Thank you for your interest in my research. Nice to meet you!

の研究にご関心をお寄せいただき、ありがとうございます。はじめまして！

Model development

The model is based on a 2-compartment model, with the schematic diagram in the figure below briefly describing the two compartments (Figure 1), following the assumptions:

1) During the UF process, pH, temperature, or centrifugal force remains constant, i.e., experimental conditions are constant.
2) Drug degradation, nonspecific binding (NSB), and protein leakage are not considered during UF.
3) It is assumed that drug-protein equilibrium is sufficiently established at the initial time point t = 0.
4) Protein conformational changes (denaturation or aggregation) and multi-protein interactions (e.g., oligomerization, complex formation) during the experimental process are not considered.

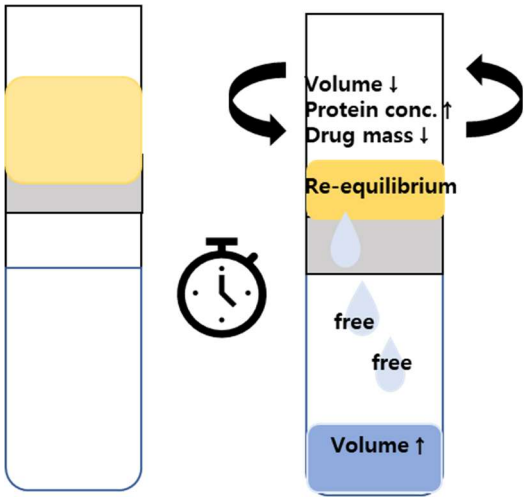

Figure 1. Graphical abstract of time-dependent UF.

Volume model

During the UF process, plasma flux decreases over time due to filter resistance and protein concentration effects. Initially, plasma is rapidly filtered, and subsequently, the rate gradually decreases. Consequently, the filtrate volume change converges in an exponential + saturation form. The Weibull function effectively describes this initial burst structure.

$$F(t) = 1 - exp\left(-\left(\frac{t}{\alpha}\right)^{\beta}\right)$$

$F(t)$ is representing the cumulative filtrate volume fraction, α is the scale parameter, and β is the shape parameter. The time-dependent filtrate volume $V_{filt}(t)$ and retentate volume $V_{ret}(t)$ can be expressed as follows:

$$V_{filt}(t) = V(0) \cdot F(t)$$

$$V_{ret}(t) = V(0) - V_{filt}(t)$$

The instantaneous filtration flow rate (flux) $v(t)$ is as follows:

$$v(t) = \frac{dV_{filt}(t)}{dt} = V(0) \cdot \frac{dF(t)}{dt}$$

Where $V(0)$ is the initial volume of retentate compartment.

Retentate model

In the simplest case, assuming that a protein molecule has one reversible binding site to which a drug can bind, the drug-protein binding reaction can be represented as follows: (Vuignier et al., 2010).

$$\mathrm{D} + \mathrm{P} \rightleftharpoons \mathrm{DP}$$

Where $[\mathrm{D}]$, $[\mathrm{P}]$, and $[\mathrm{DP}]$ are the concentrations of free drug, free protein, and drug-protein complex, respectively. The binding constant K_A, defined as the ratio of the association rate constant k_{on} and dissociation rate constant k_{off}, is the reciprocal of the dissociation constant K_d and can be expressed as follows:

$$K_A = \frac{k_{\mathrm{on}}}{k_{\mathrm{off}}} = \frac{[\mathrm{DP}]}{[\mathrm{D}] \cdot [\mathrm{P}]} = \frac{1}{K_d}$$

If the binding occupancy among all binding sites is θ and the maximum number of binding sites per protein molecule is n, then the actual number of bound drug molecules per protein molecule r is as follows:

$$\theta = \frac{[\mathrm{DP}]}{[\mathrm{P}] + [\mathrm{DP}]} = \frac{K_A \cdot [\mathrm{D}]}{1 + K_A \cdot [\mathrm{D}]}$$

$$r = n \cdot \theta = \frac{n \cdot K_A \cdot [\mathrm{D}]}{1 + K_A \cdot [\mathrm{D}]}$$

The bound drug concentration $C_{bound}(t)$ can be defined as the product of protein concentration $C_p(t)$ and r. The total drug concentration in the retentate $C_{ret,tot}(t)$ is the sum of free drug concentration $C_{ret,u}(t)$ and bound drug concentration $C_{ret,b}(t)$. Therefore, the binding dynamics in the retentate can be calculated at each time point.

$$C_{bound}(t) = C_p(t) \cdot r$$

$$C_p(t) = C_p(0) \cdot \frac{V(0)}{V_{ret}(t)}$$

$$C_{ret,tot}(t) = C_{ret,u}(t) + C_{ret,b}(t)$$

$$C_{ret,tot}(t) = C_{ret,u}(t) + \frac{n \cdot C_p(t) \cdot K_A \cdot C_{ret,u}(t)}{1 + K_A \cdot C_{ret,u}(t)}$$

At the initial sample loading time point, rapid equilibrium was assumed since sufficient time for binding equilibrium was allowed. When given values were not available, protein concentrations were set to 600 μM for albumin (Hill, 1985) and 20 μM for AAG (Blain et al., 1985), and calculations were performed using drug-specific parameters including K_A, $C_{\mathrm{ret},tot}(0)$, and n.

At a specific time point t, the total drug mass in the retentate compartment $M_{ret,tot}(t)$ is the sum of bound drug $M_{ret,b}(t)$ and unbound drug $M_{ret,u}(t)$.

$$M_{ret,tot}(t) = M_{ret,b}(t) + M_{ret,u}(t)$$

The main differential equation is as follows:

$$\frac{dM_{ret,tot}(t)}{dt} = -v(t) \cdot C_{ret,u}(t) = -\frac{dM_{filt}(t)}{dt}$$

The change in total drug mass is calculated based on the free drug concentration flowing out to the filtrate. In the dynamic model where t > 0, the binding kinetics are as follows:

$$\frac{d[\mathrm{DP}]}{dt} = k_{\mathrm{on}} \cdot [\mathrm{D}] \cdot [\mathrm{P}] - k_{\mathrm{off}} \cdot [\mathrm{DP}]$$

The binding constant K_A as well as k_{on} and k_{off} are drug-specific properties that remain constant within the system.

$$[\mathrm{D}] = \frac{M_{ret,u}(t)}{V_{ret}(t)} = C_{ret,u}(t)$$

$$[\mathrm{DP}] = \frac{M_{ret,b}(t)}{V_{ret}(t)} = C_{ret,b}(t)$$

$[\mathrm{P}]$ is the concentration of available binding sites, which is the total binding site concentration minus the already bound drug concentration.

$$[\mathrm{P}] = n \cdot C_p(t) - C_{ret,b}(t)$$

The differential equation describing the system is:

$$\frac{dM_{ret,b}(t)}{dt} = k_{\mathrm{on}} \cdot M_{ret,u}(t) \cdot (n \cdot C_p(t) - C_{ret,b}(t)) - k_{\mathrm{off}} \cdot M_{ret,b}(t)$$

$$\frac{dM_{ret,u}(t)}{dt} = -v(t) \cdot C_{ret,u}(t) - k_{\mathrm{on}} \cdot M_{ret,u}(t) \cdot (n \cdot C_p(t) - C_{ret,b}(t)) + k_{\mathrm{off}} \cdot M_{ret,b}(t)$$

When no literature values are provided for k_{on}, it was set to $6 \cdot 10^6 M^{-1} min^{-1}$ since most small molecules are concentrated in the range of $10^4 \sim 10^6 M^{-1} s^{-1}$ (Jarmoskaite et al., 2020).

Filtrate model

The filtrate compartment was designed based on the cumulative mass of free drug removed from the retentate compartment. The quantitative change in free drug is determined through the free drug concentration that exits from the retentate compartment and the flux $v(t)$.

$$\frac{dM_{filt}(t)}{dt} = C_{ret,u}(t) \cdot v(t)$$

$$C_{filt}(t) = \frac{M_{filt}(t)}{V_{filt}(t)}$$

This model mathematically integrates both drug-protein binding and filtration processes that depend on the passage of time during the UF process.

Extension to the Dynamic Multiple Site Model

Protein molecules can have multiple binding sites with different affinities coexisting. In this case, the actual number of bound drug molecules per protein molecule $r_i(t)$ for each site can be generalized as follows:

$$r_i(t) = \frac{n_i \cdot K_{A,i} \cdot C_{\mathrm{ret},u}(t)}{1 + K_{A,i} \cdot C_{\mathrm{ret},u}(t)}$$

$$C_{ret,b}(t) = \sum_{i=1}^{m} C_p(t) \cdot r_i(t)$$

The binding kinetics ODE is as follows:

$$\frac{dM_{\mathrm{ret},b}(t)}{dt} = \sum_{i=1}^{m} k_{\mathrm{on},i} \cdot M_{ret,u}(t) \cdot (n_i \cdot C_p(t) - C_{ret,b,i}(t)) - \sum_{i=1}^{m} k_{\mathrm{off},i} \cdot M_{ret,b,i}(t)$$

$$\frac{dM_{\mathrm{ret},u}(t)}{dt} = -v(t) \cdot C_{ret,u}(t) - \sum_{i=1}^{m} k_{\mathrm{on},i} \cdot M_{ret,u}(t) \cdot (n_i \cdot C_p(t) - C_{ret,b,i}(t)) + \sum_{i=1}^{m} k_{\mathrm{off},i} \cdot M_{ret,b,i}(t)$$

The site-specific k_{on}, n, and K_A values were cited from literature as input parameters.

Extension to Equilibrium Hill-based Multiple Site Model

For most small molecules, k_{on} and k_{off} are much faster than filtrate flux on the UF time scale, so it can be assumed that equilibrium is reached instantaneously at every moment (Jarmoskaite et al., 2020). In this case, the Hill equation is used to define r_i the number of bound drugs per site i. $[L]$ is the free ligand (drug) concentration, h is the Hill coefficient describing cooperativity, and K_d is the dissociation constant (Goutelle et al., 2008).

$$\theta = \frac{[L]^h}{K_d + [L]^h} = \frac{K_A \cdot [L]^h}{1 + K_A \cdot [L]^h}$$

The actual number of bound drug molecules per protein molecule $r_i(t)$ for each site as follows:

$$r_i(t) = \frac{n_i \cdot K_{A,i} \cdot \left(C_{\text{ret},u}(t)\right)^{h_i}}{1 + K_{A,i} \cdot \left(C_{\text{ret},u}(t)\right)^{h_i}}$$

$$C_{\text{ret},u}(t) = C_{\text{ret},tot}(t) - \sum_{i=1}^{m} \frac{n_i \cdot C_p(t) \cdot K_{A,i} \cdot \left(C_{\text{ret},u}(t)\right)^{h_i}}{1 + K_{A,i} \cdot \left(C_{\text{ret},u}(t)\right)^{h_i}}$$

All binding is calculated as instantaneous equilibrium, and unlike before, k_{on} and k_{off} are not used. At each time point t, the above equation is solved algebraically, and then only the unbound fraction is obtained through ODE (filtration flow). This extension enables the prediction of drug behavior with cooperative effects. The site-specific k_{on}, n, K_A, and h values were cited from literature as input parameters.

Modeling and simulation

Seven drugs were selected from previously published studies that provided filtrate and retentate concentrations at various % filtered volumes using UF. These included phenytoin (McMillin et al., 2005), disopyramide (David et al., 1983), salicylate, ibuprofen, and carprofen (Whitlam & Brown, 1981), vancomycin (Kratzer et al., 2014), and carbamazepine (Dong et al., 2013). For phenytoin, vancomycin, and carbamazepine, time-dependent % filtered volume data were available or could be inferred, and corresponding volume functions were estimated by Weibull fitting using the tidyverse package in R.

For the remaining compounds, volume functions were fitted by normalizing % filtered volume profiles to an initial retentate volume of 1 mL, based on representative data from the Centrifree® Ultrafiltration Device user guide (Millipore, 2021). by scaling % filtered volume data to an initial volume of 1 mL.

Both algebraic and differential equations were used to capture equilibrium and time-dependent dynamics of the ultrafiltration process. The system was implemented and solved using the deSolve package in R, with subsequent processing performed using dplyr and tidyr.

Thanks. If you have any questions, please feel free to contact me via email.

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