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To cite this version:
Guillaume Bastiat, Christian Pritz, Clemens Roider, Florian Fouchet, Erwann Lignières, et al.. A simple methodology to test the fluorescence-labeling stability of a nanocarrier. 9th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology, Mar 2014, Lisbon, Portugal. 2014. hal-03173096

HAL Id: hal-03173096
https://univ-angers.hal.science/hal-03173096
Submitted on 18 Mar 2021

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A simple methodology to test the fluorescence-labeling stability of a nanocarrier

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ABSTRACT

The release from loaded lipid nanocapsules (LNC) to dye-free oil medium (direct way) or from an oily 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 oil 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 intragelled 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 dye 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 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 exchange. In vitro release of various dyes was studied and new insights about dye localization in nanoparticle 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.

Objectives

- Parameters:
  - Dye nature: Nile Red, 6-Coumarine, DiI, DiO and DiD.
  - Dye concentration: 1, 0.5 and 0.1 mg/mL.
  - Oil (ratio of oil in LNC/oil in oily phase): 1/1, 1/2 and 2/1.
  - LNC hydrodynamic diameter: 25, 50 and 100 nm.
  - Oily phase nature: Labrasol®, CapteX® 200, CapteX® 300, ethyl oleate, PEG
  - LNC suspension factor dilution: 1, 2, 3, 5,

Protocol

- LNC suspension and oily phase mixture: 0.5 mL of LNC and 0.2 mL of oily phase.
- Vortex: 30 sec.
- Centrifugation: 6000 rpm, 30 min.

Fluorescence measurements in LNC suspension and oily phase.
- Size, PDI and derived count rate (DCR) measurements on LNC suspension.

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


ACKNOWLEDGMENTS

The authors wish to acknowledge European Community [Project Nanoear: NMP-2004-43151] for providing financial support for this work. Authors also thank Dr. Frederik Kalinec (Los Angeles, USA) for providing the HEI-OCl cells.

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