INDIRECT ESTIMATION OF IN-VIVO PLASMA ASSOCIATED/DISSOCIATED FRACTIONS OF DRUGS CARRIED IN NANOPARTICLES

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Background: Lipid-based nanoparticles (LNP) are composed of phospholipids and PEG formulated with drugs for targeted and/or long-acting drug delivery. One major limit in the clinical translation of these nanoproducts is the unknown in-vivo release kinetics of drugs in the carriers. Microdialysis or solid-phase micro-extraction techniques both have important limitations: Microdialysis may not represent the in-vivo condition; Micro-extractions may be confounded by the LNP composition of endogenous lipids. In the absence of a drug release measurement in-vivo, we propose a low-cost pharmacokinetic way to quantify fractions in plasma of drugs associated (i.e. stability in-vivo) and dissociated (i.e. active drug) using typical data generated in the lab.

Methods: We propose two pharmacokinetic methods using the developer's typically available pharmacokinetic data (free and LNP-formulated drugs IV).
Method 1: AUC-based method providing boundaries of time-averaged percentages of associated vs. dissociated in plasma: *max dissociated* = AUC_{free}/AUC_{LNP} %.
Method 2: based on dynamic modeling. Pharmacokinetic (PK) modeling of the physical system, in which LNPs are administered, provides predictions of associated and dissociated species time-courses. PK modeling founds on the conservation of mass-balance principle.
We computed these two methods with data from LNP formulations in primates intended for long-acting HIV therapy (Drugs: *atazanavir*, *lopinavir*, *ritonavir*, *tenofovir*). These methods were included in several IND and FIH approvals for HIV long-acting therapy.

Results: Collecting primates' pharmacokinetic data for the free and LNP drugs we estimated via Method 1 that the drugs max dissociation from LNP (over 96h experiment) as 2-13% of the dose. Hence, 87-98% was the min dose that remained associated with LNP in-vivo. In particular, hydrophilic *tenofovir* had been estimated as 10% associated with dialysis experiments, while we found it 97% associated in-vivo using our investigational tools.
Method 2 completed the analysis providing time-course simulations of the two simultaneously circulating species in the plasma. The simulated dissociated fractions, i.e. the active molecule released by LNP, resulted in a 17-20 h half-life compared to the 4-8 half-life of the free control.

Conclusions: These methods are easily and cheaply applicable to all kinds of circulating nanocarriers, such as liposomes and polymeric particles. Taking advantage of mandatory pharmacokinetic studies, the presented methods provide invaluable quantification of the in-vivo stability and the modified pharmacokinetics of the active drug as dissociates with time. **Method 1** is very easily computable to provide indicative boundaries. Although **Method 2** requires modeling expertise, once implemented, it leads to knowledge-gap-filling predictions of plasma time-courses for both associated and dissociated species.
If the developer invests in Method 2, i.e. a computer model, his drug development pipeline for long-acting formulations can leap ahead of competitors as it can be used, once validated, to skip costly and ethical concerning study about in-vivo stability and efficiency (i.e. levels of free drug released on target).