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Simulating extractables and leachables in biopharmaceutical manufacturing to support safety assessment

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ABSTRACT

The use of single-use systems in the manufacturing of biopharmaceuticals raises concerns about the accumulation of process equipment-related leachables in their production and purification processes. However, this risk is mitigated by effective sinks in the manufacturing processes and dilution of product flow, for example, in tangential-flow-filtration. This paper presents a modeling approach that combines the release and adsorption of compounds with dynamic process conditions of biopharmaceutical processes. These calculations help assess process criticality by identifying sources but also low-risk processes and components regarding extractables and leachables accumulation. This approach can significantly reduce the necessity for resource-intensive practical testing, such as leachable studies, which may be impractical from an analytical perspective due to the complex matrices in biopharmaceutical process streams.

1. Introduction

Single-use (SU) systems have been widely adopted in the biopharmaceutical industry for the production of biopharmaceuticals. Nowadays, they are increasingly applied to the production of commercial batches, though they were originally used during process development and scale-up to clinical phase production. SU technology has reached a level of maturity such that companies can implement entire end-to-end single-use production platforms for biologics, such as monoclonal antibodies, vaccines, or antibody-drug conjugates (Samaras et al., 2022) Recently, SU technology is increasingly used in research, scale-up, and scale-out such as in the production of cell and gene therapies (CGT) (Abbasalizadeh et al., 2017).

This adoption of single-use technology has led to an improved understanding of biomanufacturers regarding the interaction of plastic SU devices with complex process solutions. In qualification studies, the risk to process performance, product quality, and patient safety is evaluated (Li et al., 2015) One important qualification criterion relates to compounds—process equipment-related leachables (PERL)—that may be released into the manufacturing process from materials of construction (MoCs) in contact with the product stream and accumulate in drug substance or drug product.

Extractables studies provide information on the identity and quantity of such PERLs potentially being released. Further, data from extractables studies support scientists in deciding whether leachables studies are needed and how they should be designed and/or to initiate further toxicological assessments (Jenke, 2009) In contrast, leachable studies are designed to quantify impurities associated with contact materials that are present in the final drug product (Ball and Norwood, 2012) Both studies consider the dimensions and size of the equipment, process volumes and times, streams, and temperatures.

The concentration changes over time, the removal or accumulation, i.e., the “fate of PERLs compounds” in the process is typically not addressed. Quite often, a “worst-case” approach is chosen for predicting the quantities of different PERLs likely to be present in the process and the drug product. This approach considers the accumulation of polymer-related compounds, such as additives, their degradants, or processing aids, released into the process stream, leading to high concentrations in the final product. This assumption is questionable, and data from process validation studies often contradict this “worst-case” paradigm. Real-life data consistently show that PERLs do not contribute significantly to final drug product impurities (Magarian et al., 2016, Scherer

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et al., 2019) Instead, they are effectively removed in unit operations during downstream processing, for example, in tangential flow filtration or chromatography bioprocessing, through dilution, phase distribution, and discharge of PERLs in the waste stream.8 Studies have shown that the total mass of PERLs present in each process step actually decreases throughout the process starting from the production bioreactor as a consequence of purification mechanisms rather than accumulating in the process stream as it is assumed under “worst-case scenarios” (Magarian et al., 2016, Scherer et al., 2018, Hauk et al., 2018, Paudel et al., 2020, Hauk et al., 2017).

Therefore, it is crucial to improve the understanding of the roles that both SU systems and the biopharmaceutical process steps play as active “sinks” for PERLs. To enhance this understanding, the industry could conduct quantitative evaluations throughout the entire process, providing detailed descriptions of how each process step contributes to the formation and removal of PERLs. However, this approach presents practical challenges. Bioprocess fluids are complex mixtures containing cells, proteins, buffers, detergents, and various process-related impurities, which complicate the analysis of organic molecules like PERLs. Additionally, simulating an entire production scenario—from upstream to downstream to final filling—solely to evaluate PERL development at each process step—is cost-intensive and hardly feasible in a GMP environment.

An alternative approach is to use mathematical models or to focus on individual steps. Using models allows the development of sophisticated descriptions that mechanistically explain and estimate for bioprocess operations if PERLs accumulate or are removed from the process stream. This enables process developers to improve predictions of PERLs and, in the end, leachables concentrations in the final product. This information improves risk assessments and might streamline testing programs, i.e., reduce the need for leachables studies. A further, general advantage of modeling is to study and optimize processes already in the planning phase—here in particular the PERL load and their concentration trajectories along the process. This supports process development in a forward-looking, fast, cost-effective, and resource-friendly way. The FDA is currently working on similar mechanistic models to investigate the exposure of leachables from medical devices (polymeric implants) on tissue and their distribution in the organism (Saylor et al., 2020) One of the main arguments for establishing such models is the impossibility of analytically following the spatial and temporal leachables exposure in living organisms and the fact that all worst-case exposure models overpredict exposure scenarios substantially.

Model-based optimization methods exist for the removal of small molecules using different types of chromatography or ultra- and diafiltration (UF/DF) (Pirrung et al., 2017, Shao and Zydney, 2004, Sun et al., 2024) These approaches typically focus on impurities introduced during the cultivation process, such as nucleic acids and proteins, or during antibody-drug conjugation, including drug-linking agents and solvents. Over the past years, many modeling approaches have been developed to predict the migration of compounds from plastic materials into a contact medium, specifically for food or pharmaceutical packaging materials. Nowadays, powerful software tools are available (Ciffroy et al., 2022, Piringer and Baner, 2008, Heider and Sobańtka, 2025) The main drawback of these models is their limitation to static systems. Contrary, models have been developed to describe in dynamic flow-through systems the uptake of a compound, such as drugs, by plastic materials. However, these models do not account for the release of the compound from the material (Roberts, 1996) A recent article by Treleano et al. demonstrated how migration software can be adapted to dynamic systems by dividing the dynamic problem into several static cases (Treleano et al., 2009).

In this contribution, we selected a box model approach that adopts concepts from environmental science using a dynamic description of the sources and sinks for PERLs. In our discussion, we will focus on the most relevant topics in process qualification regarding E&L:

- Influence of the process flow on the PERL load in a bioprocess operation over time
- Impact of the interaction of PERLs with host cells on their concentration
- Identifying potential process steps or conditions that lead to an accumulation of PERLs

To the best of our knowledge, a box model approach has not yet been developed and applied to biopharmaceutical processes. The results obtained from the simulations will be compared to experimental results providing such data from extractable studies or real processes are available. The scenario of a storage bag for liquid handling is discussed, firstly as it represents a case where experimental comparative data and model validation are available. The model parameters estimated in this initial case are then applied in a second case for the in-silico modeling of cell cultivation and biopharmaceutical production in SU bioreactors, where experimental data are limited and not readily transferrable to manufacturer-specific use cases.

2. Model development

2.1. Box model of contaminants in biopharmaceutical manufacturing

A box model or compartment model is used to elucidate the fate of PERLs. It is widely used across various fields, from toxicokinetic to water-quality studies to describe the distribution of a released compound influenced by dynamic processes, such as degradation, exchange of media, and interphase transport (Ciffroy, 2018, Schwarzenbach et al., 2002, Chapra, 1997) The underlined mathematics are based on mass balance equations and solutions thereof and often require solving a system of algebraic (for steady-state problems) or differential equations (for time-variable systems) (Little, 2012).

The segmentation approach for a bioprocess using SU systems is shown in Fig. 1. The process can be regarded as a dynamic system with different compartments (liquid phase, SU material, and biological cells) connected by variables that determine the mass transport of substances between the compartments. The SU bag compartment acts as the homogeneous but transient source of PERLs. The release process is defined by diffusion and partitioning via the polymer-liquid interface and can be described with common migration calculations (see below). Inflow with already dissolved PERLs is considered a second source, e.g., via continuous input. In a continuous process, the media exchange takes place in the liquid compartment, where dissolved PERLs are removed with the outflow. The biomass compartment can act as a sink by associating PERLs via ad- or absorption. Eventually, back-diffusion into the MoC (sorption) is possible, providing another sink within the system (Gandek et al., 1992).

Besides these sinks, the PERL concentration in solution can change by chemical transformations, e.g., hydrolysis, complexation, oxidation, or through interaction, such as binding or reactions, with the biopharmaceutical drug substance. Degradation of the PERL can lead to reaction products with different properties.

Process conditions, particularly temperature, dwell times, and fluid flow influence both the migration from the MoC and the efficiency of the sinks in different ways. By knowing the temperature dependence of the state variables, it allows for incorporating its effect into the modeling (Hauk et al., 2021) Salts, pH, dissolved gases, and surfactants in solution can increase or diminish interactions, thereby affecting the adsorption properties of substances for different adsorbing phases. Process times determine whether the MoC is in short or long contact with the process solution, influencing to what extent a PERL substance can be extracted. On an abstract level, the mass transport of a PERL compound i within one compartment k of Volume V_l within a system of j joined compartments upstream to compartment k can be expressed with a differential equation (Eq. 1) where the PERL concentration $c_{l,i,k}$ is regarded as spatially constant (Little, 2012) In a static or batch system, where no

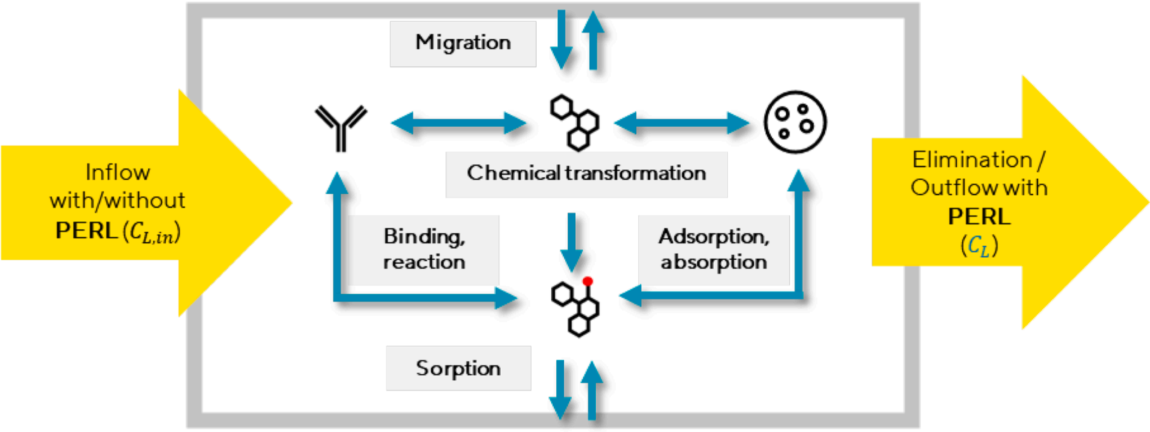

Fig. 1. Principal structure of a multi-box model used to dynamically calculate the PERL load in SU systems or unit operations with typical associated processes. Blue arrows indicate possible interaction pathways of PERLs. Yellow arrows indicate the in- and outflow of the system.

flow is applied, the terms for *Input* and *Outflow* are omitted.

$$\frac{d\left(V_{l,k}c_{l,i,k}\right)}{dt} = \sum_j (Sources + Input - Sinks - Outflow) \tag{1}$$

By employing appropriate functions to describe the terms *Input, Sources, Sinks* and *Outflow*, it is possible to construct a model balancing these effects and compute the temporal concentration profile of each PERL within the SU system. By considering that the outflow from one box can serve as the inflow for subsequent boxes, linked box systems can be generated. These systems can in principle simulate the PERL load development of the entire process from upstream processing to the final drug formulation.

2.2. Film-diffusion model

The release of plastic constituents into contacting liquids under static conditions (i.e., no exchange of liquid media) can be described as a migration process combining diffusion and partitioning. Respective models are well accepted in the field of food packaging engineering (Piringer and Baner, 2008, Poças et al., 2008) Most of these models describe the diffusion process via Fick's 2nd law of diffusion which allows to calculate local changes of concentration c as a function of time t and the diffusion coefficient D_p as relevant parameter: (Gnanasekharan et al., 1997)

$$\frac{\partial c_l}{dt} = D_p\left(\frac{\partial^2 c_l}{dz^2}\right) \tag{2}$$

Using advanced tools such as finite approximation techniques, some models reported in the literature are able to predict the migration of molecules even for complex geometric designs and multi-layer packaging (Roduit et al., 2005) In the case of perfused systems, however, such models cannot be easily applied because of non-constant boundary conditions. Furthermore, it has been shown that in most cases, simple diffusion models have often proved successful in predicting migration, as long as their input parameters can be obtained with good accuracy (Gavriil et al., 2018)

In the present model, the migration of PERLs via the polymer-liquid interface is described using a kinetic film-layer model based on Fick's 1st law of diffusion in one dimension. It is assumed that the contact layer consists of a thin polymeric film, with its matrix acting as a finite reservoir of the extractables. The substance in the liquid phase may be dilute and ideally mixed, with diffusion occurring only within a thin layer at the interface, known as the diffusion layer, between polymer and liquid phase. The mathematical description for migration can then be derived from the mass balance: (Cussler, 2009)

$$\begin{pmatrix} \textit{Substance} \\ \textit{accumulation} \\ \textit{in layer} \end{pmatrix} = \begin{pmatrix} \textit{Substance diffusion} \\ \textit{into layer} \end{pmatrix} - \begin{pmatrix} \textit{Substance diffusion} \\ \textit{out of layer} \end{pmatrix}$$

Using algebraic manipulations and considering boundary conditions, one obtains a steady-state substance diffusive mass flux during the time interval Δt (Eq. 3). For better readability, the subscripts *i, j* and *k* indicating specific PERL compounds and compartments are omitted.

$$\frac{\Delta c_l}{\Delta t} = \frac{1}{V_l}\sum \left(F(t)\cdot S_p\right) \tag{3}$$

Here, S_p represents the surface area of the polymer and $F(t)$ the diffusive mass flux. According to Fick's 1st law $F = -D_p\frac{\Delta c_p}{\Delta z}$ where c_p is the PERL concentration in the polymer and $\Delta c_p = c_{p,interface} - c_p$ (< 0) is the concentration gradient that drives diffusion accross the diffusion path of length $\Delta z = z_2 - z_1 = z_p$.

The precise value of z_p cannot accurately be described, as it is influenced by factors such as temperature and the age of the material. However, for thin films, it can be reasonably assumed that z_p falls within a range that allows the compound to diffuse through the material during the extraction period.

A scheme of the migration process is depicted in Fig. 2. The system is approaching equilibration due to the concentration gradient with the PERL diffusing from the higher concentrated polymer phase into the less concentrated liquid phase. This leads to a decline of the concentration gradient and in turn of the mass flux F until equilibrium conditions are achieved. The maximum achievable concentration of a PERL in solution under phase equilibrium is determined by the polymer-liquid partition coefficient $K_{p/l} = c_{p,interface}/c_l$ which is characteristic for a given polymer material, the migrating compound, and the composition of the solution.

Substituting $K_{p/l}$ into Eq. (3), considering only one source term (SU device) and rearranging yields:

$$\Delta c_l(t) = F(t) = \frac{S_p D_p}{V_l z_p}\left(c_p(t) - K_{p/l}\cdot c_l(t)\right)\Delta t \tag{4}$$

To simplify the model and calculations, the time-dependence of z_p (Fig. 2, blue arrow) is excluded, treating them as constant for a given temperature. The film-layer theory results in a linear concentration profile. This underestimates diffusion fluxes at the beginning of the migration (within the first hours) while it results in higher fluxes at the end of the process. A constant diffusion length is accepted for film migration modelling as discussed in Chapter 4.1. It has almost no influence on the overall modelling results (Denberg et al., 2007).

To describe the MoC-liquid interface appropriately, the geometry of the SU system needs to be included in the calculations. It is sufficient for

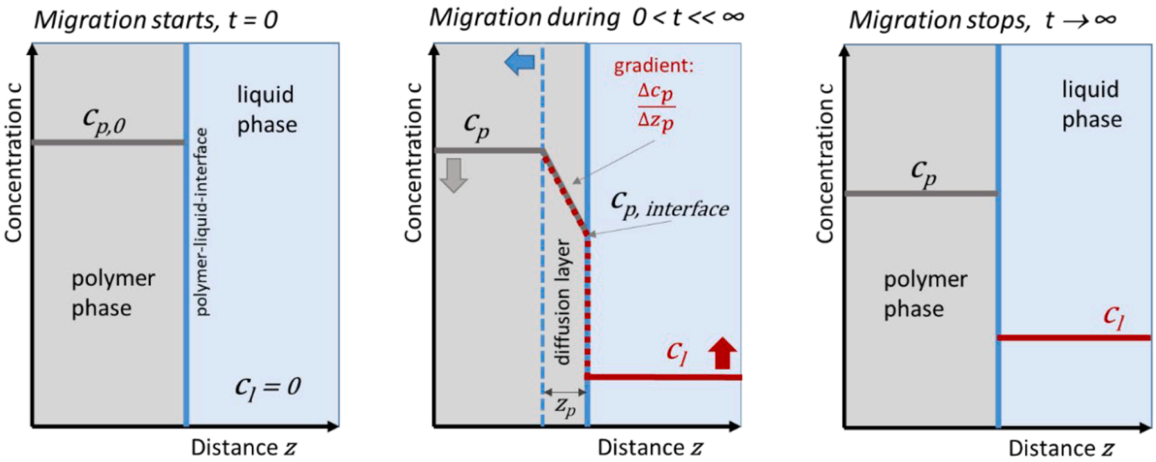

Fig. 2. Schematic description of an extraction for a film diffusion model: From left to right: a two-phase system in dis-equilibrium ($t = 0$) causes a diffusion-controlled migration (0<t<<∞), and ends in phase equilibrium (t →∞). This means, PERL concentration in solution rises (red arrow), while the PERL load in the plastic phase decreases (grey arrow). Reproduced with permission from Hauk et al., "Using extractables data from single-use components for extrapolation to process equipment-related leachables: The toolbox and justifications", European Journal of Pharmaceutical Sciences, 2021 (Hauk et al., 2021).

most calculations to assume a planar or circular shaped geometry for the interface, nevertheless more complex designs can be considered, if necessary.

2.3. Physicochemical considerations for PERL release in SU devices and operations

For a realistic description of the fate of leachables in SU systems, comprehensive knowledge of the process parameters, such as the temperature, residence or contact time, and for perfused systems, the flow rate is necessary. In general, low process temperatures correlate to slower PERLs release (smaller D_p) and lower partitioning of the PERL towards the solution (higher $K_{p/l}$) and are thus beneficial from a risk point of view. Further, information about individual PERLs is required, such as its initial concentration in the MoCs, solubility in the process liquid, diffusion, and partition coefficients. Diffusion coefficients D_p of PERLs for a specific MoC can be obtained from kinetic extraction studies or approximated by means of semi-empirical expressions (Hauk et al., 2021, Piringer, 1994) In cases where experimental data are lacking, upper-bond values can be estimated (Elder et al., 2025, Elder and Saylor, 2023) Diffusion constants for any temperature can be obtained by extrapolation using an Arrhenius-type equation if data from extraction experiments conducted at different temperatures is available (Welle, 2013) The temperature effect on the partition coefficient $K_{p/l}$ can be expressed with a van't Hoff-type equation when equilibrium concentrations of PERLs $c_{l,eq}$ are known (Hauk et al., 2021, Tehrany and Desobry, 2004) The initial PERL concentration in the polymer $c_{p,0}$ and, correspondingly, the total amount of PERL in the MoC m_{tot} is often not known. However, its value can be derived from extraction studies with long contact times (typically for extraction times > 21 days), when the maximum PERL concentration in solution $c_{l,eq}$ is reached: (Piringer and Baner, 2008, Pahl et al., 2019).

$$c_{l,eq} = \frac{m_{tot}}{V_l + K_{p/l} \cdot V_p} \tag{5}$$

In this equation, V_l and polymer V_p denote the volumes of the liquid and the polymeric phase, respectively. Another way to obtain information on D_p, $K_{p/l}$ and $c_{l,eq}$ simultaneously from one extraction curve is to use solutions of differential Eq. (2) for a plane sheet suspended in a well-mixed solution, as described in literature (Piringer and Baner, 2008, Heider and Sobańtka, 2025, Saylor and Young, 2023, Crank, 1975) Such calculations reflect the mechanistic nature of migration and are commonly used to estimate the release of packaging constituents in the food industry. They are accepted by EU regulators for safety assessments of food packaging material (Hoekstra et al., 2015, Begley et al., 2005) In case that the extracting solvent is not replaced during extraction, the mass ratio of PERLs m_l migrated into solution at time t and at infinite time $t = \infty$ can be computed by:

$$\frac{m_l(t)}{m_l(t = \infty)} = (1+\alpha)\left[1 - \exp\left(\frac{\tau}{\alpha^2}\right)\mathrm{erfc}\left(\sqrt{\tau}\ \Big/\ \alpha\right)\right] \tag{6}$$

Dimensionless variables α and τ include the volumes of the liquid (V_l) and polymer (V_p) and the thickness of the film layer (d_p), parameters which are known or can easily be assessed:

$$\alpha = \frac{1}{K_{p/l}}\frac{V_l}{V_p} \text{ and } \tau = \frac{D_p t}{d_p^2} \tag{7}$$

Using multiparametric solvers, D_p, $K_{p/l}$, and $c_{l,eq}$ can be approximated by fitting experimental extraction data to Eq. (6) (Pahl et al., 2019, Saylor and Young, 2023).

Extraction protocols typically consider a constant surface area to volume ratio for the test item and the extraction solutions, for example kept at 6 or 1 cm^2/mL. This allows scaling for short extraction times (diffusion-controlled extraction). However, scaling is limited for extraction into equilibrium (long-contact time) to PERLs with a small $K_{p/l}$ value (when V_p can be neglected). In all other cases, the polymer volume has to be considered (Jenke and Rabinow, 2017) It should be noted that the magnitude of m_{tot}, the amount of PERL accessible during an extraction, can be estimated from extraction studies. When two different MoCs are combined (e.g. a LDPE bag with a connected PVC tube), a second $K_{p/l}$ parameter will be added to the calculation, altering the PERL concentration-time profile. For example, a PERL with a low solubility in one polymer can have a higher solubility to a second polymer leading to accumulate within this polymer after sufficient contact time. Likewise, the impact of the PERL solubility in solution on migration has to be considered. While a hydrophilic PERL, such as ε-caprolactam, will migrate in both aqueous and organic solutions, a more hydrophobic PERL, like an alkane, will only migrate into organic solutions but almost not into aqueous ones. It should be emphasised that certain solutions or solutes can lead to swelling or even dissolution of the polymer. As extraction studies aim to avoid any swelling, it is not relevant for the film-layer model (Dorey et al., 2018).

Organic suspended matter (OSM) can lead to adsorption of organic molecules including PERLs, often referred to as biosorption. This changes their concentration in solution. In biopharmaceutical manufacturing, OSM can include host cells, cell debris, protein fractions, as well as therapeutic cells, viral vectors, or microcarriers in a CGT cell-culture process. The adsorption of PERLs on OSM is significant for safety evaluations for two main reasons. Firstly, the separation and removal of OSM during downstream processing, e.g., filtration and/or centrifugation, can serve as a terminal sink for PERLs. Secondly, if the OSM undergoes further processing as product in case it is cells and viral vectors in CGT, the PERLs are transferred along with this matrix (Budde et al., 2021) It has been demonstrated that PERLs can impact cell growth but can also be removed from the production fluid during cell harvest (Paudel et al., 2020) The biosorption will be incorporated in the calculation as a reversible process. The biomass-PERL affinity coefficient K_d (Eq. 8) is introduced to quantify the interaction strength. It describes the ratio of the PERL concentration adsorbed on solid biomass c_s and in solution c_l with the unit mL/g. Absorption into the OEM is not considered.

$$K_d = \frac{c_s}{c_l} \tag{8}$$

In bioreactors, the host cells can either be suspended in solution or bound to microcarriers. The first case is assumed for this study. Consequently, it can be assumed that diffusion of the PERL in the cell broth is not kinetically limited and that adsorption equilibrium conditions are achieved in much shorter time than the residence time of the PERLs in the bioreactor (Pagnanelli et al., 2003) The magnitude of K_d for a specific PERL and biomass type can be obtained from the adsorption isotherm and can be considered constant for a narrow concentration range. By modifying Eq. (5) the maximum PERL concentration in solution with biomass $c^*_{i,l,eq}$ can be expressed with the amount of solid biomass m_s to give Eq. (9). The asterisk indicates the presence of two contacting phases.

$$c^*_{l,eq} = \frac{m_{tot}}{V_l + K_{p/l} \cdot V_p + K_d \cdot m_s} \tag{9}$$

The fraction of PERLs remaining dissolved in solution f_{diss} can be defined as: (Schwarzenbach et al., 2002).

$$f_{diss} = \frac{V_l}{V_l + K_d \cdot m_s} \tag{10}$$

Introducing the solid-liquid phase ratio $r_{sl} = m_s / V_l$ one obtains:

$$f_{diss} = \frac{1}{1 + K_d \cdot r_{sl}} \tag{11}$$

This indicates that the equilibrium concentration of PERLs in solution can be reduced by a strong affinity towards the biomass (high K_d) or a high amount of biomass (high m_s). The concentration of PERLs that distribute to the biomass $c_{i,s}$ can be calculated:

$$c_s = (1 - f_{diss}) \cdot c_l \tag{12}$$

The growth kinetics of biomass during cultivation can be described mathematically with logistic and Gompertz functions (Tjørve and Tjørve, 2017) Relevant input parameters are the maximum biomass concentration at the end of the cultivation $c_{bio,\max}$, the biomass concentration at inoculation $c_{bio,0}$, and the growth rate constant μ and the lag phase t_{lag}:

$$c_{bio}(t) = \frac{c_{bio,\max}}{1 + \frac{c_{bio,\max} - c_{bio,0}}{c_{bio,0}} * \exp\left(-\mu \cdot (t - t_{lag})\right)} \tag{13}$$

The incoming flowrate Q_{in} and the take-off flowrate Q_{out} are crucial parameters for any SU device or operation that is perfused or filled. They can be defined by using a mass balance for the respective device:

$$\Delta m_l = c_{l,in} \Delta t Q_{in} - c_l \Delta t \mathrm{Q}_{out} \tag{14}$$

In perfused cell cultures, common description for the media exchange rate is the perfusion rate P, expressed as volumes of media per bioreactor volume per day ($L_{\mathrm{media}} / L_{\mathrm{reactor}}$/day (Bielser et al., 2018)

3. Data sources and model application

3.1. Materials and methods

The extraction protocols and data used as reference were published previously or were retrieved from extractable data provided for the manufacturer, for example, for the SU bag component (Hauk et al., 2021) This SU bag consists of a thin multiple-layered film with a thickness of approximately 400 μm. The main MoC is made from Linear Low-Density Polyethylene (LLDPE), which is also the material in liquid contact. The content of additives in the film, e.g., antioxidants, are below concentrations 1000 ppm and meet the requirements of the European Pharmacopeia [(European Pharmacopoeia (Ph. Eur.) 2017, Jurkiewicz et al., 2014, Blaschczok et al., 2016)] Film samples with a surface-to-volume ratio of approximately 1 $\mathrm{cm}^2/\mathrm{cm}^{-3}$ were used. The samples were gamma irradiated at 50 kGy prior to extraction with pure ethanol at 40 and 60 °C for a total contact time of 120 days. Samples were taken from the extracts after 1, 7, 21, 70, and 120 days and analysed via HPLC-UV/Vis for target screening. The analytical protocol is described in detail elsewhere (Hauk et al., 2021) An K_D value, of 0.02 L/g is applied for the OSM, which is an approximate taken from published values for CHO cells and T-cells in contact with PERLs including the common extractables 2,4-Di-*tert*-butyl phenol (D*t*BP) (Bossong et al., 2023)

For consistency of the units, most volumes and areas of materials and liquids are given in mL or cm^3. A density of 1 g/mL was assumed. The nomenclature, abbreviations and units used in this publication are given in chapter 6.

3.2. Simulation of PERLs in a SU unit operation (storage) with experimental data

A SU bag is addressed in the modelling. Several common and basic assumptions are required for migration calculations: (Limm and Hollifield, 1995)

a) The SU system is a flat sheet containing one layer of the MoC.
b) One dimensional diffusion calculation can be applied.
c) Only one PERL compound is considered. Multi-substance effects on mass transfer exist but are excluded here for simplicity.
d) The PERL is equally distributed within the respective MoC at time t=0.
e) The liquid is ideally mixed and does not permeate into the polymer (no polymer swelling).
f) The PERL is stable and does not decompose during process time and conditions. It is not volatile and does not distribute in the container head space.
g) D_p and $K_{p/l}$, are only a function of the temperature.
h) The polymer is in amorphous state and its properties, D_p and $K_{p/l}$ values, remain constant.

With assumption (f), a reaction or degradation term is not required in the model calculation. Because the magnitude of $K_{p/l}$ will vary with respect to the composition of the liquid media (e.g. by presence of surfactants, salts, organic solvents), it was decided to use values obtained from the experimental extraction data in pure ethanol. This will mimic situations with higher solute propensities of the respective PERL in solution compared to solubilities in aqueous solutions. In most cases, this will overestimate the PERL concentrations under realistic process conditions. Not only will it reproduce worst-case situations, but it will also facilitate the comparison of the simulation results with spiking experiments. As surrogate PERL compound, 2,4-Di-*tert*-butyl phenol (D*t*BP), a degradation product of an antioxidant commonly detected in extraction studies of polyolefins, was chosen (Pahl et al., 2014)

D_p, $K_{p/l}$, z_p and $c_{p,0}$ were obtained from fitting extraction data from chapter 3.1 to Eq. (1). For this purpose, Excel Solver was used which is an optimization tool available within Microsoft Excel™ that allows users to find the optimal mathematical solution by adjusting multiple variables. Table 1 lists all relevant physical parameters and those that were used as input for the simulations.

The storage of cell culture media in a SU bag represents a simple yet relevant process step that can be modelled and compared to experimental data from extraction studies. The system represents a one - or – in case of adsorption - two-box model (Fig. 3). The mass flux of PERLs between the film and the liquid phase depends on the diffusion and partition coefficients and the concentration gradient between the phases. During storage there is no flow through the system and the stored solution shall not contain any impurities during the bag filling step, i.e. $c_{l,t=0} = 0$. The SU device considered is a 2D bag consisting of one layer of LDPE (0.04 cm in thickness) filled with 1.4 L of solution. The LDPE layer contains different leachable compounds but only one PERL representative DtBP is considered (Hauk et al., 2021) For the diffusion layer z_p, lengths of 0.5 μm were assumed. This is based on the Einstein-Smolukowski relation which relates the mean free path of a molecule to the square root of the diffusion coefficient $\overline{x}(t) = \sqrt{2Dt}$ (Einstein, 1905)

3.3. *PERLS simulation for a unit operation with OSM for stagnant and dynamic conditions with limited experimental data*

The measurement of PERLs in a stirred SU bioreactor cell culture suspensions is challenging due to the complex matrix and requires advanced sample preparation techniques, such as a stir bar sorptive extraction (Scherer et al., 2018) Model approaches can be applied to support the analytical test. The simulation of PERL migration in a SU storage bag (chapter 3.2 and 4.1) was extended by parameters that describe the flow in the system, the cell growth and the adsorption of PERLs on the growing biomass.

By keeping the surrogate DtBP and all other data and parameters from section 3.2. constant, the setup allows for a direct study of the impact of sorption on PERLs concentration in the liquid. The interaction between biomass and PERLs affects both, the PERL concentration in solution and the concentration gradient for their release from the MoC (s). For the simulation, a hypothetical OSM phase with a ratio of 50 g/L is considered to be present in the liquid. The following examples show the two-box model for two common cultivation methods i.e., batch, and perfusion mode. The same working volumes were assumed for the batch and perfusion mode examples. In the simulation of the perfusion mode, complete cell retention was assumed. The diffusion constants and the polymer-liquid partition coefficients are taken from the previous chapters and correspond to a process temperature of 40 °C, closely aligning to the physiological temperatures of biological cells (37 °C). The biomass-PERL affinity constant K_d obtained from off-line batch experiments at room temperature is included as described in chapter 3.1 (Bossong et al., 2023) The different temperatures have minimal impact on the K_d value due to its low temperature dependency, as found in other studies (Malik, 2004) For simplicity, no destinction was made between viable and dead cells. Furthermore, the interaction of the PERLs with the biopharmaceutical product, media and other device components was neglected. Process times and maximum viable cell densities (VCD) were selected based on literature (Willard et al., 2017) Table 2 lists all relevant parameters for the simulation.

Table 1
Parameters of the PERL surrogate DtBP and the polymer device used to model a SU storage bag.

	Parameter	Value
Migrating compound D*t*BP	M [g/mol]	206
	$\log K_{o/w}$ [-]	4.8
	D_p [cm^2/*sec*]	2.74×10^{-10} (60 °C) |2.84×10^{-10} (40 °C)
	$K_{p/l}$ [-]	25 (60 °C) | 56 (40 °C)
	$c_{p,0}$ [μg/ cm^3]	120
Device Film	S_p [cm^2]	860
	d_p [cm]	0.04
	V_p [cm^3]	34.4
	V_l [cm^3]	1000
	z_p [μm]	0.5

The maximum wet cell weight $c_{bio,max}$ was estimated from reported cell densities, using an adapted and modifyed empirial correlation of viable cell densities and wet cell weight according to the following formula: (Metze et al., 2020)

$$c_{bio} = 2.76 \frac{\text{mg}}{10^6 \text{ cells}} \cdot VCD + 2.55 \frac{\text{mg}}{\text{mL}} \tag{15}$$

3.4. *Numerical method*

The different equations were solved using the software package of MATLAB 9.1. The algorithm is described below:

1) The process time is split in equal intervals of time Δt from start to finish.
2) Under flow-through conditions, a change of volume in the system V_l is included. The mass gain and loss from the inflow and outflow must be considered for mass conservation (see Eq. 14). The concentration of PERLs in the feed solution $c_{l,in}$ is regarded constant.
3) In each iteration, the mass flux F in the polymer-liquid diffusion layer is calculated numerically based on Eq. (3) using the Forward Euler algorithm with the initial condition $t = 0 \rightarrow c_l = 0$ and considering the partition coefficient $K_{p/l}$.
4) The concentration of the PERL substance in solution and in the polymer is updated after each iteration.
5) In the presence of biomass, the fraction of PERLs that is dissolved f_{diss} is calculated via the solid-liquid phase ratio r_{sl} and the biomass-PERL affinity coefficient K_d according to Eq. (11).
6) In order to account for the change in biomass and thus the amount of potential adsorbent phase during cell cultivation, the biomass concentration is adjusted for every time step Δt using Eq. (13).

4. Results and discussion

4.1. *Simulation of PERLs for a stagnant SU system with experimental data*

The mechanistic *migration model* and the kinetic *film-diffusion model* can replicate the experimental results for extraction times until 70 days and provide a satisfactory approximation for both temperatures when interpolating from $t = 0$ up to approximetly 40 days. During this period, the slope and absolute concentration values do not significantly deviate from the experimental data (Fig. 4). A key difference between the trajectories of Eqs. (3) and Eq. (6) is that they approach different equilibrium concentrations. Unlike the *film-diffusion model*, the *migration model* does not achieve equilibrium within the 120 days of experiments. Eq. (3) cannot reliably be used for extraction times beyond this time frame

The differences between the mechanistic *migration model* and the *film-diffusion model* arise primarily from their underlying assumptions. The *migration model* explicitly accounts for time-dependent diffusion coefficient and material thickness, thus capturing dynamic changes in transport pathways within the polymer matrix. In contrast, the *film-diffusion model* applies a simplified representation of diffusion and partitioning at the polymer–liquid interface, assuming constant parameters and a steady-state flux across a thin diffusion layer. As a result, the *migration model* predicts a slower and more gradual approach toward equilibrium, whereas the film-diffusion model tends to reach equilibrium earlier and at lower concentrations. Importantly, within the

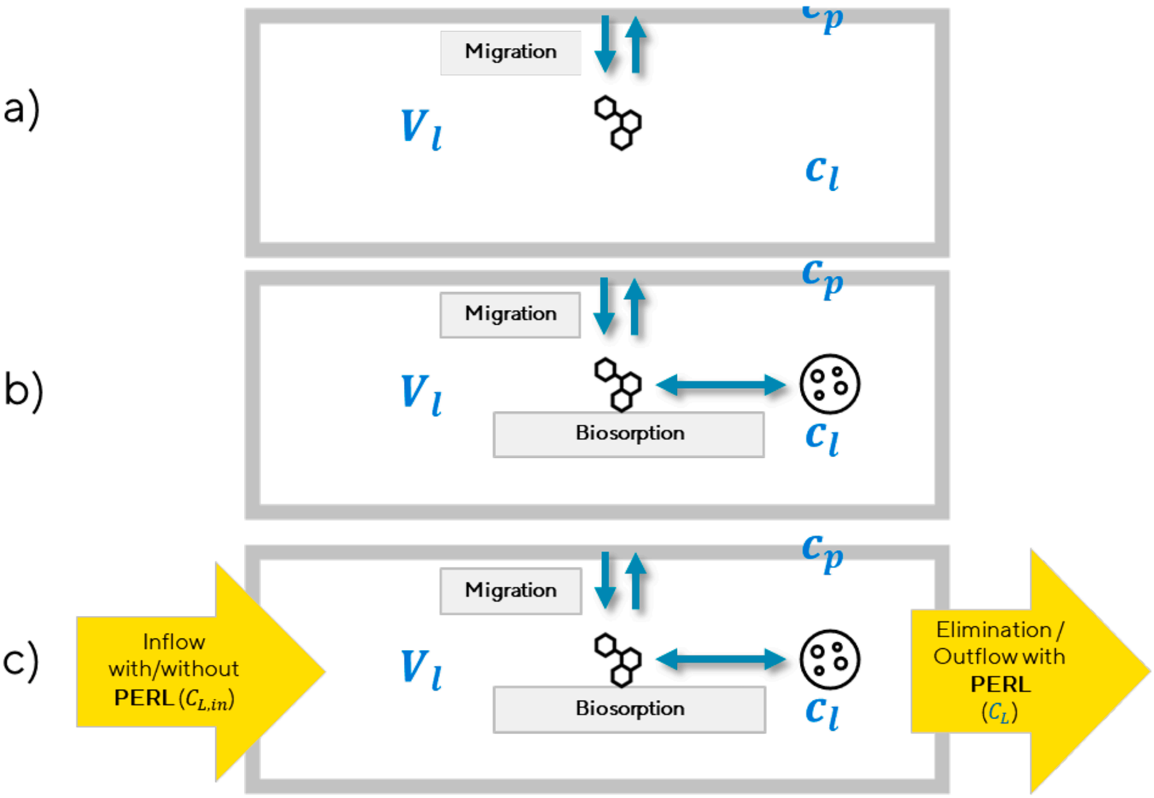

Fig. 3. Box models and relevant parameters for simulation of the PERL sources and sinks for (a) a SU bag (b), a batch bioreactor, and (c) a perfusion bioprocess.

Table 2
Additional input parameters for the simulation of PERL migration in different bioprocesses. Other input parameters are taken from Table 1 (40 °C).

Input parameters	Batch	Perfusion
K_d [L/g] *	0.02	0.02
$c_{bio,max}$ [g/L]	50	50
VCD_{max} [10^6 cells/mL$_{reactor}$]	17	17
$c_{bio,0}$ [g/L]	1	1
μ [1/days]	0.8	0.8
t_{lag} [days]	5	5
Q [mL$_{media}$/day]	0	1000
P [mL$_{media}$/mL$_{reactor}$/day]	/	1

*Data taken from Bossong et al., 2023

relatively large error margins of the experimental data, both models fit the observed trends equally well, particularly at early extraction times where the impact of a time-dependent D_p is limited

The *film-diffusion model* describes the PERL release kinetic fitted to Eq. (6) sufficiently for further evaluation. It resulted in a similar flux at the beginning of the extraction for temperature of 60 °C and a lower flux for 40 °C. This is because the experimental data for 7 days were omitted for the multiparametric fit. Simulated results from the *film-diffusion model* equilibrium reach equilibrium earlier, on a lower concentration level. In dynamic exposure calculations, the initial flux is crucial for determining maximum exposure, whether under steady-state conditions or within a full dynamic timeframe. These states are vital for dynamic safety assessments, whereas evaluating the system equilibrium concentration is less relevant, as equilibrium is unattainable in a dynamic environment.

Based on these simulation results for a stagnant SU system, it can be concluded that the selected input parameters for the given temperatures and for contact times up to 40 days are suited to be adapted to a non-stagnant and perfused systems, as described in chapter 3.3.

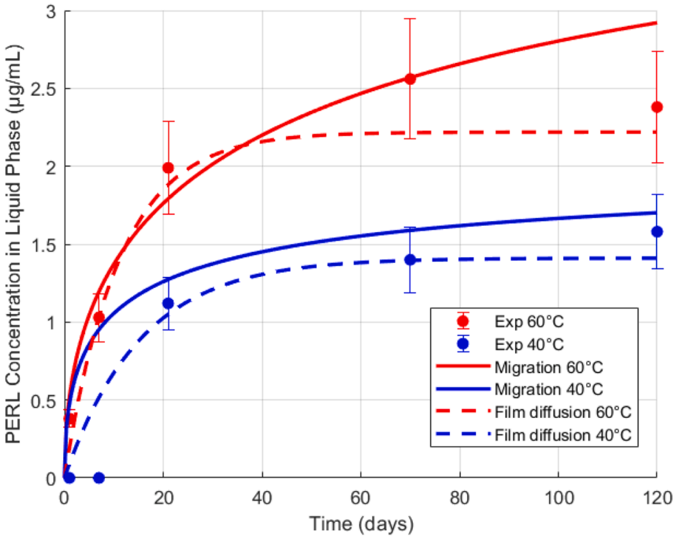

Fig. 4. Simulated and experimental concentrations of DtBP in solution after migration from a one-layer LDPE polymer bag for two temperatures 40 and 60 °C. The initial concentration of DtBP in the liquid was zero. An analytical error of ± 15 % (CoV) is assumed for the experimental data.

4.2. PERLs simulation for stagnant and perfused systems and OSM

4.2.1. PERL concentration stagnant conditions with constant OSM

In the presence of suspended OSM it can be expected that a fraction of the released PERLs is adsorbed leading to a lower effective dissolved PERL concentration compared to a case without OSM. This trend is correctly reflected by the film-diffusion model which aligns with the equilibrium concentration for long contact times $c^*_{l,eq}$ (Fig. 5). The

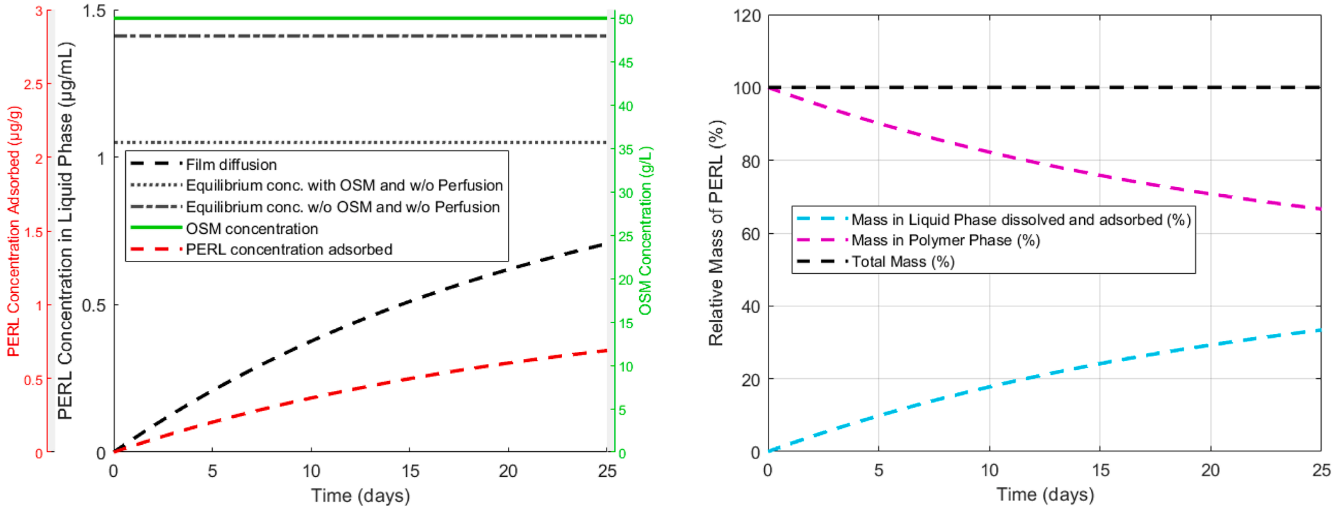

Fig. 5. Temporal development of PERL release from a SU bag in a suspension with OSM. Left: The black dashed curve refers to the dissolved PERL concentration in solution while the dotted lines show the equilibrium PERL concentrations with and without OSM using Eqs. (9) and Eq. (5), respectively. The red dashed curve displays the adsorbed PERL concentration per gram of biomass and the solid green line the OSM concentration. Right: Temporal development of the amount of PERL remaining in the polymer phase (magenta) and released into the cell suspension (cyan).

calculated equilibrium concentration is approximately 1.1 µg/mL for the scenario with OSM while it is 1.4 µg/mL when OSM not part of the system. The mass balance in Fig. 5 shows that approximately a total of 30 % of the PERL is extracted from the MoC.

4.2.2. *PERL concentration stagnant conditions and growing OSM*

In next step, the cell growth is also incorporated into the simulated system. The simulation of the PERL release during cell cultivation in batch mode is depicted in Fig. 6. Compared to the previous case, the growth of biomass impacts the dissolved PERL concentration in solution leading to a decline after 8 days. This is plausible as a high cell growth rate at that time results in more PERLs being adsorbed from the solution than are replenished by release from the material. Towards the end of cultivation, as cell growth slows and most adsorption sites are occupied, further PERL release from the MoC increases the DtBP concentration in the solution. The mass balance in Fig. 6 shows that approximately a total of 30 % of the PERL is extracted from the MoC. This suggests that the final OSM concentration, rather than the growth kinetics, influences the amount of PERL extracted. Eventually, for longer contact times, the system approaches a lower equilibrium concentration $c^*_{l,eq}$ as in the example before (calculated using Eq. 9).

4.2.3. *PERL concentration dynamic, perfused conditions and growing OSM*

Finally, a constant exchange of liquid is included in the modelling of a cell cultivation process in perfusion mode. The exchange of liquid leads to a discharge of PERLs with the outflow resulting in veryl low peak concentration of dissolved and adsorbed PERLs of below 0.1 µg/mL compared to 0.7 µg/mL for the cell cultivation in batch mode, see Fig. 7. For process configurations that contains a higher concentration of dissolved and adsorbed PERLs, it can be assumed that this dilution effect leads to a decrease in the dissolved PERL concentration and via desorption in turn to a decreased concentration of adsorbed PERLs. Compared to the previous modes, this wash-out in the perfused system on the biomass by constant in- and outflow is a beneficial effect. This is especially important if cells are used multiple times after harvest for the production of biopharmaceuticals (so-called “intermediate harvest”) or

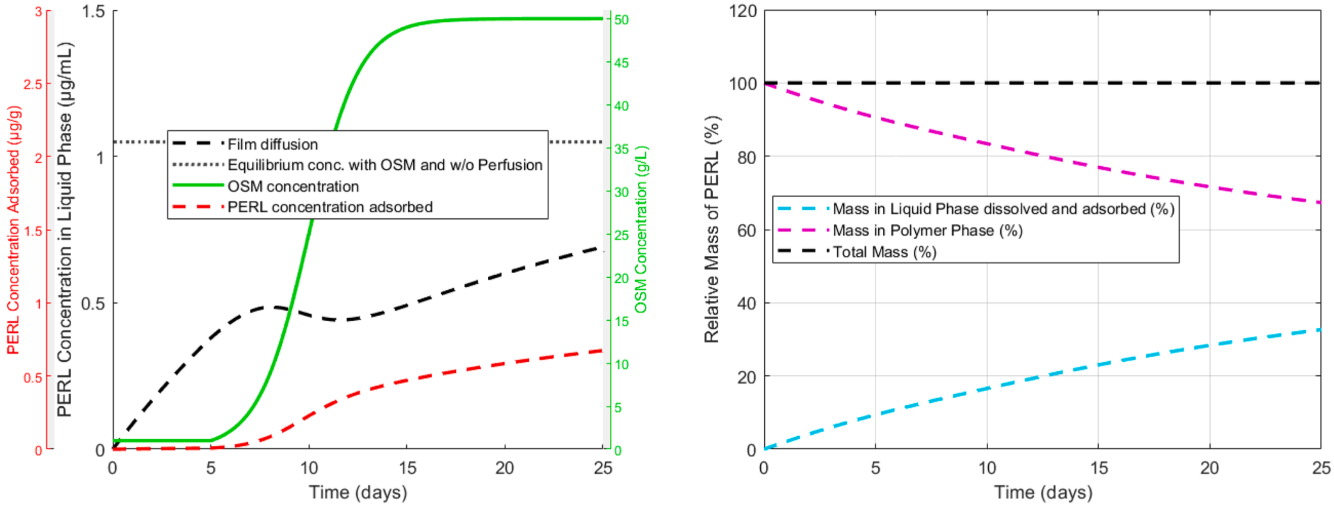

Fig. 6. Temporal development of PERL release from a SU bag during cell cultivation in batch mode. Left: The black dashed curve refers to the dissolved PERL concentration in solution while the dotted line shows the equilibrium PERL concentrations with OSM using Eq. (9). The red dashed curve displays the adsorbed PERL concentration per gram of biomass and the solid green line the OSM concentration. Right: Temporal development of the amount of PERL remaining in the polymer phase (magenta) and released into the cell suspension (cyan).

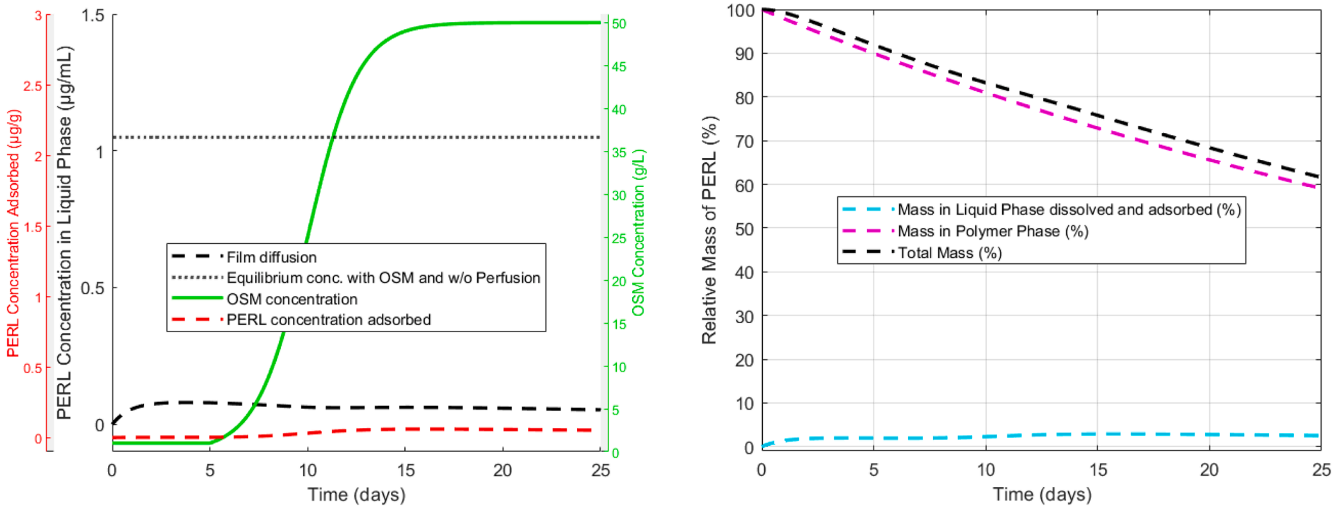

Fig. 7. Temporal development of PERL release from a SU bag during cell cultivation in perfusion mode. Left: The black dashed curve refers to the dissolved PERL concentration in solution while the dotted line shows the equilibrium PERL concentrations with OSM using Eq. (9). The red dashed curve displays the adsorbed PERL concentration per gram of biomass and the solid green line the OSM concentration. Please note that the y-axis limits are slighly shifted to make the low values better visible. Right: Temporal development of the amount of PERL remaining in the polymer phase (magenta) and released into the cell suspension (cyan). The removal of substance wih the outflow leads to lower total mass and thus lower PERL concentration adsorbed on cells and dissolved in solution.

if the cells itself represent the drug product, such as in cell therapy applications). The final PERL concentration in solution of < 0.1 µg/ml remains well below the equilibrium concentration $c_{l,eq}^*$ of 1.1 µg/mL without an applied flow. Considering that perfusion systems are commonly equipped with membrane-based cell retention devices which can act as adsorptive surfaces for PERLs, the PERL concentration in the outflow will be further reduced (Hauk et al., 2018) The mass balance in Fig. 7 accounts only for substances within the SU system (polymer, liquid, and biomass phases) and shows a decline with the applied flow due to PERL exiting the system. It indicates that approximately 40 % of the PERL is extracted from the MoC after 25 days, and for hypothetical longer cultivation times, the total mass, mass in the polymer phase, and mass in the liquid phase (both adsorbed and dissolved) converge to zero. Due to the wash-out effect, less than 3 % of the extracted substance remain in the suspension either dissolved in the liquid or adsorbed to cells for the time frame simulated.

5. Conclusion

This study has shown that the dynamic box model approach is a promising tool to predict the PERL concentration profiles in bioprocesses. In particular, it allows the evaluation of PERLs in process steps, where an analysis of PERLs is not feasible due to the complexity of the process solution. While some input parameters such as the thickness of the diffusion layer are based on approximations, others such as the diffusion and partition coefficients were obtained from extraction experiments under stagnant conditions studying the sources of PERLs only. Application of the box model to storage scenarios leads to predictions that agree well with experimental findings. Consequently, the input parameters were regarded as generally valid and sufficient to be adapted to non-stagnant systems, too.

The input parameters chosen for the simulation of the storage bag and the two modes of cell cultivation vary according to the process conditions and the MoCs used and their dimensions. The diffusion and partition coefficients as well the initial PERL concentration D_p, $K_{p/l}$, z_p and $c_{p,0}$ are the most crucial parameters for the release of PERLs from the MoC. D_p and $K_{p/l}$ are temperature-dependent which was demonstrated in section 4.1. However, for some systems, $K_{p/l}$ may show little or no dependence on temperature. Therefore, while temperature effects can be an important factor, the influence of temperature on $K_{p/l}$ should be evaluated on a case-by-case basis, taking into account the specific material and process conditions.

During cell cultivation, the fraction of PERLs dissolved in solution depends on the biomass-liquid ratio r_{sl} and the PERL-biomass affinity coefficient K_d. As for CHO cell lines used for monoclonal antibody production, the variability of K_d of the most common PERL substances encompasses a range of two orders of magnitude (Paudel et al., 2020, Bossong et al., 2023) Cell densities, and thus the size of r_{sl}, can be very different as well - according to the cell densities in the bioprocess. The simulation of the perfusion process indicates that perfusion rate has the highest impact on PERL concentration compared to the release-specific parameters D_p, $K_{p/l}$, $c_{p,0}$, the adsorptive, and cell-specific parameters K_d or r_{sl}. Considering that the dilution-effect caused by perfusion can be modelled with high accuracy makes such model calculation robust and reliable. Although the variability of some input parameters on the process outcome was assessed, a more general study on uncertainty analysis should be part of future work.

Using the experimentally obtained biomass-PERL affinity constant K_d, allowed to add a sink term to the model and to predict the behaviour of PERLs in bioprocesses under both stagnant and dynamic conditions. As a result, it could be revealed that a significant amount of PERLs can be removed from the cultivation broth due to biosorption. The magnitude of K_d, i.e., the strength of interaction between the PERL compound and the biomass, as well as the solid-liquid phase ratio influence the substance concentration in solution. It should be noted that the K_d parameter was obtained from a batch spiking experiment in buffered solution without complex media components. Further interaction studies of cell culture components will help to better understand the effect on the adsorptive strength.

While it seems beneficial that a large amount of PERLs leaves the process, an impact on the cell productivity and viability cannot be excluded. In cell therapy applications, there is a risk that PERLs may be administered to the patient, which could have unforeseeable effects on therapeutic success and patient health. Perfused systems on the other side, seem to not only reduce the PERL concentration in solution but also the adsorbed PERL concentration on the biomass compared to batch systems. The simulation of a perfused bioreactor showed that the dilution rate of the system terminated by the flow through surpasses the release rate of PERLs from the MoC, making accumulation of PERLs in such systems impossible – neither in the process fluid nor on the cells. Increasingly intense processes, i.e., higher cell densities will further reduce concentrations of PERLs dissolved per liter and adsorbed per wet cell weight. From a risk assessment point of view, this makes bioprocesses with perfusion mode and high cell densities beneficial for both production of biopharmaceuticals as well as for the cultivation of therapeutic cells. Despite the valuable insights from our simulation studies, the results should be validated by probing of real bioreactors during cell cultivation. Possible events not considered in our simulation yet, such as sink effects of cell retention devices, the influence of PERLs on cell growth and viability, the interaction of PERLs with each other and with other compounds in the process fluid, e.g., media components, biopharmaceutical or host cell proteins, might have an impact on the respective concentration-time profile. These factors may also affect the outcomes of the simulation. Further development of the presented model could include such additional effects and lead to even more suitable simulation outcomes.

Thanks to the modular principle of the model, it should also be possible to include the description of further unit operations such as filtration or chromatographic steps and their respective mechanistic models. Future research should therefore concentrate on the proper description on how unit operations downstream to the production stage impact the PERL concentration in the process fluid.

6. Nomenclature

List of symbols*

c_s	Concentration of PERLs adsorbed to biomass [mg/g]
c_{bio}	Concentration of biomass in solution (wet cell weight) [mg/mL]
c_l	Concentration of dissolved PERLs in solution [μg/mL]
$c_{l,in}$	Concentration of dissolved PERLs in feed solution [μg/mL]
c_p	Concentration of PERLs in polymer matrix [μg/mL]
$c_{p,interface}$	Concentration of PERLs in polymer matrix at the polymer-liquid interface [μg/mL]
D_p	Diffusion coefficient in polymer phase [cm^2/s]
d_p	Thickness of polymer layer [cm]
F	Diffusive mass flux [μg/s]
f_{diss}	Fraction of PERLs dissolved in solution [-]
K_d	Biomass-PERL affinity coefficient [L/g]
$K_{o/w}$	Octanol-water partition coefficient [-]
$K_{p/l}$	Polymer-liquid partition coefficient [-]
M	Molecular weight of PERL [g/mol]
m_l	Mass of PERL in suspension (dissolved and adsorbed) [g]
m_s	Mass of solid biomass [g]
m_{tot}	Total mass of leachable compound [μg]
P	Perfusion rate [$mL_{media}/mL_{reactor}$/day]
Q	Volumetric flow rate (feed and outflow) [mL/s]
r_{sl}	solid-liquid phase ratio [g/mL]
S_p	Surface area of polymer [cm^2]
t	Time [s]
t_{lag}	Time of lag phase [s]
V_l	Volume of solution [mL]
V_p	Volume of polymer phase [mL]
VCD	Viable cell density [10^6 cells/$mL_{reactor}$]
$\bar{x}$	Mean free path [cm]
z_p	Thickness of film-layer at the interface [cm]
Indices *i*, *j* and *k* refer to PERLs substances, joined compartments upstream to compartment *k* and the compartment *k* for which the mass transfer is calculated.	
Greek symbols:	
α	dimensionless variable [-]
μ	Biomass growth rate [1/s]
τ	dimensionless variable [-]

*For better comparability and ease of calculations, most units of volumes (e.g., mL) are given as cm^3 or weights (e.g., g) by assuming a uniform density of 1 g/cm^3.

CRediT authorship contribution statement

Maximilian Bossong: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Armin Hauk:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Formal analysis, Conceptualization. **Ina Pahl:** Writing – review & editing, Methodology. **Roberto Menzel:** Writing – review & editing, Methodology. **Peter Langguth:** Writing – review & editing, Supervision, Project administration, Funding acquisition, Conceptualization.

Data availability

Data will be made available on request.

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