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Extractables and Leachables Assessment for Single-Use Systems in Intensified and Continuous Bioprocesses

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Abstract

Efforts to introduce process intensification and continuous processing in biopharmaceutical manufacturing arise from the demand to enhance production capacity in a resource-efficient, sustainable, and economically viable way. This can be achieved by optimizing drug substance and product manufacturing with single-use solutions. However, a dynamic production environment requires additional considerations concerning the qualification of the process equipment. For example, the temporal development of process equipment-related leachables should be addressed. This can be accomplished by specifically developed algorithms capable of predicting their temporal development under kinetic conditions and by applying a production media flow to the system.

Benefits of an Intensified and Continuous Bioprocess

Efficiency and productivity in drug substance manufacturing can be increased by applying intensified and continuous bioprocesses (ICB), while maintaining stringent quality standards required for therapeutic products.[1,2] Combining single-use systems (SUS) in multiple unit operations into a cohesive, intensified system can address both demands, resulting in an enhanced production capacity combined with lower consumption of auxiliary materials and resources. However, to move from batch production, i.e., from process intensification (PI) level 0 to hybrid or even full ICB (Figure 1) with SUS is challenging.[2] In this white paper, we discuss the extractables and leachables (E&L) assessment in ICB, which represents one key element of the qualification of SUS for biomanufacturing processes.

Extractables Assessment and PERL Exposure Estimation

SUS made from polymeric materials are successfully used in the manufacturing processes of various approved biopharmaceuticals and, recently, in the production of advanced therapies and medicinal products (ATMPs).[1,4] This qualification process includes an E&L assessment with the exact scope defined by a previous risk assessment.[5]

For SUS, the first step is conducting standardized extractables studies. The extractables data generated is quantitatively extrapolated to process equipment-related leachables (PERLs) and their projected exposure to host cells, therapeutic cells, proteins, and patients. Finally, safety margins are calculated by comparing the exposure with relevant thresholds and limits (Figure 2).

Figure 1: *Different Levels of ICB from Mclaughlin et al.*[3]

L0 – Standard Batch	Stand-alone unit operation.
L1 – Intensified, Stand-alone Unit Operation	Increases the individual step productivity by, e.g., rapid cycling, multiple columns, in-line buffer generation, operating at higher binding capacity, switching to SU.
L2 – Connected Process	At least two (standard or intensified) unit operations running simultaneously, including pool tanks with varying fill levels. Software orchestration is beneficial. Also called a clustered or linked process.
L3 – Continuous Process	Fully integrated with steady-state flow, small intermediate tanks, software orchestration, long run times, and closed processing. Also called a semi-continuous or pseudo-continuous process.

Figure 2: *Workflow of an Extractables Assessment to Evaluate the Potential Influence of PERLs on Process Performance, Product Quality, and Patient Safety*

Extractables Data
- Quantitative substance list

Expected Exposure
- Host cells
- Products
- Patients

Thresholds & Limits
- EC_{50} values
- PDE-values
- TTC-Values

Safety Margin
- ≥1
- <1

Standardized extraction protocols, such as USP , are established for extractables testing.[6] Typically, defined extraction time points are tested in the extraction study. These are subsequently used for exposure extrapolation, reflecting only static conditions of use.[6–9] In contrast, evaluating PERLs in ICB requires a dynamic extrapolation, and more attention must be paid to PERL exposure as a function of time (t). Besides processing and dwell time, the process media flow—the volume change over time ($V_{l/t}$)—becomes a highly relevant parameter.

An E&L assessment in continuous and intensified production needs to account for PERL exposure changes over time.

Prior Knowledge

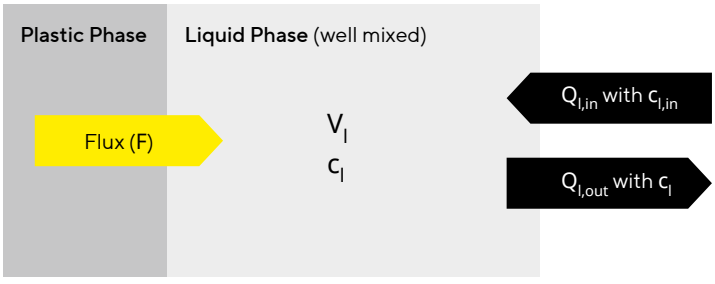
The standardized extraction protocols provide consistent data sets for defined solutions, extraction times and temperature, and SUS surface-to-extraction volume ratios typically obtained for small representatives of the device families.[6,7] The data is made accessible to end-users in extractables reports, named 'Extractables Guides' for Sartorius products and components. For SUS, there are only a few publications on the dynamic PERL release and exposure calculations.[10–12] Algorithms for static exposure calculations (Table 1) are used to leverage the assessment process as shown elsewhere.[11,13] For Sartorius devices, scaling and combining component E&L data can be done in silico with an elegant solution, the proprietary ExSim software.[14,15] All Sartorius extractables data are stored in the ExSim data repository to allow retrieval and aggregate data scaled to respective static in-use conditions.

Exposure Calculation Under Dynamic Conditions

Principles of Dynamic Exposure Calculation

The main processes most relevant to modeling a dynamic exposure of PERLs in a SUS are shown in Figure 3. Here, the SUS is described as a box-shaped compartment with a given volume (V_l), a given polymer-liquid contact surface (S_p) and a defined flow of process liquid (Q_l) through the compartment.[16] The exposure is expressed as the actual concentration of PERL in the system (c_l). For the following discussion, we consider the system volume as constant (V_l = const) without a headspace. Further, an equal in and out-flow is considered $Q_l = Q_{l,in} = Q_{l,out}$. It is possible, that the $Q_{l,in}$ already contains PERLs in a defined concentration ($c_{l,in}$) from previous operations, for example, from a media storage bag. This results in two potential sources of PERLs, the plastic material (migration via the flux, F) and the PERLs already present in the inflow ($c_{l,in}$).

Figure 3: *Dynamic Box-Model of a SUS*



Note. PERLs are delivered by diffusion in and partitioning at the plastic | liquid interface resulting in a release (flux) into the liquid phase over time. Additionally, a flow of liquid through the system constantly dilutes the PERLs. The incoming liquid may already contain PERLs from previous operations.

Table 1: *Algorithms for the Static Scaling and Combination of Extractables Data.*

Contact Times	Single-Use Component		Single-Use Assembly (Multiple Components)	
Short, e.g., 24 h	$c_l = \frac{(\hat{F}_a \times S_p)}{V_L}$	Eq. 1	$c_l = \frac{\sum_{i=1}^{n} (\hat{F}_{a,i} \times S_{p,i})}{V_L}$	Eq. 2
Long, e.g., > 21 days (equilibrium)	$c_l = \frac{C_{p,0}}{V_l / V_p + K_{p/l}}$	Eq.3	$c_l = \frac{\sum_{i=1}^{n} (m_{p,i})}{\sum_{i=1}^{n-1} (K_{i,n} \times V_i) + V_n}$	Eq. 4

A dynamic extrapolation requires balancing sources, here the normalized flux $\hat{F}$ of PERLs and the dilution with the flow ($Q_l/V_l \times c_l$). This can be achieved by setting up differential equations for the temporal development of PERLs in the system (dc_l/dt). Three relevant dynamic exposure scenarios can be established and are described in Table 2. They are independently addressed in the following section.

Table 2: *Three Typical Use Cases for Dynamic PERL Exposure Calculations*

SUS	Devices used for media preparation, storage, or a bioreactor	Perfused devices, e.g., perfusion bioreactors	Flushed devices like single-use filters and tubing
Processes to be Considered	Migration of PERLs from the SUS	Decreasing flux of PERLs from the SUS and a constant dilution with the flow	Constant flux of PERLs from the SUS and a constant dilution with the flow
In-Use Scenario	Dynamic but no flow (stagnant conditions)	Dynamic, low-flow rates	Dynamic, high-flow rates
Differential Equation	$\frac{dc_l}{dt} = \hat{F}(t)$ Eq. 5	$\frac{dc_l}{dt} = \hat{F}(t) - \frac{Q_l}{V_l} \times c_l$ Eq.6	$\frac{dc_l}{dt} = \hat{F} - \frac{Q_l}{V_l} \times c_l$ Eq. 7

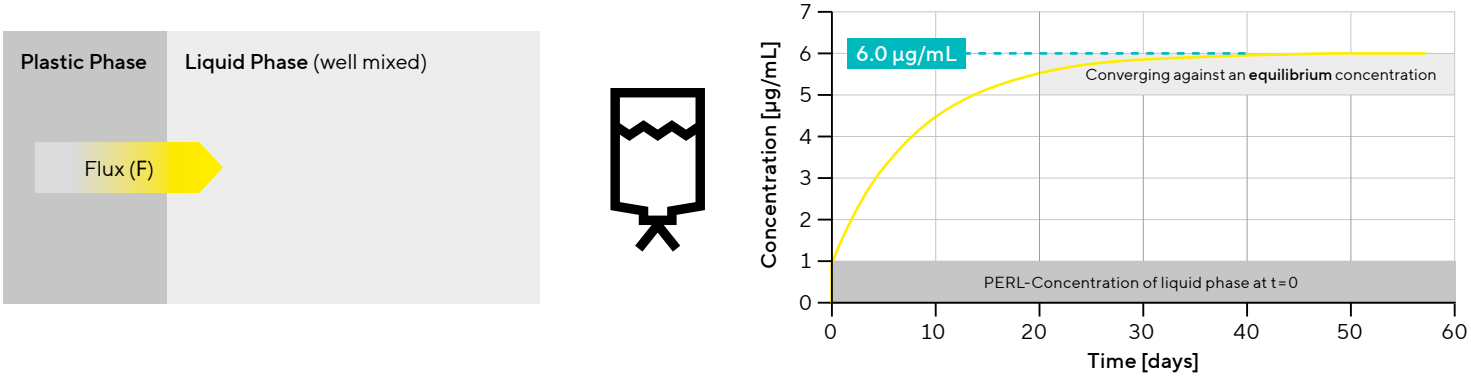
Storage of Liquids in Single-Use Bag Systems With Accumulation of PERLs Over Time

The temporal development of PERLs in single-use (SU) bags is of great interest for the majority of ICB processes (Figure 1) as process liquids and media are stored in these bags before being applied to the process streams. Additionally, they represent a relevant source of PERLs that are introduced into the subsequent process steps. Analytical solutions for **Eq. 5** are already used to calculate the exposure for food-contact materials for notifications in the EU and are used in the FDA's CHRIS model for medical device exposure evaluations.[17–20]

Figure 4 illustrates the typical concentration development over time in a dynamic but stagnant closed system until equilibrium concentration is reached.

In this example, the PERL concentration converges to a maximum of 6.0 µg/mL at phase equilibrium within the system. The equilibrium concentration of an SU assembly manufactured from several SU components can be directly calculated with **Eq. 4**.

Figure 4: *Development of the Concentration of a PERL Over Time in a SU Bag System*



Note. The concentration is calculated with Eq. 5, considering only the migration of the PERL out of the polymer without flow through the system (i.e., dynamic but stagnant conditions). In this example, the liquid used to fill the bag contained already the PERL. Input parameters: $c_{l,0} = 1$ µg/mL, $c_{p,0} = 150$ µg/g, $\alpha = 29$, and $D_p = 1 \times 10^{-9}$ cm²/sec.

PERLs Over Time in a Slowly Perfused SUS

Slowly perfused SUS can be found at different unit operations in ICB. Examples are perfusion bioreactors or SUS systems for continuous virus inactivation. Run times can be up to several weeks with liquid exchange or dilution rates ($Q_I/V_I=k_Q$) of ≤ 1/day for an in-use scenario. Plastics are limited sources of PERLs with an almost exponential decay of the 'extractability' of PERLs from the plastic material over time (Figure 4). Therefore, the migration flux $\hat{F}(t)$ —the release of PERLs— can be approximated with a first-order decay function. The decrease rate of the migration-flux k_F can be incorporated in **Eq. 6**. Together with the migration flux $\hat{F}_0$ at the beginning of the exposure, and $c_{I,in}$ as the PERL concentration in the inflow, the time-dependent PERL concentration can be obtained with:

$$c_I(t) = \hat{F}_0 \frac{e^{(k_F \times t)} - e^{(-k_Q \times t)}}{k_F + k_W} + c_{I,in} \qquad \text{Eq. 8}$$

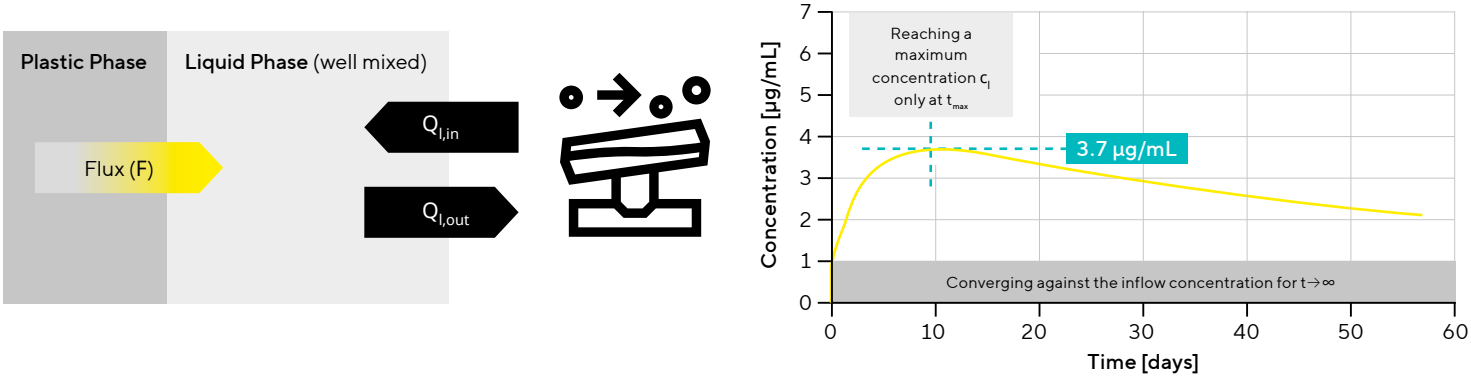
Modeling results are shown in Figure 5. The PERL concentration decreases after reaching an initial maximum ($c_I^{\#}$) and eventually converges to the inflow PERL concentration. This concentration development is explainable and plausible, as the release of PERLs decreases over time, while the diluting flow through the system remains constant. The highest PERL exposure in this system occurs at a time point t_{max} when $c_I^{\#}$ reaches its maximum. t_{max} is obtained from the zero points of the first derivative of **Eq. 8**; with $k_F + k_0 > 0$ we can write:

$$t_{max} = \frac{\ln\left(\frac{-k_Q}{k_F}\right)}{(k_F + k_Q)} \qquad \text{Eq. 9}$$

In our example, the maximum PERL exposure $c_I^{\#}$ is 3.7 µg/mL reached at t_{max} = 9.7 days. This is significantly lower compared to the equilibrium concentration under stagnant conditions (Figure 4) for identical polymer input parameters. For longer operation times, the PERLs released from the bag will be removed entirely from the system (wash-out effect) with c_I eventually converging against the inflow concentration $c_{I,in}$. The trajectories calculated with **Eq. 8** are characteristic of any perfused SUS and valid for any PERL. It should be noted that $\hat{F}_0$ input values can simply be obtained from extractables study data for short contact times, e.g., at t = 24 h.

In perfused systems, the wash-out effect limits PERL accumulation, keeping exposure below that of non-perfused systems.

Figure 5: *Development of the PERL Concentration Over Time in a Slowly Perfused SUS*



Note. A decreasing release of PERL in full dynamic conditions, calculated with Eq. 8, t_{max} = 9.7 days is calculated with Eq. 9. As in the previous example, the liquid entering the SU device already contains the PERL. Input parameters: $c_{I,in}$ = 1 µg/mL, $\hat{F}_0$ = 1 µg/mL/day, k_F = -0.02/day and k_Q = 0.3/day.

PERLs in SUS With High Flow Rates (Rinsing or Flushing)

SUS, such as tangential flow devices or sterile filters and tubes used in fill-and-finish operations, comprise flow rates that are significantly higher compared to perfused systems discussed in the previous section. Considering high flow rates over a short time period of only a few hours allows the flux to be set as a constant value, as opposed to decreasing over time ($\hat{F}$ = const). Integration and using the steady-state concentration (c_l^∞) for the integration constants yields:

$$c_l(t) = c_l^\infty - c_l^\infty \times e^{(-k_Q \times t)} + c_{l,in} \qquad \text{Eq. 10}$$

The steady-state concentration (c_l^∞) can be obtained by determining the zero points of the first derivative $d_{c_l}/d_t = 0$, when PERL release equals its removal. After rearrangement the steady state concentration can be obtained with the following equation:

$$c_l^\infty = \left(\frac{\hat{F}}{k_Q}\right) \qquad \text{Eq. 11}$$

It was previously shown that it is suitable to differentiate between extractables and rinsables, as shown for filtration devices.[12] Extractables are released by migration, while rinsables are a subset of extractables located on the surface of the material, making them directly accessible to the process fluid.

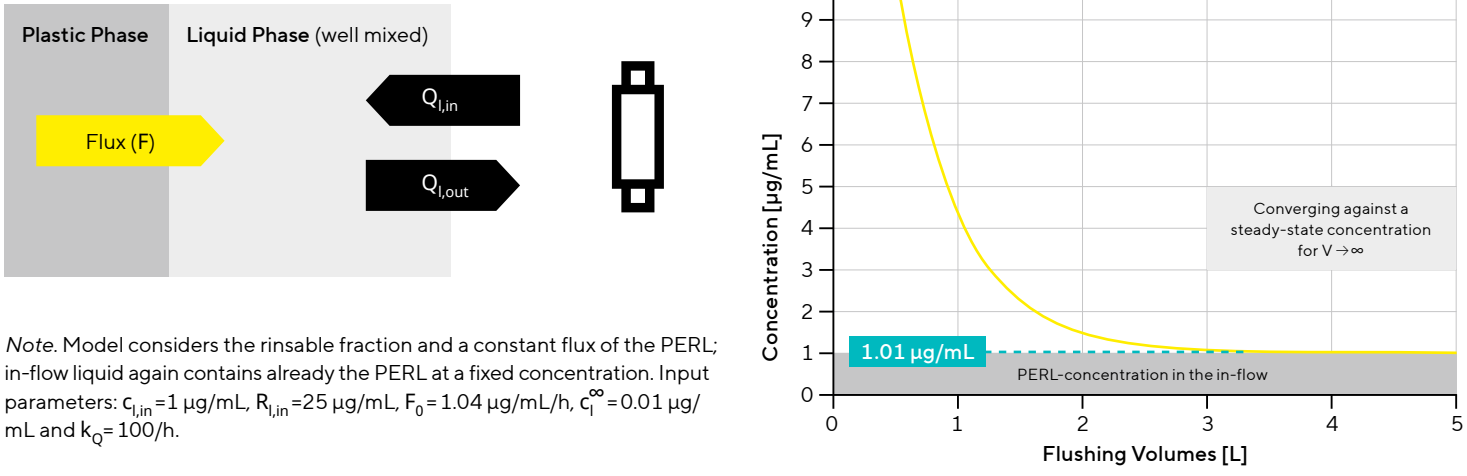
Consequently, rinsible PERLs are instantaneously dissolved and dispersed in the void volume of the filter expressed by introducing an additional dynamic dilution term $R \times e^{(-k_Q \times t)}$ for the rinsables in **Eq. 10**:

$$c_l = R \times e^{(-k_Q \times t)} + c_l^\infty - c_l^\infty \times e^{(-k_Q \times t)} + c_{l,in} \qquad \text{Eq. 12}$$

Notably, determining a PERL exposure with **Eq. 12** reflects a worst-case scenario. It takes the extractables concentration into account twice: first, as rinsables (R), which are just the scaled SUS extractables data, and second, via a constant flux of PERLs from the SU plastics. Transforming **Eq. 12** into a function of the eluate volume rather than time results in "rinsing" or "flushing" curves (Figure 6). Such curves are characteristic of filtration devices and valid for any dissolvable PERL.[12,21] In the example in Figure 6, the eluate concentration converges to the combined steady state of migration-flux and the inflow concentration, resulting in 1.01 µg/mL PERL. As a rule of thumb, it was demonstrated that rinsing a filter with approximately one to two void volumes already reduces the PERLs to concentrations close to the steady state concentration of the respective device.[12]

The flow-through SUS dynamically dilutes PERLs. Since dilution is a well-accepted physical principle, it can be used in risk assessments without the need for additional validation.

Figure 6: *PERL Concentration Depending on Filtration Volume*



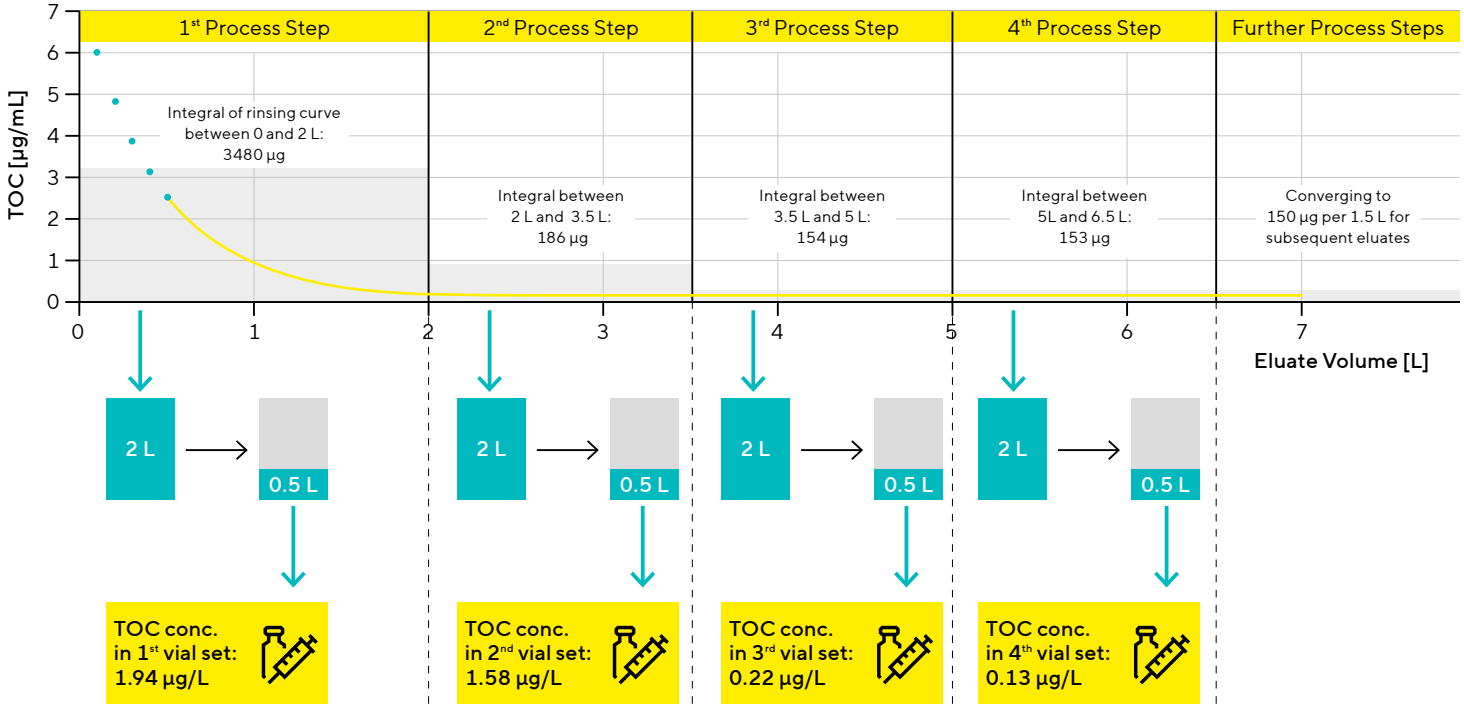
Note. Model considers the rinsable fraction and a constant flux of the PERL; in-flow liquid again contains already the PERL at a fixed concentration. Input parameters: $c_{l,in}$ = 1 µg/mL, $R_{l,in}$ = 25 µg/mL, F_0 = 1.04 µg/mL/h, c_l^∞ = 0.01 µg/mL and k_Q = 100/h.

A Fill and Finish Operation as a Use Case for a Dynamic Exposure Calculation

The last example addresses a continuously operating fill and finish line with a filter and a buffer bag combining the two use cases storage and rinsing with high flow rates. The batch size of the drug product is 300 L, which is filtered through a 10" sterile filter with a void volume of approximately 0.5 L into a 2 L buffer bag. For this example, the total organic carbon (TOC) as a sum parameter of all organic compounds is used instead of an individual PERL concentration. TOC presents an appropriate and often used parameter for rinsing studies, cleaning validations, and extractables studies for aqueous solutions. The sequence of the filling process is as follows: a) Filtration into the buffer bag up to 2 L and intermediate stop of filtration, b) Filling of 1,500 vials with 1 mL from the buffer bag leaving approximately 0.5 L remaining in the bag, c) Filtration into the buffer bag continued until it is filled again to 2 L, d) filling of the 1,500 vials as described before. The procedure is repeated until the entire batch is processed and filled into the vials. The filtration flow rate is approximately 70 L/h.

The TOC development over time for this fill and finish scenario is shown in Figure 7. It was assumed that the drug product (DP) before filtration already contains a TOC of 0.1 µg/mL. The TOC in the subsequent processing steps, filtration into the buffer bags, and vial filling decreases over time due to the flow through the system. The most relevant factor is the early rinsables, which mainly affect the first filling cycle. As the wash-out effect dominates the system, the TOC load nearly converges to the inflow concentration of 0.1 µg/mL by the fourth filling cycle in this example. Such considerations provide valuable insights and can, for example, justify when the filling process should start if rinsing is possible, or determine after which steps the TOC or any other PERL falls below a certain threshold in DP, making it safe for patient use.

Figure 7: *Total Load of PERLs (TOC) in Vials From a Fill and Finish Line*



Note. Repetitive processing scenario to fill sets of 1,500 vials per fill and finish fill cycle. Input parameters: $c_{I,in}$=0.1 µg/mL, R=7.5 µg/mL, c_I^{∞}=0.02 µg/mL, k_Q=144/h.

Conclusion

The E&L assessment of SUS used in ICB can be performed using available extractables data. It requires the inclusion of dynamic PERL exposure calculations for plausible exposure evaluations. In most cases, this can be achieved by including the flow through the SUS and using simple algorithms derived from the basic dynamic dilution equation.

Abbreviations

V_l	Volume of liquid phase [cm^3 or mL]
V_p	Volume of plastic [cm^3]
S_p	Polymer surface area exposed to extraction fluid [cm^2]
$c_{p,0}$	Initial extractables concentration in the plastic [$\mu g/cm^3$]
$m_{p,i}$	Initial quantity of extractables in the phase i [µg]
c_l	Extractables concentration in the liquid phase [µg/mL or $\mu g/cm^3$]
$k_{p,l}$	Partition coefficient between plastic and liquid phase [dimensionless]
F	Flux of compound through an interface [$\mu g/cm^2/sec$]
$\hat{F}_a$	Surface related extractables concentration [$\mu g/cm^2$] after a defined time point
$\hat{F}$	SUS-size and -volume normalized flux [µg/mL/sec]
$\hat{F}_0$	$\hat{F}$ at beginning of the exposure, i.e., derived from extractables data after 24h exposure
t	Time [sec]
k_F	Decrease rate of flux [sec^{-1}]
Q_l	Flow of liquid [cm^3/min]
k_Q	Dilution rate due to flow through system [sec^{-1}]
D_p	Diffusion coefficent in plastic
α	Material ratio in migration equation

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