# Albumin and Uptake of Drugs in Cells: Additional Validation Exercises of a Recently Published Equation that Quantifies the Albumin-Facilitated Uptake Mechanism(s) in Physiologically Based Pharmacokinetic and Pharmacodynamic Modeling Research

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**ABSTRACT:** The impact of albumin concentration on the uptake of drugs in cells might involve mechanisms going beyond the free drug concentration hypothesis. Proceeding from the assumption that both the unbound and protein-bound drug fractions can be available for uptake, several authors have argued that the uptake of highly bound drugs in cells might be driven mainly by the albumin-facilitated uptake mechanism(s). Hence, a novel approach quantifying the additional contribution of the protein-bound drug complex and pH gradient effect in diverse *in vitro*-to-*in vi*vo extrapolation (IVIVE) procedures of drug uptake and clearance has been proposed and extensively validated by Poulin et al. (2015. J Pharm Sci. Epub ahead of print); this approach consisted of replacing the unbound fraction in plasma ($fu_p$) with an adjusted $fu_p$ value ($fu_{p\text{-adjusted}}$). After a second review of literature, the objective of the present study was to perform further validation exercises of the concept of $fu_{p\text{-adjusted}}$ by using additional case examples of IVIVEs that covered diverse drug properties and experimental settings with varied albumin concentrations (e.g., perfused liver, isolated and suspended hepatocytes, and cultured cells overexpressing transporters). Again, the novel IVIVE method based on $fu_{p\text{-adjusted}}$ was the best-performing prediction method of the uptake rate (or clearance) as a function of protein binding compared with the conventional method based on the $fu_p$ theory (absolute average fold error of 1.4 vs. 7.4). Therefore, the present study confirms the utility of $fu_{p\text{-adjusted}}$ compared with $fu_p$ in IVIVE procedures for drugs highly bound to albumin, and the improvement was observed particularly in the higher range of albumin concentrations. From these findings, we may conclude that uptake of these drugs in cells is primarily driven by the albumin-bound form. Consequently, it is suggested to estimate the uptake kinetic parameters with cell-based assays incubated in 100% human serum or to make a correction while the experimental data are generated either without albumin or with varied albumin concentrations, in order to predict more accurately the *in vivo* conditions in physiologically-based pharmacokinetic and pharmacodynamic (PBPK/PD) modeling research. Overall, the protein-facilitated uptake mechanism(s) could be another paradigm shift in addition to a previous paradigm related to the pH gradient effect.  J Pharm Sci 104:4448–4458, 2015

**Keywords:** ADME; clearance; disposition; drug safety; hepatocytes; IVIVEs; isolated perfused liver; PKPD; pharmacokinetics; pharmacodynamics

## INTRODUCTION

An organ (e.g., liver) may remove a diverse group of drugs from the circulation with remarkable efficiency, despite extensive binding of these solutes to the extracellular proteins (e.g., albumin). Poulin et al.[1] have recently argued that uptake of highly bound drugs in cells might be driven by the albumin-facilitated uptake mechanisms. Several published experimental studies confirmed that for several highly protein-bound drugs, the "real" free drug concentration taken by the cells in the presence of extracellular binding proteins could be substantially higher than the calculated free drug concentration from the unbound fraction in plasma ($fu_p$) measured *in vitro*.[2–20] Hence, the hepatic removal of drugs in the isolated perfused rat liver (IPRL) (and hepatocyte suspensions) was faster than expected when serum (plasma) is used as the perfusate compared with serum-free perfusate.[3–11] This is also true for the removal of drugs in the isolated perfused rat kidneys.[12,13] In addition, recent drug–drug interaction (DDI) studies at the transporter level were performed in cultured Madin–Darby Canine Kidney epithelial (MDCK) cells overexpressing organic cation (OCT2) or anion transporters (OAT1B1); thus, the later DDI studies also indicated that for highly protein-bound inhibitors, the measured IC50 (the concentration of an inhibitor where the response or binding is reduced by half) measured in 100% human serum was significantly lower than those estimated based on $fu_p$ and the intrinsic transporter IC50 assessed in a serum-free medium.[14] Conversely, for drugs with a low degree of albumin binding, the albumin-mediated effects were not observed in these experimental settings.[1,9,17–20] From these observations, the authors concluded that the impact of albumin concentration on the estimation of uptake of drugs in cells might involve mechanisms going beyond the free drug concentration hypothesis. Therefore, it was suggested that the protein-bound drug concentration was also involved in the cellular uptake but for the compounds that are highly bound to albumin particularly in the higher range of albumin concentrations, which supports the notion of albumin-facilitated uptake mechanism(s). The net

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result of the albumin-facilitated mechanism(s) is that more unbound drug may become available at the cell surface for uptake, that is, drug can be transported in cells in two ways: one way is the unbound form and the other way a cell surface-mediated enhanced dissociation from the bound form.[1,21]

The extracellular protein-mediated uptake mechanism(s) of drugs has mostly been observed with the hepatocytes, cardiac myocytes, and cells from kidneys. Similarly, different experimental conditions either performed under *in vitro* or *in vivo* conditions with rat and human materials have demonstrated the presence of an albumin-facilitated uptake effect.[1–21] The mechanism(s) was not specific only for serum albumin but also for other serum proteins (e.g., globulins).[4,6,7] In this case, the general trend is that the albumin-facilitated uptake mechanism(s) is predominant for an extracellular binding protein with a net positive charge compared with a net negative charge, and, hence, the experimental studies using alpha-acid-glycoprotein (AAG) or negatively charged albumin showed no protein-mediated uptake mechanism(s) compared with positively charged albumin and globulins.[1,6,7,21] However, the exact mechanism by which these binding proteins may facilitate the uptake of a drug in cells is still unknown, but a recent review of literature compiled the mechanisms that have been proposed by diverse authors.[1] To date, the most plausible mechanism seems to be related to the presence of ionic attractions between the protein–drug complex and cell surface to result in a marked reduction in the diffusional distance of the extracellular protein–drug complex.[1,21] Whichever mechanism explaining the protein-facilitated uptake process, it is almost certain that more drug is delivered into the cells than expected from the conventional pH gradient effect theory of the unbound drug.[1]

The additional protein-facilitated uptake mechanism(s) needs, therefore, to be quantified in the *in vitro*-to-*in vivo* and *in vitro*-to-*in vitro* extrapolation (IVIVE) procedures, as the concentrations of extracellular binding proteins used in cell-based *in vitro* assays and/or perfused organ studies are usually different from the physiological concentrations of these proteins *in vivo*, or simply, no proteins are added in these assays, which can be problematic because the intracellular drug concentration may differ between the experimental and *in vivo* conditions, particularly in the presence of protein-facilitated uptake mechanism(s).[1–16] Accordingly, inaccurate IVIVEs of the intracellular drug concentration in physiologically-based pharmacokinetic and pharmacodynamic (PBPK/PD) modeling exercises could potentially induce unexpected toxicity and/or inefficacy in preclinical and clinical trials, which can be of concern in pharmacology studies.[1] Moreover, the most recent prediction models of drug disposition only considered the uptake of the unbound drug in cells, and, hence, the uptake effect of the protein–drug complex was disregarded.[1,22–27]

Poulin and coworkers,[1,17–20] however, have recently proposed diverse equations to quantify the uptake in cells of both the unbound and bound drug forms by combining an albumin-facilitated uptake mechanism and pH gradient effect. The main equation consisted of replacing the measured $fu_p$ value with an adjusted $fu_p$ value ($fu_{p\text{-adjusted}}$). This novel parameter considered additional processes that may potentially occur between the experimentally determined *in vitro* conditions used to estimate $fu_p$ and the real *in vivo* conditions, namely, (1) the pH gradient effect on the unbound drug fraction between plasma and the intracellular compartment, and (2) the albumin-facilitated uptake mechanism(s), which quantifies the uptake of the albumin-bound drug fraction at the cell surface. This novel equation was based on the binding isotherm as presented below.[1,17–20]

$$fu_{p\text{-adjusted}} = \frac{PLR \times fu_p \times \frac{funionized_{plasma}}{funionized_{cells}}}{1 + (PLR - 1) \times fu_p \times \frac{funionized_{plasma}}{funionized_{cells}}} \quad (1)$$

where PLR is the plasma-to-tissue concentration ratio of extracellular binding proteins under *in vivo* conditions (e.g., albumin), whereas $funionized_{plasma}$ and $funionized_{cell}$ is the corresponding fraction of unionized drug in each matrix. The ionization parameters can be estimated from the Henderson–Hasselbalch equations, the p*K*a value of the drug and the physiological pH values on both sides of the membrane (e.g., about 7.0 for cells and 7.4 for plasma). Therefore, the above Eq. (1) converted the protein-bound drug concentration from the plasma to tissue (cell surface) assuming that the protein-bound drug fraction is also available for uptake in cells, and, that, in the case of albumin, this protein was found mainly outside the cells based on literature[18]. Eq. (1) also considered that the drug at the cell surface is affected by the pH gradient. In other words, it was assumed that each drug molecule bound to an extracellular binding protein (e.g., albumin) in plasma, and, hence, in the interstitial space, may interact with the cell surface to deliver the additional bound drug into cells. Consequently, the intracellular drug concentration could be substantially higher than expected based solely on the free drug concentration, which is in accordance with the albumin-facilitated uptake mechanism(s). The net result will be that values of $fu_{p\text{-adjusted}}$ will be greater than $fu_p$. Eq. (1) is sensitive in the low range of $fu_p$ values (i.e., for the highly bound drugs), and the change of $fu_{p\text{-adjusted}}$ with $fu_p$ is not proportional based on the binding isotherm, which might be of concern in PBPK/PD research as the plasma concentration used as a reference matrix could significantly deviate nonproportionally to the cell concentration.[1,18] To apply Eq. (1) to diverse experimental settings, the PLR value can simply be adjusted to reflect the real concentration ratio of albumin between the perfusate (or buffer) and organ materials. Similarly, the pH values can be adjusted to estimate the drug ionization parameters.

Equation (1) was extensively and successfully validated with several drug examples for which their hepatic clearance was governed either by a metabolic, transporter, and/or permeation limitation effect.[17–20,23] By considering several drugs that bind mainly to albumin or AAG, the hepatic drug CL *in vivo* was more accurately predicted by correcting the microsomal and hepatocyte *in vitro* data with $fu_{p\text{-adjusted}}$ compared with $fu_p$. In other words, the novel concept of $fu_{p\text{-adjusted}}$ demonstrated a significantly reduced prediction bias compared with the conventional $fu_p$ approach, particularly for those drugs highly bound to albumin. Furthermore, it has been demonstrated that the PLR effect (i.e., the correction for the albumin-bound drug effect) was predominant compared with the pH gradient effect according to Eq. (1).[17–20] Overall, the hepatic *in vivo* clearance was more accurately predicted by incorporating the calculated value of $fu_{p\text{-adjusted}}$ in IVIVE procedures compared with the actual value of $fu_p$ measured *in vitro*. Also, the *in vivo* tissue–plasma and tumor–plasma partition coefficients at the whole organ level of several acidic and neutral drugs that bind mainly to albumin, were also accurately predicted by considering a pH gradient