFERENCES

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