# Evaluation of *in vitro* stability of large unilamellar liposomes coated with a modified polysaccharide (O-palmitoylpullulan)

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Aiming at encapsulation of a hydrosoluble drug, large unilamellar liposomes (LUV) of egg phosphatidylcholine (PC) were coated with a natural polysaccharide derivative, O-palmitoylpullulan (OPP), and its *in vitro* stability evaluated using fluorescent probes. This coating (in OPP/PC weight ratio of 3) improved significantly the *in vitro* stability of LUV by decreasing both the permeability and fluidity of the liposomal membrane.

## 1. Introduction

Much effort has been made in order to improve the *in vitro* and *in vivo* stability of liposomes as drug delivery systems. Within this context, Sunamoto *et al.* [1] developed a strategy which consists in coating the liposomal surface with natural modified polysaccharides such as pullulan. The aim of this present work was the synthesis and characterization of a modified polysaccharide, OPP, and the evaluation of its effect on membrane permeability and fluidity of LUV.

## 2. Materials and methods

OPP was prepared by reaction of pullulan dissolved in dry dimethylformamide with palmitoyl chloride in the presence of dry pyridine [1], and characterized by infrared (IR) and proton nuclear magnetic resonance spectroscopy (NMR).

PC LUV were prepared in a solution containing Tris-HCl 20 mM and NaCl 200 mM, pH 8.6, by a combination of reverse evaporation and extrusion methods. PC small unilamellar liposomes (SUV) were obtained by sonication with a titanium probe. For permeability studies, liposomes were prepared in the same medium containing 5,6-carboxyflourescein (CF) 50 mM. The non-encapsulated CF was removed by filtration through a Sephadex G-25 medium column. Coating of previously purified liposomes was performed by addition of OPP (corresponding to OPP/PC weight ratios from 1 to 4) dissolved in the medium referred to above to the liposome suspension, and subsequently submitted to magnetic stirring at about 20 °C for 1 h before use. Permeability studies were carried out by measuring changes in the fluorescence signal of released CF ($\lambda_{em}$ = 515 nm) at 50 °C from a liposome suspension. Fluidity studies were performed by measuring the fluorescence polarization of 1,6-diphenyl-1,3,5-hexatriene (DPH) and 3-[p-(6-phenyl)-1,3,5-hexatrienyl] phenylproprionic acid (DPH-PA), at 37 and 50 °C, previously incorporated into lipid membranes (lipid/ probe molar ratio of 400).

## 3. Results and discussion

### 3.1. OPP characterization

The OPP IR spectrum revealed the existence of an ester bond between pullulan and the palmitoyl group indicating that the two compounds were covalently bounded. From a detailed analysis of proton NMR spectra, the substitution degree of palmitoyl residues in OPP was computed to be 0.4 per 100 glucose units (0.4%).

### 3.2. Permeability studies

The influence of OPP on the permeability of liposomal membrane has already been studied by Sunamoto *et al.* [1] with SUV obtained by sonication. Since we aim at prospective encapsulation of a hydrosoluble drug, attention was focused on LUV which are specially suitable for the delivery of these drugs. Thus, the effect of coating with the synthesized OPP on the stability of LUV was evaluated on the basis of CF release and compared to that of SUV. Fig. 1 shows

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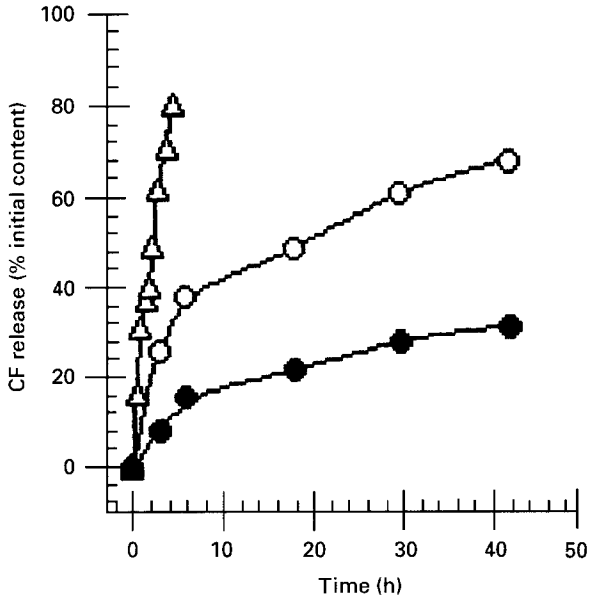


*Figure 1* CF spontaneous release from (△) SUV (with a mean size of 53.9 nm) and from (○) non-coated and (●) coated LUV (with mean sizes of 247.3 and 242.8 nm, respectively) at 50 °C.

that non-coated LUV present a significantly lower membrane permeability than SUV. In fact, after 42 h, LUV release 67% of their initial CF content, whereas SUV release 80% of their initial amount of CF in only 5 h. This difference may be due to the higher bilayer curvature of SUV, as compared to LUV, which make the former thermodynamically less stable than the latter [2]. Thus, besides the advantage of LUV to encapsulate a higher fraction of aqueous volume than SUV, the former present higher stability than the latter (in terms of membrane permeability). Fig. 1 also shows that coating LUV with OPP remarkably suppresses CF release, as compared with non-coated LUV. This result demonstrates that coating LUV with OPP improves their stability, since it decreases membrane permeability.

Moreover, the effect of OPP coating on the *in vitro* stability of LUV depends on the OPP/PC weight ratio. Fig. 2 shows that an increase of OPP/PC weight ratio from 0 to 4 lead to a significant decrease in the rate (Fig. 2a) and in the percentage CF release after 2 h (Fig. 2b). As an example, for an OPP/PC weight ratio of 3 there is a decrease of about 20% in the percentage CF release after 2 h relative to a control sample (without coating) (Fig. 2b). The selected OPP/PC weight ratio for further studies was 3, since it represents the lowest amount of polysaccharide required to obtain the highest stability of liposomes. These results are qualitatively similar to those obtained by Sunamoto *et al.* [1] with SUV, when the OPP/PC weight ratio was increased from 0 to 3.

## 3.3. Fluidity studies

Coating liposomes with OPP did not induce significant changes in the fluorescence polarization of DPH (Table I), suggesting that lipid organization of the hydrophobic region, where the probe is located, was

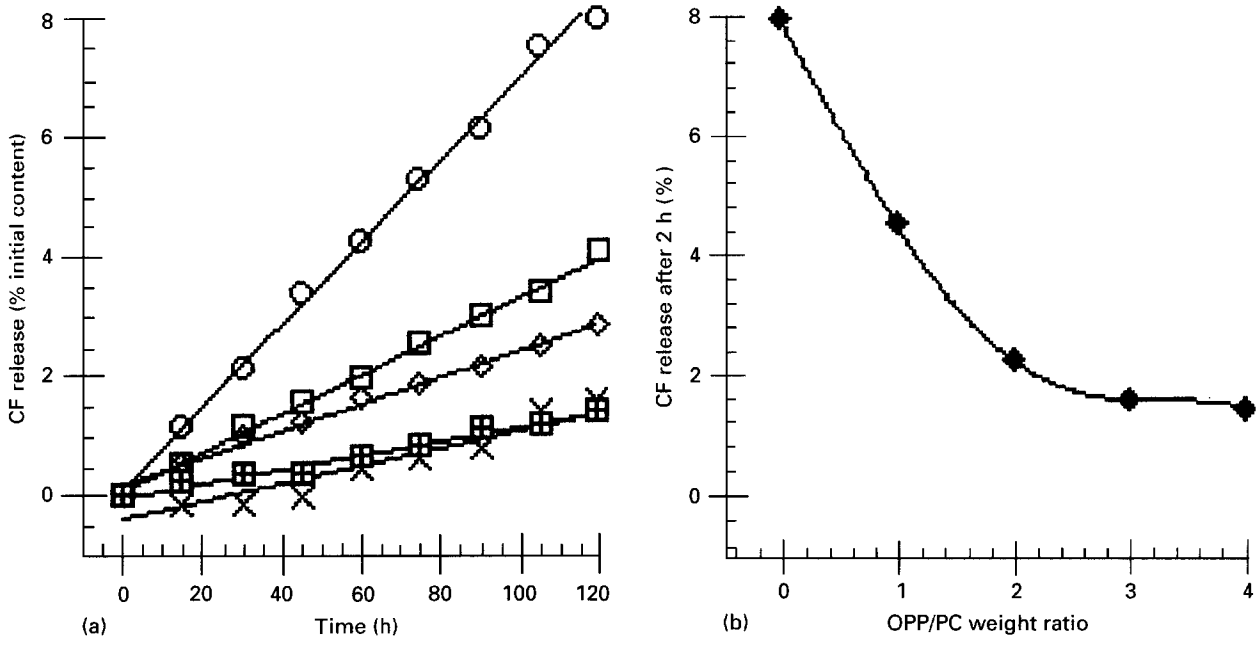


*Figure 2* CF spontaneous release from non-coated and coated LUV with different OPP/PC weight ratios at 50 °C, as a function of time (a) and after 2 h of incubation (b). (a) OPP/PC weight ratio: ⊖ 0; ⊟ 1; ⟡ 2; ⋇ 3; ⊞ 4.

TABLE I Fluorescence polarization values of DPH and DPH-PA incorporated in non-coated and OPP-coated LUV

| Sample | Fluorescence polarization ($p$) | | | |
|---|---|---|---|---|
| | DPH | | DPH-PA | |
| | 37 °C | 50 °C | 37 °C | 50 °C |
| Non-coated LUV | 0.119 (±0.003) | 0.085 (±0.009) | 0.186 (±0.002) | 0.159 (±0.006) |
| Coated LUV (OPP/PC = 3) | 0.108 (±0.002) | 0.092 (±0.003) | 0.190 (±0.004) | 0.186 (±0.004) |

not apparently perturbed by the polymer. However, for the same OPP/PC weight ratio (3), fluorescence polarization of incorporated DPH-PA (Table I) (probe displaced to the external region of the lipid bilayer) increases significantly (15% at 50 °C, relative to the control sample without coating) indicating that OPP perturbs this domain of the bilayer, leading to a decrease in lipid fluidity.

## 4. Conclusions

These results confirm the lower membrane permeability of LUV as compared to SUV and clearly indicate that coating LUV with an OPP/PC weight ratio of 3 improves their *in vitro* stability by decreasing the permeability and fluidity (of the external region) of the liposomal membrane. This suggests that the delivery of hydrosoluble drugs might be significantly improved by using OPP-coated LUV.

## References

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