Modified Gemcitabine-loaded lipid nanocapsules: when a drug participates to the structure of a nanomedicine

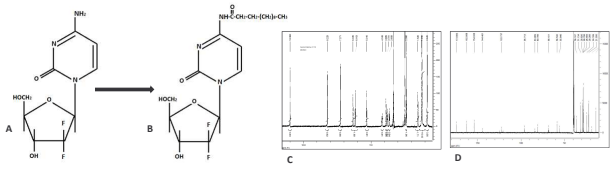
E. Moysan , G. Bastiat , A. Cieślak , J. Bejaud , N. Lautram , J.-P. Benoit

Micro et nanomédecines biomimétiques INSERM 1066, IBS-CHU, Angers, 49933 Cedex 9, France

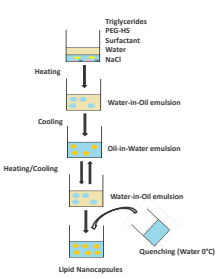
guillaume.bastiat@univ-angers.fr

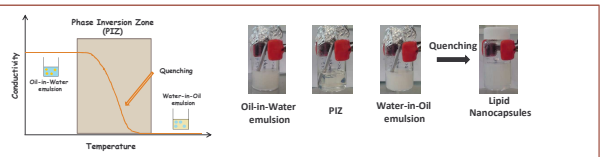
Lauryl-modified gemcitabine (GemC12) was encapsulated in lipid nanocapsules (LNC) with a high entrapment efficiency and a hydrogel was spontaneously formed, depending on LNC concentration and drug loading. GemC12 was localized in the surface layer of LNCs and the gemcitabine moieties of GemC12, exposed to water medium, formed inter-LNC H-bonds, and therefore an association between LNCs like a pearl necklace occurs. In diluted phases, GemC12-loaded LNCs presented a cytotoxic activity with regard to various cancer cell lines, higher than the native drug.

Lipophilic analogue of gemcitabine, 4-(N)-lauroyl-gemcitabine (GemC12) was synthesized. The addition of an alkyl chain on the amine group allows (i) to protect the drug from deaminase, (ii) to improve lipophilicity, thus leading its encapsulation in lipid nanoparticles, (iii) to increase its half-life, (iv) to increase its cytotoxic effect.



A. Gemcitabine. B. 4-(N)-lauroyl-gemcitabine. C-D. ^{1}H NMR and ^{13}C NMR Spectrum of 4-(N)-lauroyl-gemcitabine



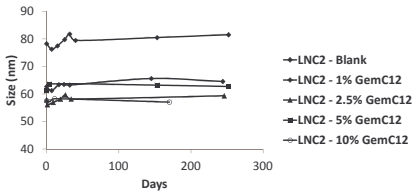


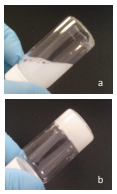
LNC formulation is based on at least three principal components: an oily phase. an aqueous phase and a nonionic surfactant. The formulation of lipid nanocapsules consists of heating and cooling cycles leading to the formation of water-in-oil emulsions at high temperature, and oil-in-water emulsion at low temperature. In the third cooling, an irreversible shock was induced by sudden dilution of the medium with cold water. This quenching was performed to break the microemulsion system obtained in the PIZ, and led to the formation of stable nanocapsules.

LNCs were formulated with different concentrations of GemC12. The LNCs were analysed for size distribution by photon-correlation spectroscopy and zeta potential using a Malvern Zetasizer®.

LNC presented great stability over time in terms of size, PdI and zeta potential.

A gel state of LNC with GemC12 was obtained.





Picture 1: Blank lipid nanocapsules in liquid state (a) and gel state (b : LNC2 5% GemC12)

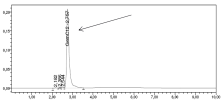
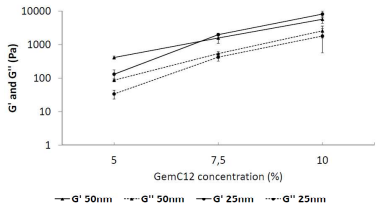


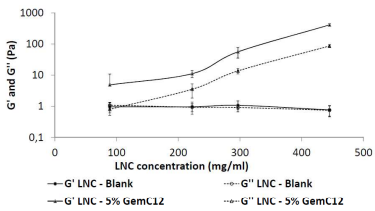
Fig 2: A typical chromatogram of gemcitabine-C12 exhibiting a retention time of about 2.75 min

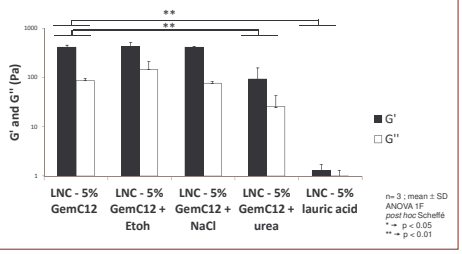
High-performance liquid chromatography (HPLC) was performed to determine the payload. **The encapsulation efficiency was 100%** for all LNC-GemC12 dispersions and did not change over the time (tested until 8 months).

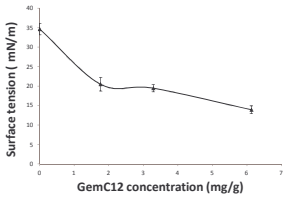
	LNC 1% Gem-C12	LNC 2.5% Gem-C12	LNC 5% Gem-C12	LNC 10% Gem-C12
Encapsulation rate (%)	100	100	100	100

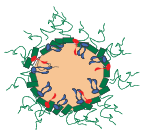
The viscoelastic properties of different formulations were studied using a Malvern rheometer with a fixed shear strain and frequency. **A GemC12 dose and LNC concentration dependent gelation process was observed, and this is independent of the size**. The replacement of Gemcitabine-C_{12} by lauric acid has confirmed the essential role of Gemcitabine-C_{12} for the gelation. The addition of urea in the LNC 5% GemC12 showed **the existence of hydrogen bonds between the hydrophilic head group of GemC12**. The addition of ethanol and salt allowed us to discard any electrostatic and hydrophobic interactions.





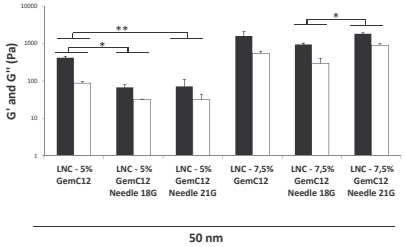


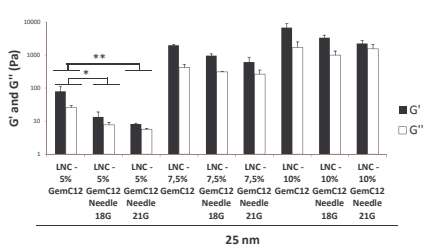




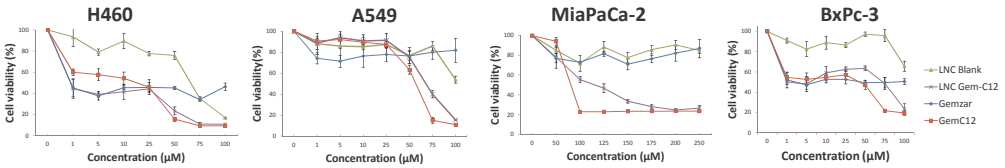
Drop tensiometer measurements were realized to characterize the localization of gemcitabine inside the LNCs (n=3; mean ± SD). Drop tensiometer showed a decrease in surface tension of triglyceride / water interface when the lipophilic prodrug was added. **This supports an active participation of GemC12 as a co-surfactant in the LNC formulation, and may explain the high rate of encapsulation obtained.**

The viscoelastic properties of different formulations after injection with a syringe with different sizes of needle were studied using a Malvern rheometer with a fixed shear strain and frequency (n=3; mean ± SD, ANOVA 1F, *post hoc* Scheffé, * → p < 0.05, ** → p < 0.01) . **LNCs loaded with GemC12 can be injected using syringe with different needle sizes without loss of the gel-state.**





Cytotoxic activity of free and loaded GemC12 versus gemcitabine hydrochloride (Gemzar®) were screened on different cell lines (H460 and A549: two human lung cancer cell lines ;MiaPaCa-2 and BxPc-3: two human pancreatic cancer cell lines). This part was assessed by MTS assay after 48 hours of incubation (n=3; mean ± SD). **In comparaison with Gemzar®. GemC12 free and encapsulated induced greater cytotoxicity in H460 , A549, MiaPaCa-2 and BxPc-3 cell lines.**



Encapsulation of lipophilic gemcitabine in nanocapsules, the high level of antitumor efficacy observed and the new rheological properties obtained offer great potential for innovative uses.

- **The liquid suspension of LNCs** can be used IV to target tumors with or without ligands grafted on the nanocarrier surface.
- **The gel of LNCs**, obtained with the most concentrated formulations can be regarded as a sustained gemcitabine delivery system implantable in the vicinity of a tumor site.