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On the "Fate of Leachables" in biopharmaceutical up-stream and down- stream processes

Armin Hauk
Sartorius Stedim Biotech GmbH, armin.hauk@sartorius.com

Ina Pahl
Sartorius Stedim Biotech GmbH

Roberto Menzel
Sartorius Stedim Biotech GmbH

Samuel Dorey
Sartorius Stedim Biotech GmbH

Isabelle Uettwiller
Sartorius Stedim Biotech GmbH

On the Fate of Leachables: An Introduction of a Concept to Investigate Leachables with a "Holistic" or System Approach

Armin Hauk, Ina Pahl, Roberto Menzel, Samuel Dorey and Isabelle Uettwiller;
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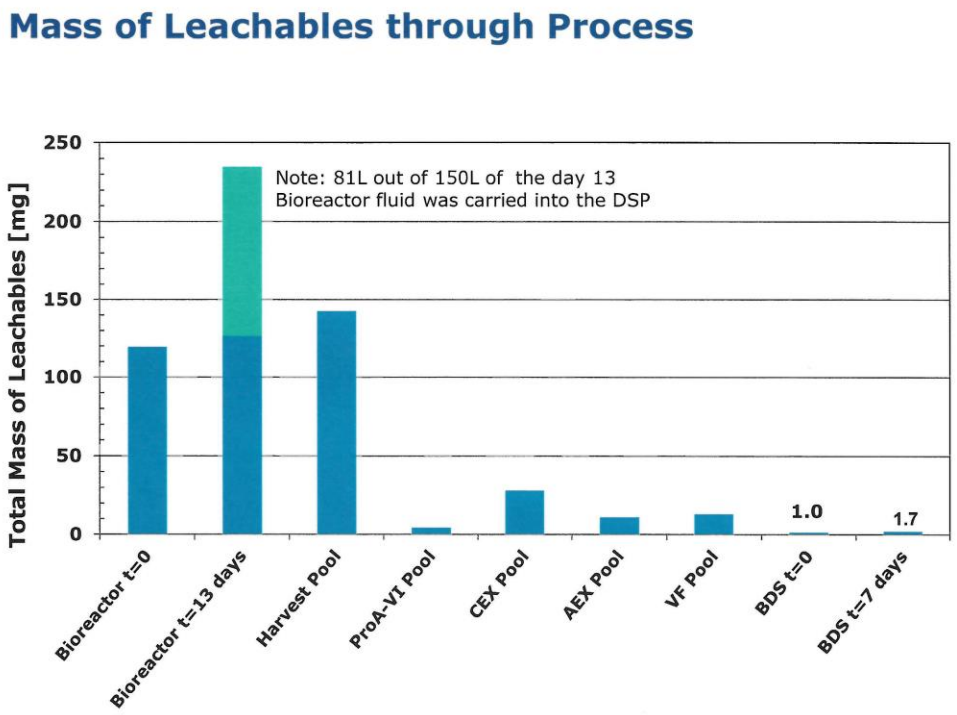
Agenda "On the Fate of Leachables"

- Introduction of the "Fate of Leachables" concept
- Sources of leachables in bio-pharmaceutical processes
- Distribution and sinks of leachables in down-stream process steps ("clearance" of leachables)
- Modelling the "Fate of Leachables" throughout a down-stream process

The current "worst case" approach to predict process leachables

- Today leachables are solely regarded as compounds *released* from polymeric materials into the process liquid; the common understanding is → SUS are sources of leachables
- The typical extrapolation from extractables to leachables (e.g. required in risk assessments) applies simple, conservative and cumulative models to "predict" leachables throughout a process
- The observation in process validation studies stands in contradiction to the above given: Process leachables - although "predicted" to be present in high concentrations - do very often *not contribute significantly to final drug impurities*
- On the other hand our current understanding does not allow a quantitative evaluation of leachables throughout an entire process; missing → proper description of process steps and SUS as sources, the leachables distribution in the process steps and the sinks of leachables (also addressed as "clearance" of leachables)

Leachables load along a process chain; a published example



Jessica Shea (EDM-Millipore) presentation at Rapra E&L-Europe Conference 2016, Dublin

The "holistic" or system approach; the Fate of Leachables concept

- Bio-pharmaceutical processes can be regarded as systems of different coupled sub-systems (bioreactor, centrifugation-, filtration-, chromatography-devices etc.)
- The processes in these sub-systems can be described based on physical and chemical principles (mass flow, dilution & concentration, sedimentation, adsorption & desorption, separation, dissolution & precipitation etc.)
- Leachables are intrinsic elements of these processes
- The sources, the behavior and the sinks of leachables throughout a process should be described analogous to other process related impurities based on process parameters and the underlying phys.-chem. mechanism (→ Fate of Leachables concept)
- Knowing the Fate of Leachables in a given process, a better and more realistic prediction of the leachables concentrations in risk assessments and in planning a reasonable Leachables Study for final products should be possible

Learning form other disciplines; the system approach in environmental engineering

- The environment is regarded as a system of coupled sub-systems or compartments (air, soil, aquifers, rivers, the oceans and biota etc.)
- The processes in the compartments are described based on physical and chemical principles (mass flow, energy flow, dilution & concentration, bioaccumulation, evaporation, sedimentation, adsorption & desorption, separation, dissolution & precipitation, degradation etc.)
- Environmental contaminants are regarded as intrinsic parts of the system
- The fate of environmental contaminants take into account the sources, the distribution and the sinks of these compounds based on the underlying phys.-chem. principles throughout all compartments
- Knowing the fate of the contaminants allows to predict the concentration in the different compartments including biota and potential exposure to humans. It is even possible to describe the fate of virtual compounds, based on estimated phys.-chem. properties
- Any modern risk assessment of chemicals is based on exactly these principle

Sources of leachables from SUS in bio-pharmaceutical processes

Sources are in general the materials of construction, e.g.

- bioreactors and storage bags
- tubes and connectors
- filtration devices
- adsorbents / chromatogr. mat.
- etc.

Leachables from polymers are

- monomers & oligomers
- additives
- processing aids
- reaction products thereof
- plus NIAS*)

*) NIAS: **N**on **I**ntentionally **A**dded **S**ubstances

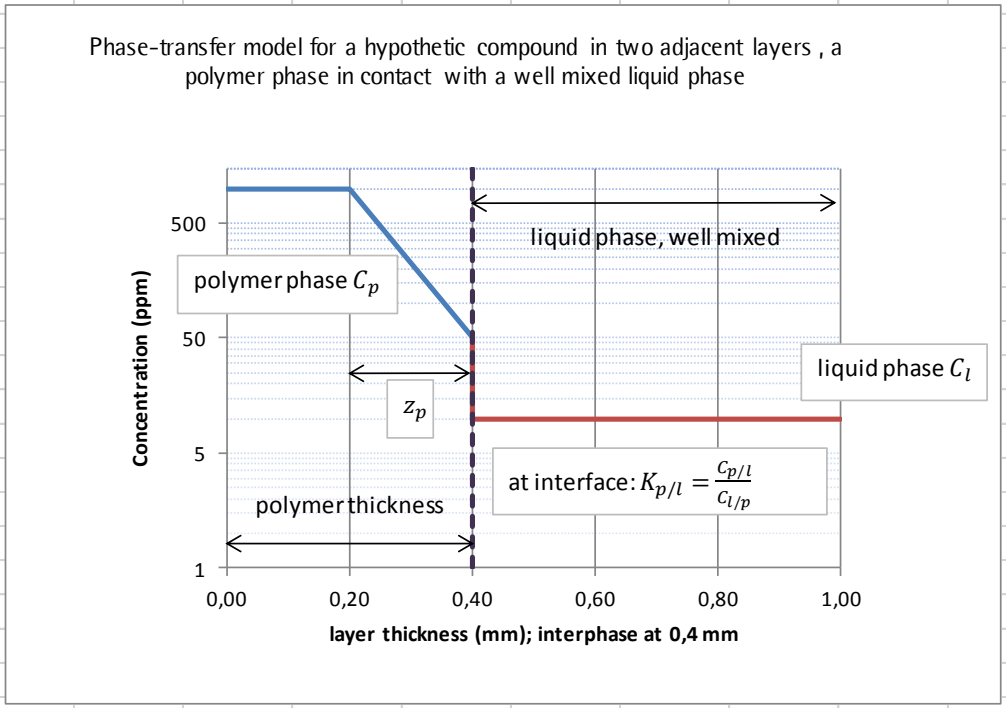
Mechanisms responsible for release of leachables are

- desorption from polymer surface
- solubility in the liquid phase
- diffusion/migration out of the polymer
- partition between polymer phase and liquid-phase

more quantitative information; relevant parameters can be found in literature: e.g. Piringer & Baner (2008)

more qualitative information; good overviews can be found in literature: Jenke (2009), Pahl & al (2014), Marghitoiu & al (2015)

Sources of leachables in bio-pharmaceutical processes; diffusion controlled release of extractables



Ficks 2nd law of diffusion:

$$\frac{\partial c}{\partial t} = D\left(\frac{\partial^2 c}{\partial x^2}\right)$$

Exact mathematical solution for the differential equation (Piringer & Baner 2008 or Crank 1975):

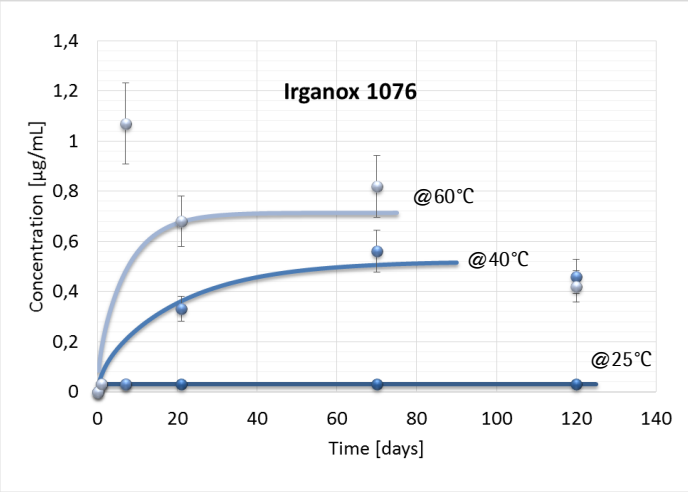
$$\frac{m_t}{m_{tot}} = 1 - \sum_{n=1}^{n} \frac{2\alpha(1+\alpha)\exp(-q_n^2 T)}{1+\alpha+\alpha^2 q_n^2}$$

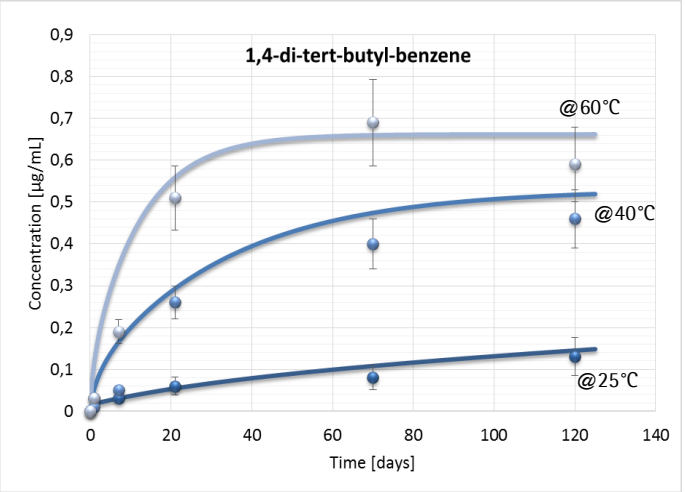
- with $\alpha = \frac{V_l}{V_P K_{p/l}}$ and $T = \frac{Dt}{z_p^2}$
- values for q_n are tabulated

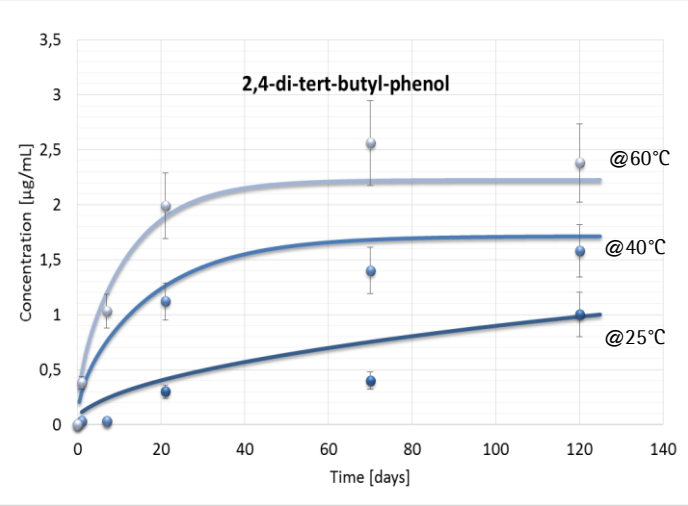
Piringer O.-G. and Baner A.L.: Plastic Packaging; Interaction with Food and Pharmaceuticals 2008; Wiley-VCH, Weinheim, New York

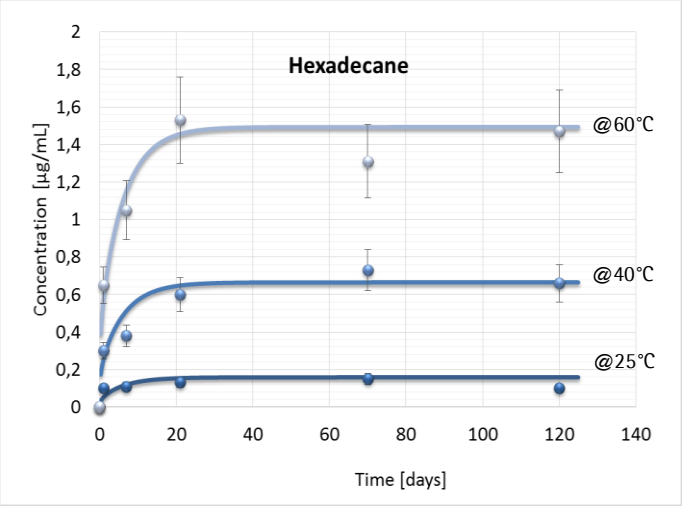
Crank J.: Mathematics of Diffusion, 2nd ed. 1975, Oxford Science Publication, Oxford Univertsity Press

Sources of leachables in bio-pharmaceutical processes; examples for diffusion controlled release of individual extractables









Comparison of measured (dots) and calculated data (solid line) at 25°C, 40°C and 60°C:

- Measurement with GC/MS or HPLC of individual EtOH-extractables, i.e. additives, additive-degradants and alkanes, from a bag.
- Diffusion calculation assuming simplified a LDPE monolayer with 0,4 mm thickness; the partition- and diffusion- constant were taken from literature or were estimated.

Sources of leachables in bio-pharmaceutical processes; summary and outlook

Comparison of measured and calculated extractables data indicates :

- Release kinetics and final equilibrium concentrations in an extraction-experiment can be calculated
- The parameters describing the extractables release and load are diffusion constants for the polymer and equilibrium constants for specific polymer/liquid phase system
- Principle can be transferred to leachables

Therefore, a prediction of leachables released – even under dynamic conditions - should be possible; it requires knowledge of extractables, their diffusion constants in the polymer plus equilibrium partition constants for polymer/process liquids

Sinks of leachables, where is a removal of leachables conceivable?

Sinks are related to process steps, like

- separation
- product isolation
- UF/DF filtration
- sterile filtration
- polishing
- etc.

Mechanisms responsible for removal of leachables are

- split of fractions or phases
- adsorption on materials
- diffusion/migration into a polymer-phase
- partition between liquid phase and solid phase

These sinks of leachables in a process can be considered as,

- scavengers (valid for most adsorptive and partitioning steps)
- terminal sinks (valid for phase separation or UF/DF steps)

Sinks of leachables, what can be anticipated & are there things we already know?

- Chromatographic systems: Application is intended to isolate e.g. a protein from undesired compounds based on different polarity, pKa values, molecular size, chemical moieties – some of these techniques are very selective (e.g. protein-A columns)
- Polymeric contact materials can be regarded as scavenger for hydrophobic compounds; filter-membranes, tubes etc. provide high surface areas for interaction
- Purification systems (e.g. based on membrane-adsorber); this technique is intended to remove undesired components from e.g. a protein solution
- Ultra-filtration / dia-filtration (based on cross-flow principle), this technique is intended to remove undesired components from e.g. a protein solution or replace the fluid system
- Harvest step with the split of protein fraction and cell debris, leachables may be removed adsorbed at the cell surface

Sinks of leachables, experiments to check the scavenger effect of polymeric membranes

SartoScale 47, Sartopore-2 and Sartobran-P, each with 0,45µm+0,20 µm membrane; filter area (nominal surface) is **17,3 cm2**

Materials of construction (membrane, fleece & housing) of the filter is similar/identical to membranes used in the larger Sartopore-2 (PESU) and Sartobran-P (CA) filters

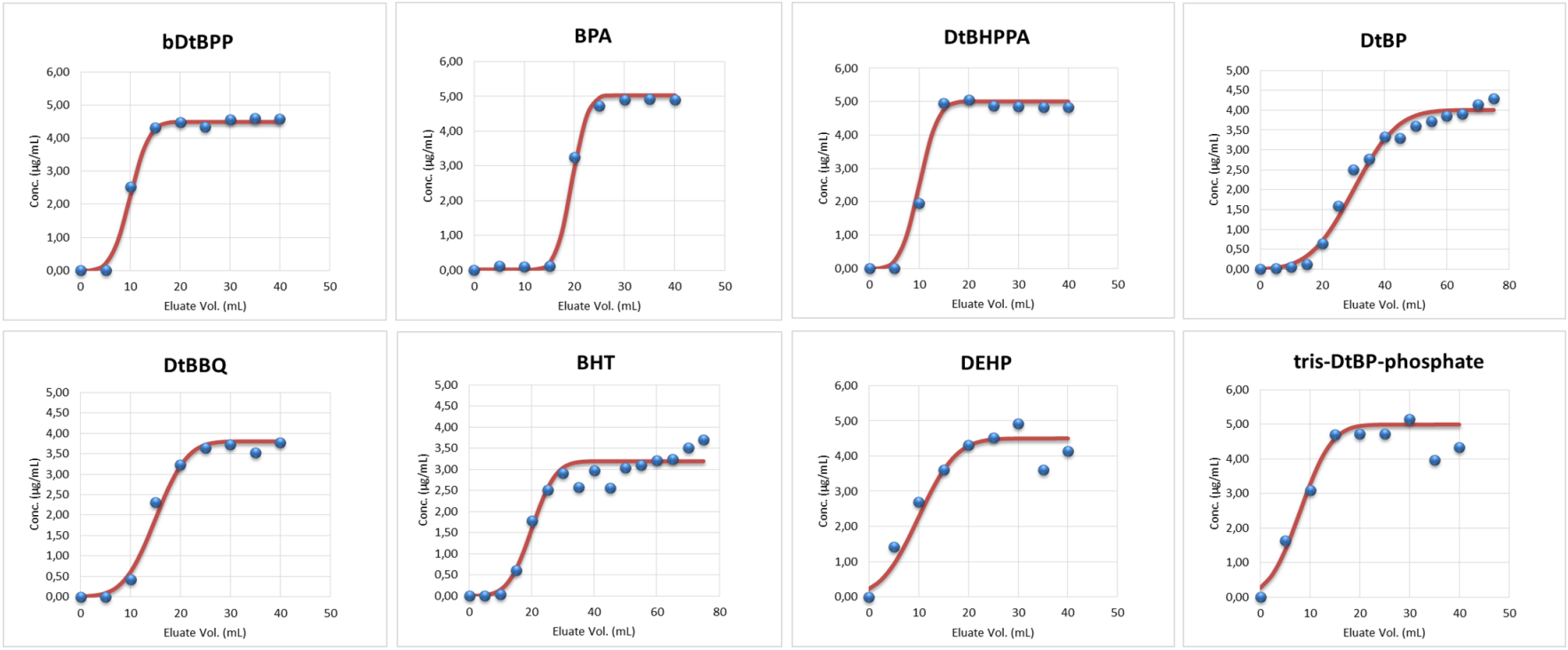
- Test scenario: Preparation of an aqueous buffer solution (plus 10% EtOH) spiked with 5 µg/mL of 8 typical leachables model compounds (LMC-Mix)
- The solution is filtered trough the filters after conditioning of the filters
- During filtration fractions of 1 mL are sampled, directly in Autosampler vials
- The eluate fractions are subjected to a subsequent analysis with HPLC-UV
- After the filtration experiments the filters are rinsed with 10 mL EtOH and analysed (check for LMC substance balance)

Sinks of leachables, experiments to check the removal of leachables

Leachables-Model Compound-Mix (LMC-Mix) containing some typical AO degradation products plus a monomer and a common phthalate – all compounds can easily be analyzed - they represent a variety of molecular weights, polarities and water solubilities including acidic compounds. The abbreviation used for the LMCs in this presentation, their CAS numbers, molecular weights and logKow values are given below

Name	Compound Class	abbreviation	CAS	MW	Log-Kow
Bisphenol-A	Phenolic monomer	BPA	80-05-7	228	3,32 (gestis)
3,5-Di-tert-butyl-4-hydroxyphenyl-propionic acid	AO-degradation product, acid	DtBHPPA	20170-32-5	378	4,77 (echa)
bis-(2,4-Di-tert-butyl-phenol)-phosphate	AO-degradation product, phosphate	bDtBPP	69284-93-1	457	approx. 2,7 (estimated from HPLC RT
2,4-Di-tert-butyl-phenol	AO-degradation product, phenol	DtBP	96-76-4	206	4,8 (calculated, ACD software)
2,6-Di-tert-butyl-1,4-benzoquinonen	AO-degradation product, quinonen	DtBBQ	719-22-2	220	4,42 (calculated, ACD software)
2,6-Di-tert-butyl-4-methylphenol	AO, phenol	BHT	128-37-0	220	5,1 (gestis)
Di(2-ethylhexyl)phthalate	Plasticizer, phthalate	DEHP	117-81-7	391	7,88 (gestis)
Tris-(2,4-Di-tert-butyl-phenyl)-phosphate	AO-degradation product, phosphate	tris-DtBPP	95906-11-9	663	>8 (estimated from HPLC RT)

Sinks of leachables, scavenger effect associated with filtration; results for a PESU membrane filter



Eluate volume versus eluate concentration by filtration of LMC-Mix through a PESU membrane ⇒ filter is an effective & specific scavenger for all 8 LMCs

Sinks of leachables, scavenger effect associated with filtration; results for a PESU membrane filter

Summary:

- Breakthrough volumes can be determined by curve fitting with Gaussian cumulative distribution function (Eq 1); inflection point μ returns the break-through volume

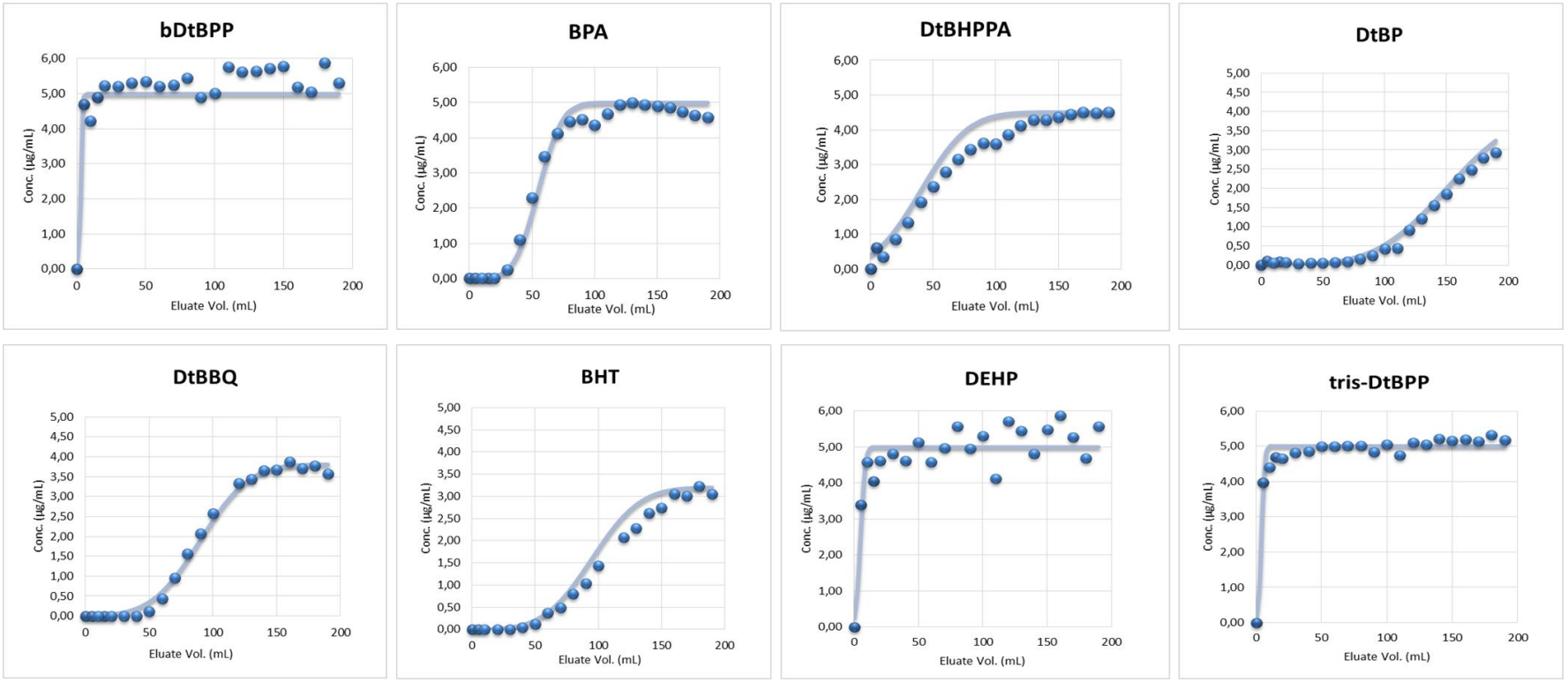
$$F(x) = \Phi\left(\frac{x-\mu}{\sigma}\right) = \frac{1}{2}\left[1 + \operatorname{erf}\left(\frac{x-\mu}{\sigma\sqrt{2}}\right)\right]$$

- Specific scavenger capacity ranges between 3 µg/cm² and 13 µg/cm²
- Recovery (LMC mass balance of eluate fractions plus EtOH rinsing fraction) of LMCs was acceptable - high

Substance from LMC-Mix (abbreviation see slide 14)	Calculated break-through volume (µ from Eq1) [mL]	Specific capacity calculated from rinsing data [µg/cm²]	Total LMC recovery [%]
bDtBPP	10	6,8*)	101
BPA	20	6,9	100
DtBHPPA	10	3,0	95
DtBP	30	11	93
DtBBQ	15	6,5	86
BHT	20	13	96
DEHP	10	5,6	93
tris-DtBPP	10	4,0	93

*) corresponds well to a value of 6,5 µg/cm² determined for bDtBPP determined in a bio-comp. study conducted at SSB

Sinks of leachables, scavenger effect associated with filtration; results for a CA membrane filter



Eluate volume versus eluate concentration by filtration of LMC through a CA membrane ⇒ filter is an effective & specific scavenger for 5 LMCs; 3 others show no or nearly no interaction with CA

Sinks of leachables, scavenger effect associated with filtration; results for a CA membrane filter

Summary:

- Breakthrough volumes can be determined by curve fitting with Gaussian cumulative distribution function (Eq 1); inflection point μ returns the break-through volume

$$F(x) = \Phi\left(\frac{x-\mu}{\sigma}\right) = \frac{1}{2}\left[1 + \operatorname{erf}\left(\frac{x-\mu}{\sigma\sqrt{2}}\right)\right]$$

- Specific scavenger capacity ranges between 0 µg/cm² and 45 µg/cm²
- Recovery (LMC mass balance of eluate fractions plus EtOH rinsing fraction) of LMCs was acceptable - high

Substance from LMC-Mix (abbreviation see slide 14)	Calculated break-through volume (μ from Eq1) [mL]	Specific capacity calculated from rinsing data [µg/cm²]	Total LMC recovery [%]
bDtBPP	<5	0	101
BPA	55	17	97
DtBHPPA	40	20	97
DtBP	150	45	96
DtBBQ	90	26	81
BHT	95	33	83
DEHP	<5	11	115
tris-DtBPP	<5	1,0	96

Sinks of leachables, experimental set-up to check leachables adsorption effects on HIC membrane-adsorber

LMC-Mix is applied to the device

Purging filter with air - then rinsing with 100% EtOH

Step 1: Filtration of LMC-Mix

Step 2: Rinsing solution is pressed through the filter to desorb the adsorbed LMC

Eluates are sampled for analysis

Rinsing eluate for analysis (HPLC-UV)

- Experiments with HIC membrane adsorber (Sartobind Phenyl; Pico 0,08 mL) with a similar experimental set-up as above but adjusted to adsorption capacity of the device
- Application conditions (i.e. buffer and salt concentration) are similar to a *polishing (flow through)* or a *protein desorption* step

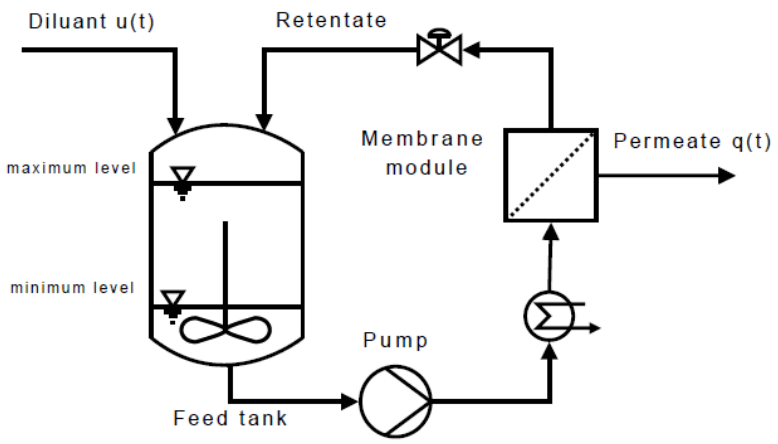
Sinks of leachables, results of experiments to check leachables adsorption effect on HIC membrane-adsorber

Summary:

- HIC membrane adsorber is an effective & specific scavenger for 7 LMCs; only one compound showed nearly no interaction with HIC adsorber membrane (i.e. DtBHPPA)
- Specific absorption capacity ranges between 17 µg/cm³ and 290 µg/cm³ (bed-volume)
- Recovery (LMC mass balance of eluate fractions plus EtOH rinsing fraction) of LMCs was acceptable – high; for some compounds (e.g. bDtBPP) rinsing step has to be optimized

Substance from LMC-Mix (abbreviation see slide 14)	Specific capacity calculated from rinsing data; referenced to bed-volume of the tested device [µg/cm³]	Total LMC recovery [%]
bDtBPP	64	64
BPA	28	101
DtBHPPA	17	105
DtBP	140	97
DtBBQ	110	76
BHT	>190	81
DEHP	>290	88
tris-DtBPP	>250	87

Sinks of leachables, experiments published demonstrating the removal of leachables with UF/DF (cross-flow)



Experiments from Magarian & al (2016) and Jahn & Stebler (2016):

Test of removal of leachables with permeate, dilution in retentate; mathematical expression with the "dia-filtration-equation":

$$C_l = C_l^0 \times e^{-z(V_{add}/V_0)} \qquad \text{with } 0 < z < 1$$

Retention factor z = 0 for compounds which remain 100% in the retentate (e.g. the target molecule); z = 1 for compounds which are perfectly diluted and removed with the permeate

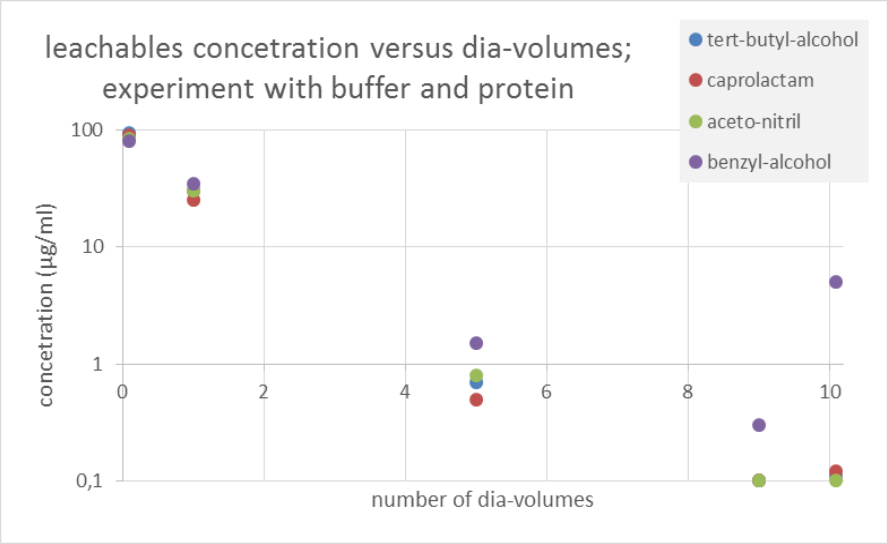
Magarian & al (2016): Clearance of Extractables and Leachables from Single Use technology via Ultrafiltration/Diafiltration; AIChE Publication

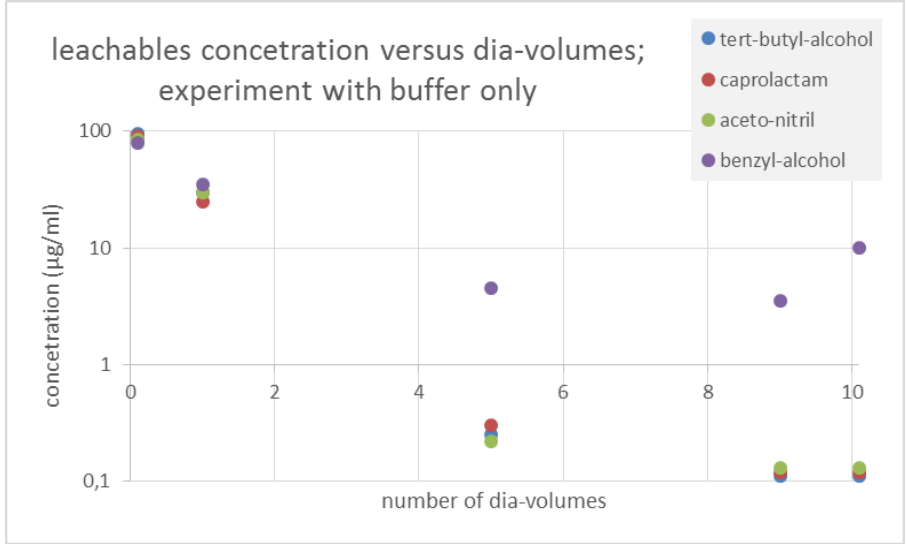
Jahn & Stebler (2016): The Fate of Leachables During Biotechnol. DS Downstream Processing; Rapra E&L Europe Conference; Dublin

Kovacs, Fikar, Czermak (2009) Mathematical modeling of dia-filtration; Hungarian Journal of Industrial Chemistry

Sinks of leachables, experiments published demonstrating the removal of leachables with UF/DF (cross-flow)

- Fig. below with example of results from Magarian & al (2016): Removal of investigated compounds in buffer and protein solution are obvious (log-scale plot!)
- Jahn & Stebler (2016): Leachables removal depends on Kow value of leachables and potentially on protein adsorption





Magarian & al (2016): Clearance of Extractables and Leachables from Single Use technology via Ultrafiltration/Diafiltration; AIChE Publication

Jahn & Stebler (2016): The Fate of Leachables During Biotechnol. DS Downstream Processing; Rapra E&L Europe; Conference; Dublin

Sinks of leachables in bio-pharmaceutical processes; summary and outlook

Review of scavenger effects and the existing publications indicates :

- There are sinks of leachables, some of them seem to be quite efficient (i.e. considering the high surface area of filter-membranes and adsorber in a multi-step bio-pharmaceutical down-stream process
- The parameters describing leachables-sinks are, scavenger- or adsorption capacity, UF/DF-dilution rates and partition-coefficient between different phases

Therefore, prediction of leachables removal – even under dynamic conditions - should be possible; it requires knowledge of extractables their distribution- and adsorption-constants

Modelling the Fate of Leachables; the concept

Quantitative description of sources of leachables for contact materials or devices

Quantitative description of distribution of leachables for devices and/or process steps

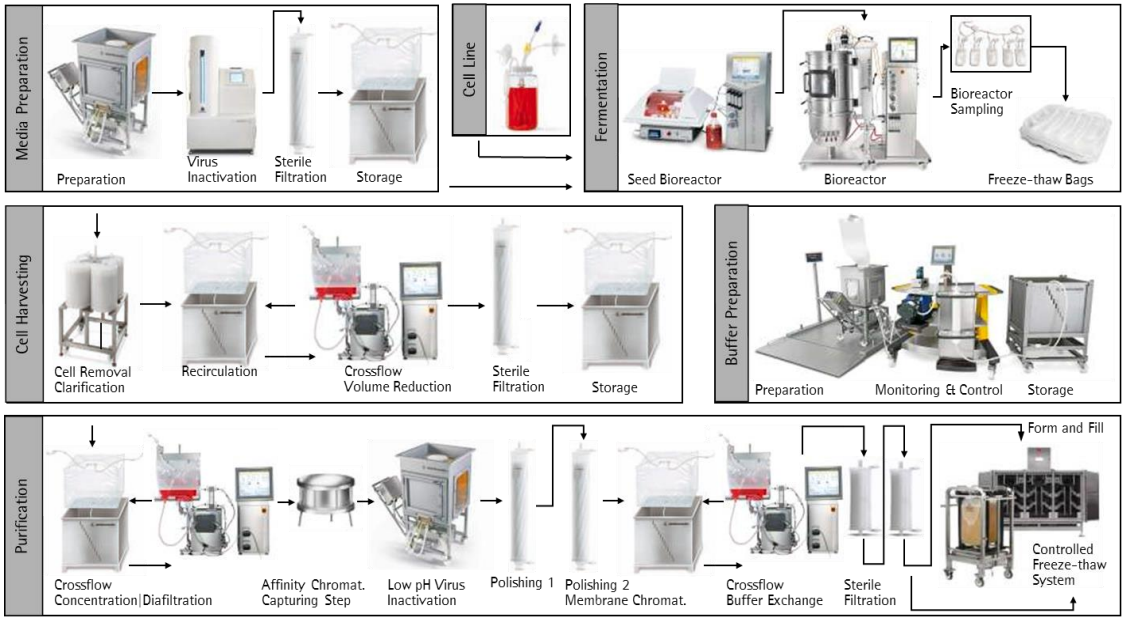
Quantitative description of sinks of leachables for devices and/or process steps

Quantitating the load of leachables throughout an entire process using a model approach

⇒

Fate of Leachables

Modelling the Fate of Leachables; the principle approach



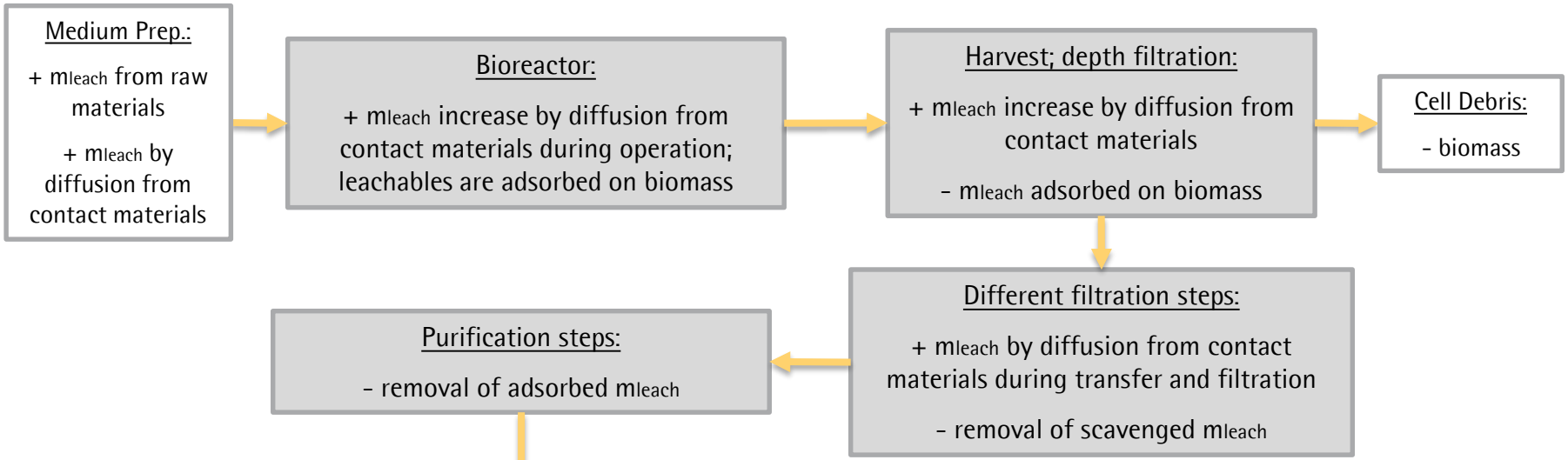
A bio-pharmaceutical process:

- It is a well defined system of components (devices) and subsequent process steps
- Process steps are dynamic but carried out in closed systems
- The boundary conditions for all process steps are well known (e.g. volumes, composition, mass flow, temperature etc.)

Applying the Dynamic Box Model approach to a bio-pharmaceutical process:

- The different process steps (devices) can be described as individual compartments
- In each compartment sources, distribution and sinks of leachables can be calculated or modelled based on the underlying phys.-chem. mechanism
- Strict mass balance conditions have to be applied for all processes in all compartments
- Exchange between compartments or discharge can be modelled with the flow of liquid phases

Modelling the Fate of Leachables; structure of a dynamic box model



In each compartment (box) the $\Sigma_{leachables}$ are calculated with phys.-chem. methods:

- Diffusion from contact materials
- Adsorption on materials (biomass, membranes etc.)
- Dilution and/or concentration e.g. in cross-flow steps

Modelling the Fate of Leachables; required model input data

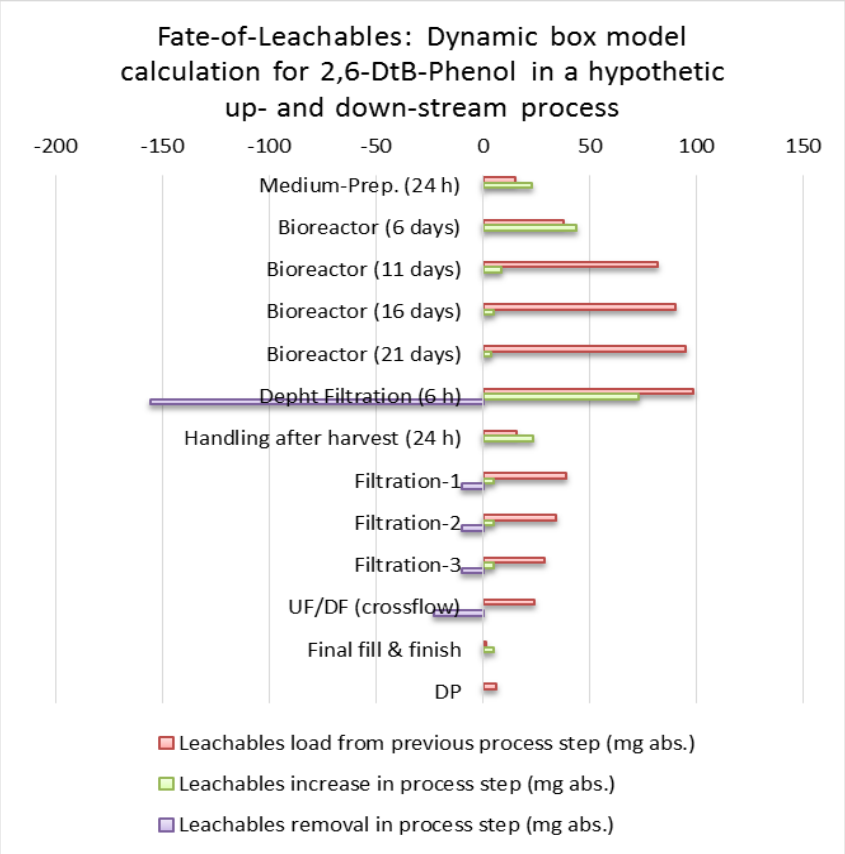
Input data for the different compartments (corresponding to process steps and/or devices):

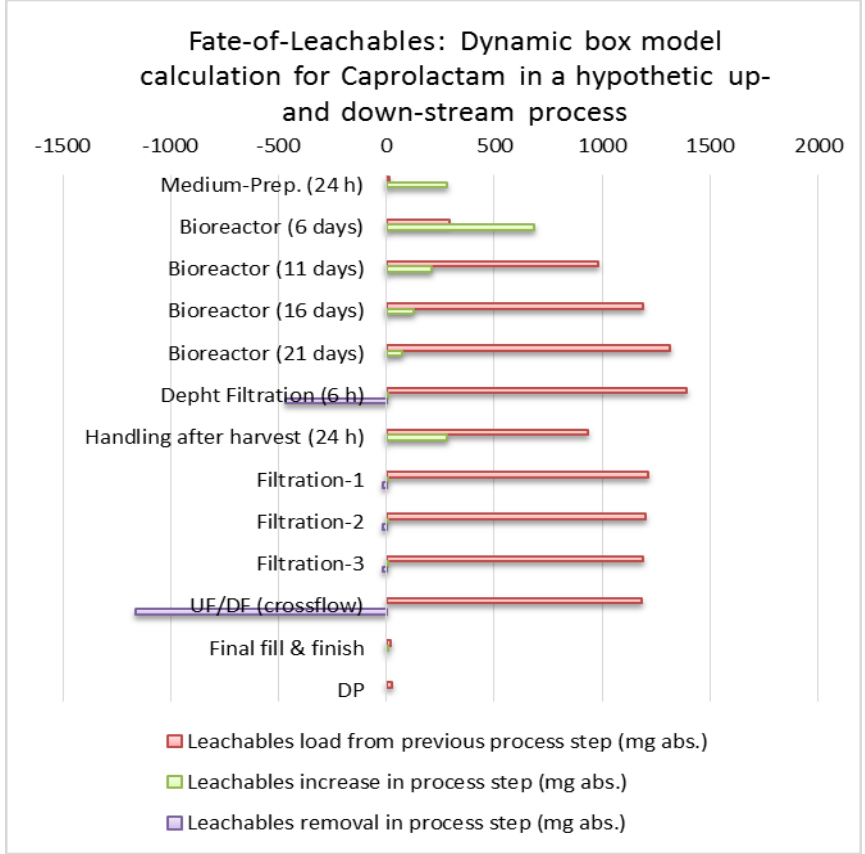
- Total volume (or mass) of liquid phase
- Total mass (or volume) of polymer phase
- Thickness and surface area of polymer
- m_0 of leachables compound in polymer
- Biomass in bio-reactor
- Mass and/or surfaces of adsorbents
- Temperature and dwell time
- Number of dia-volumes in UF/DF steps

Input data for Leachables compound:

- Diffusion constants (D)
- Partition coefficient between polymer and liquid phase ($K_{P/L}$)
- Partition coefficient between biomass and liquid phase ($K_{D\text{-}bio}$)
- Specific capacity of filters and purification devices (Kap_{fitr})
- The UF/DF-factor

Fate of Leachables; modelling results for the dynamic box model





Model input data for the leachables 2,6-DtB-Phenol (as a additive degradant):

D =	2,0E-10 cm^2/s	$K_{P/L}$ =	1000
$K_{D\text{-}bio}$ =	100	Kap_{fitr} =	5 µg/cm^2
UF/DF-factor =	0,5		

Model output for 2,6-DtB-Phenol:

m_{DP} = approx. 6 mg

Model input data for the leachables Caprolactam (as a film/laminate monomer):

D =	8,0E-10 cm^2/s	$K_{P/L}$ =	1
$K_{D\text{-}bio}$ =	5	Kap_{fitr} =	5 µg/cm^2
UF/DF-factor =	0,7		

Model output for Caprolactam:

m_{DP} = approx. 28 mg

Fate of Leachables investigations; summary and outlook

- The current understanding that SUS and devices are solely sources of leachables in combination with a risk assessment build on cumulative extrapolations to process leachables stands in contradiction to empirical findings in leachables measurements
- Of course SUS are sources of leachables, but it is easily conceivable – and could be shown experimentally - that there are also distribution processes and sinks of leachables in down-stream processes → Fate of Leachables
- Investigating the Fate of Leachables requires a holistic or system approach based on the underlying phys.-chem. mechanism (diffusion, adsorption, dilution, concentration, phase separation etc.)
- First attempts to apply a dynamic box model for a hypothetic process to estimate the Fate of Leachables for 2,6-DtB-Phenol and Caprolactam showed that a quantitative prediction of the load of leachables is possible, which reflects the empirical findings quite well

- Further research is required:
  - To complete and fine tune the model (e.g. to better define the compartments, boundary conditions, to include phase separation steps and/or reaction of leachables).
  - Studying the influence of proteins on leachables and leachables-protein interaction.
  - Model validation comparing model results and measurement in technicum-scale

Fate of Leachables investigations; acknowledgement

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- Samuel Dorey
- Isabelle Uettwiller
- and our lab teams

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- Kate Lee (Genentech)
- Michael Jahn (Lonza)

Questions and Discussion

Dr. Armin Hauk

Lead Scientist E&L

Phone: +49.551.308.1189 | Fax: +49.551.308.2062

Mobile: +49.160.6421.345