Basics in Single-Use Bioprocessing

→ Single-Use Bags

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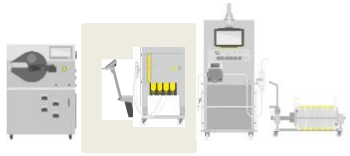
Drug Manufacturing Process

Upstream

Media Preparation

Cell Culture

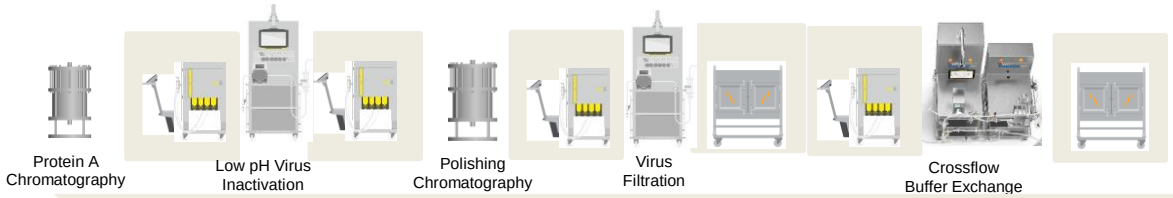


Cell Harvest/Removal/Clarification

Crossflow/ Volume Reduction

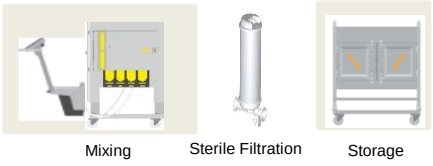
Downstrea

Buffer Preparation



Purification Steps

Form & Fill



Excipient/Buffer Preparation

Final Formulation

Final Filling

Stainless Steel vs. Single Use

picture: stainless steel

- Sterilization, cleaning
- Possible cross contamination
- Low flexibility

→ Sterilized, only hardware-cleaning

→ Avoid cross contamination

→ High flexibility: set of different designs

→Critical: extractables & leachables

Picture stainless steel [News and innovation in the pharmaceutical manufacturing industry (fluidhandlingpro.com)]

Challenges

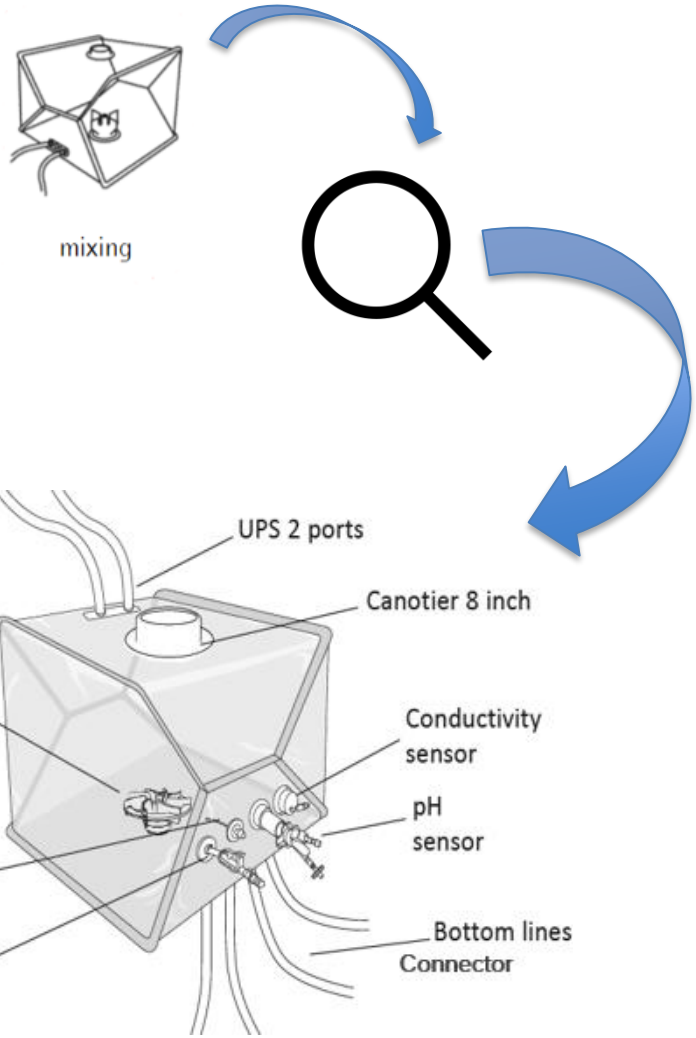
- **Supply chain**
  for SUS is complex and significantly increases the reliance on supplier quality system, change control & business continuity
- **Biocompatibility**
  industry reports on variability of cell growth performance in bags
- **Purity**
  Unexpected E | L can lead to production stop
- **Integrity**
  bag failures often combine a lack of robustness with difficult manipulations

Bag manufacturing

Bag details

- Complex assembly of **different components** made of **different material**
- HDPE, PE, EvOH, PP, EVA PSU, LLDPE, Si,
- Film material

- Extractables & Leachables



Bag manufacturing

Extractables & Leachables

Extractables

- Extractables are **compounds that can be extracted from a container closure system**,
  drug **packaging component** or any other **contact surface**.

  This **extraction** is happening during **harsh conditions**
  such es **heat**, **extraction solvents**
  or any other **highly-concentrated buffers or solutions**.

  Extractables **can be potential Leachables**.

Bag manufacturing

Extractables & Leachables

Leachables

- Leachables are **compounds that passively migrate or leach into the drug product over time** as a result of direct contact of the drug formulation with the surfaces (packaging, container closure system, etc.)

  Leachables are generally **a subset of Extractables** - but not always.

Bag manufacturing

Extractables & Leachables

- Tests and studies usually **done by the manufacturer**
  results stated in validation / E&L guides for the customers

  Sartorius provides **in house studies** and **risk assessment** via an extractable simulator.

- Customers do their **own tests**

- **independent laboratories**

Bag manufacturing

Extractables & Leachables

- **Typical solvents** – examples

1M Hydrochloric Acid	1M Sodium Hydroxide	4M NaCl
1% Polysorbate 80	Ethanol	WFI
10 % Dimethyl sulfoxide (DMSO)	4M Ammonium Sulfate	

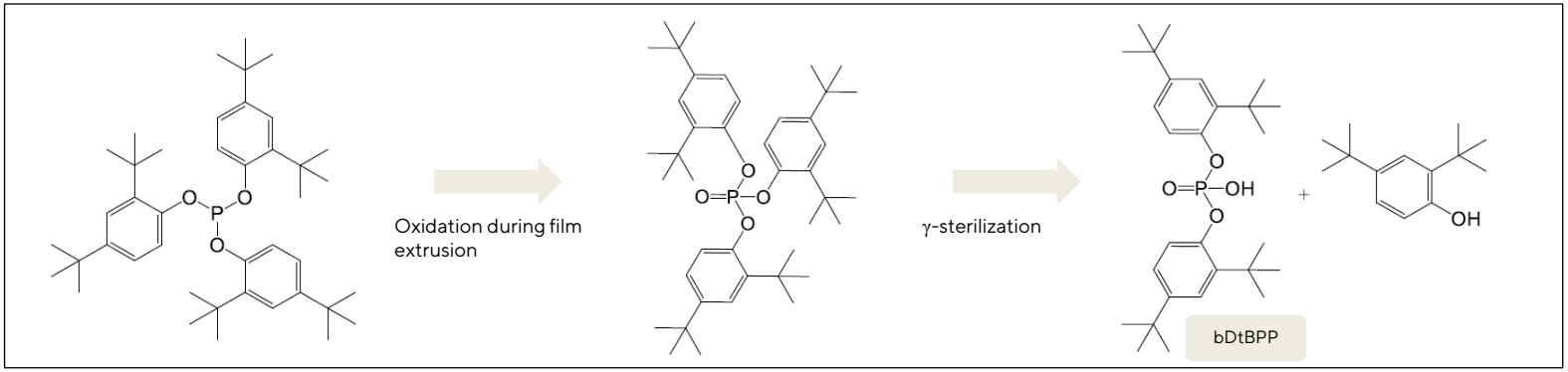
- **Test method** e.g.:

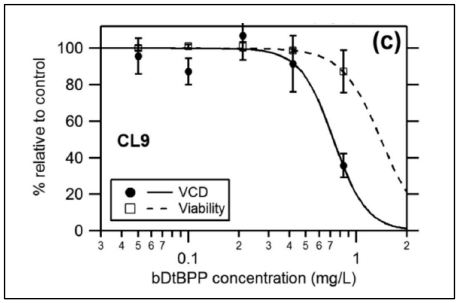
  gas/liquid/ion chromatography

  & mass spectrometry & flame ionization detector

- **E&L examples:** Silicon -> anti blocking agent for PE
  Linear and branched alkane -> from PE resins

Leachable Studies from Bags Show that Trace Amounts of Degradation of an Antioxidant Can Impact Cell Growth





- The Tris (2,4-di-tert-butylphenyl) phosphite is a common antioxidant used for PE resins to protect the films from oxidative degradation during extrusion, γ-radiation and storage.
  - Referenced in the European Pharmacopoeia
  - Irgafos 168® is one of the trade names
- The bis (2,4-di-tert-butylphenyl) phosphate (bDtBPP), a breakdown product is a common leachable released from bags and have been shown to be detrimental to cell growth*.

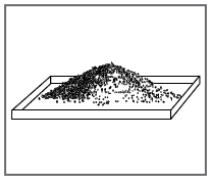
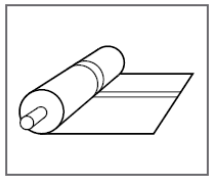
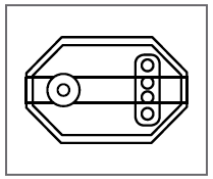
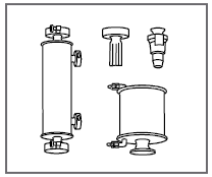
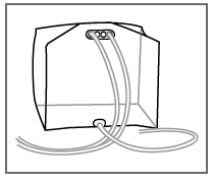
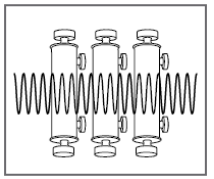
* Ref - Matthew Hammond, Heather Nunn, Gary Rogers, et al., Identification of a Leachable Compound Detrimental to Cell Growth in Single-Use Bioprocess Containers, PDA J Pharm Sci and Tech 2013, 67 123-134.

Bag manufacturing

Production Process

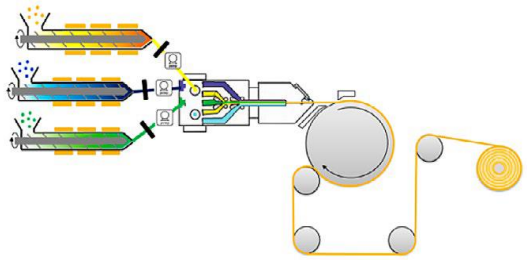
Gamma and x-ray sterilization
(Supplier) Integrity Testing
Point of use Leak Testing

Resin ▶ Film ▶ Bag ▶ Component ▶ Assembly ▶ Sterilization

Material Control | Process Control | Quality Control

DoE on Critical Film Extrusion
Process Parameters



3 orthogonal parameters

- Extrusion temperature –melting of raw materials
- Chill roll temperature –cooling of the film
- Extrusion Speed

Manufacturer documentation:
Extractable & Leachable guide
Validation guide

Bag manufacturing

Film material

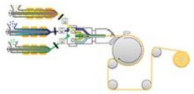
Two main types of film materials on the market
regarding product contact

- EVA → ethyl vinyl acetate
  - Main characteristics: robust, elastic but poor barrier to gases
  - Well known, long time on the market

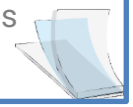
- PE → polyethylene
  - Main characteristics: robust, flexible, good water vapor and alcohol barrier properties
  - Well known from packaging industry and clinical use

Specifications and control of raw material

Control of the critical process parameters

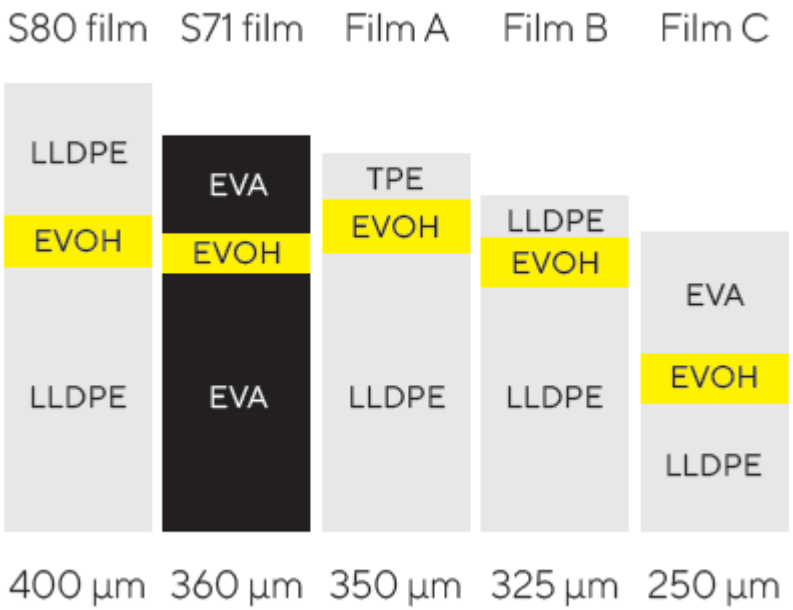


Specifications and control of film quality attributes



Bag manufacturing

Film material



- EVOH → ethyl vinyl alcohol
- LLDPE → linear low-density polyethylene
- TPE → thermoplastic elastomer

Bag manufacturing

Film material

Process & application requirements

- Robustness
- Ease of use
- Biocompatibility
- Purity
- Cleanliness
- Compatibility
- Adsorption
- Stability
- Sterility

→

Raw material science, film & bag expertise

S80 film | PE 400 µm

Backbone	LLDPE	Flexsafe® Bags & Cultibags
	EVOH	
Contact Layer	LLDPE	

S71 film | EVA 300 – 360 µm

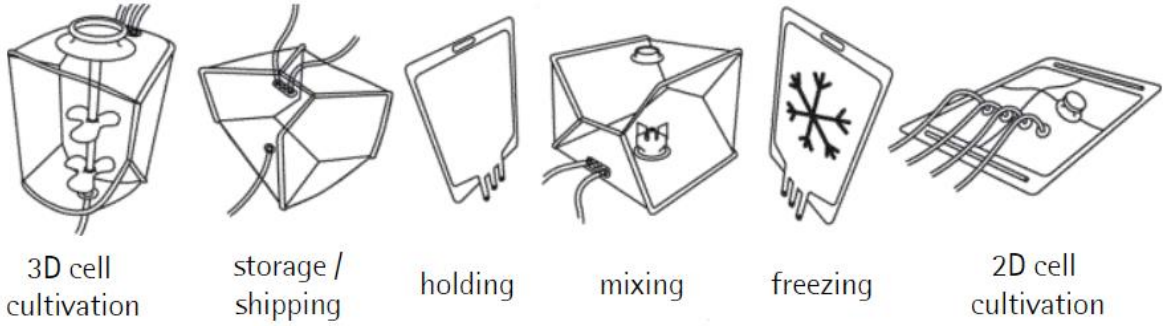
Backbone	EVA	Flexboy® & Celsius® Bags
	EVOH	
Contact Layer	EVA	

→

Bag quality attributes

- Strength
- Flexibility
- Weldability
- Cell growth
- Extractables
- Particles
- Gas Barrier
- Chemical resistance
- Bioburden

Single-Use bag types & Hardware → live demonstration



References

- Pictures from Sartorius if no reference is stated below the picture
- Sartorius documentation: data sheets and application notes, further information
  → contact tanja.sedlacek@sartorius.com or visit www.sartorius.com for more details

Thank you.