



## EX VIVO IMAGING OF CURCUMIN AND DOCETAXEL LOADED POLYSORBATE 80 COATED PLGA NANOPARTICULATE SYSTEMS

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### INTRODUCTION

Delivery of drugs in Central Nervous System (CNS) is a major challenge for researchers. The blood-brain barrier (BBB) significantly inhibits the delivery of systemically administered therapeutic agents to the brain and limits the distribution and longevity of the locally distributed agents in brain extracellular matrix. On the other hand, for drug delivery systems to achieve the desired benefit and reach the therapeutic effect, it must remain in the blood circulation for a long time enough. Polysorbate 80 (Poly 80) coated Poly (lactic-co-glycolic acid) (PLGA) nanoparticles (Poly 80-PLGA NPs) offer a promising solution to these problems. Polysorbate 80, used as a coating agent, reduces the removal of drugs from the blood by avoiding phagocytic uptake by the reticuloendothelial system (RES) thus increasing the circulation time of the nanoparticle in the blood and its uptake into malignant cancer cells. Furthermore, it enables drugs encapsulated in nanoparticles to cross the blood-brain barrier and enter the brain. Docetaxel was chosen as

the active ingredient in the scope of the thesis because of its physicochemical (e.g. solubility) properties and its unsuitability for oral use. Curcumin is an active ingredient of natural origin and well tolerated orally. Recently, it has been found to have an anticancer effect. It was decided to be used in combination with docetaxel due to its chemical sensitivity (chemosensitizing) and chemical protective (chemoprotective) properties.

### OBJECTIVE

In this study, PLGA NPs containing curcumin and docetaxel active ingredients in combination were optimized. By selecting the best nanoparticle formulation obtained, the antitumor effect of docetaxel in the presence of curcumin and the penetration efficiency of the obtained nanoparticulate system after polysorbate 80 coating were investigated.

### MATERIAL & METHODS

CD1 mice were anesthetized with an i.p injection of 50 mg/kg ketamine and 4 mg/kg xylazine before *in vivo* distribution of the developed formulations to the brain. For the *ex vivo* follow-up of nanoparticles with the small animal imaging system 3 experimental groups were created : uncoated PLGA NPs

(Group1 - without polysorbate 80, n = 3) and Polysorbate 80 coated PLGA NPs (Group 2 – with Polysorbate 80, n = 3) were loaded with Flamma 774 dye and prepared in the same way as the optimum formulation (see publication) and Control Group (n=1) where only autofluorescence was imaged. With the help of a catheter placed in the tail vein, the mice were i.v. injected a volume of 150µl at a concentration of 175 mg/kg. 2 hours after the injection, the mice were decapitated under anesthesia. All organs and brain tissues were removed for *ex vivo* fluorescence imaging with an excitation wavelength of 774 nm and emission wavelength of 806nm with the imaging system, Newton 7.0 (Vilber Lourmat, France).

## RESULTS

### Figure 1. *Ex vivo* representative fluorescent intensities of the brains of CD1 mice 2hrs after injection.

The images show drug distribution of uncoated PLGA NPs (A, n=3), polysorbate 80 coated nanoparticles (B, n=3) both flamma 774 loaded. The autofluorescence of the control group (n=1) 2hrs later can also be observed. Fig.1 shows that the intensity of the nanoparticles in Group B (coated with polysorbate 80) was denser, with more accumulation in the brain tissue, than uncoated nanoparticles Group A.

### Figure 2. *Ex vivo* drug distribution fluorescent intensities of uncoated PLGA NPs and polysorbate 80 coated PLGA NPs 2hrs after injection.

Otofloresan = autofluorescence of the Control group. A = Uncoated PLGA NPs (without polysorbate 80) flamma 774 loaded; and B = Coated PLGA NPs (with polysorbate 80) flamma 774 loaded. Liver (Karaciger), Lungs (akciger), Heart (kalp), Kidneys (bobrekler), Spleen (dalak). Fig2 shows that nanoparticles that were not coated with polysorbate 80 (GroupA) were more concentrated in the liver (Karaciger). The image also demonstrates that there was accumulation in the lungs (akciger) and in the kidneys (bobrekler) in group B – coated polysorbate 80. This demonstrates that polysorbate 80 coated PLGA NPs are better protected by RES organs and may have increased the duration of stay in the blood. This situation is thought to be an important factor in reducing side effects of polysorbate 80 coating.

## CONCLUSION

In order to develop passive tumor targeting strategies, polysorbate 80 coating has been tested on the outer surface of PLGA-based NPs in an *in vivo* CD1 mice model. These hydrophilic molecules on NPs provide stealthy properties, avoiding phagocytic uptake by the reticuloendothelial system (RES) and remaining in the systemic circulation for a long time. The effectiveness of the polysorbate coating *ex vivo* was evaluated using the small animal imaging system Newton 7.0 (Vilber Lourmat, France). This study enlightens the use of the Newton 7.0 as a powerful measuring tool for imaging coated nanoparticles *ex vivo*.

