Impact of material and geometry on pH stability of CO_2-buffered media for cell therapy applications

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Congrès / Congress

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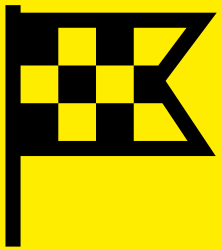
Institut Charles Sadron, Strasbourg

25-26 mars
25-26 march
2026

With the support of:

Objective

1	Introduction to CAR T-cell culture and **complementing storage in PET bottles with flexible polymer bags**
2	Explore **sustainability challenges**
3	Understand the impact of **polymer materials & geometry** on cell culture media
4	**Feasibility study** on optimizing pH stability and sustainability



Agenda

Example: Introduction to CAR-T cell Therapy

Sustainability

pH stability optimization in cell culture media

- CO2 diffusion
- Surface to Volume ratio
- Sterilization

Feasibility study

Conclusion



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Example: Introduction to CAR-T cell Therapy

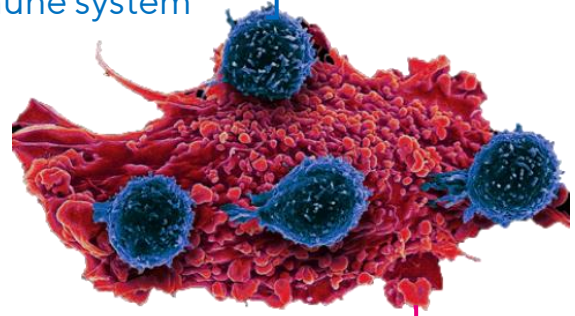
Cell culture media storage

Media for cell culture are used for mABS and CAR-T cell therapy
CAR-T cell therapy example : a breakthrough in cancer treatment

T-cells are genetically modified to target and destroy cancer cells

T-cell
= white blood cells
essential to the imnune system

- Patrol the body
- Eliminate harmful cells



Cancer cell

CAR-T cells persist and proliferate in vivo, targeting cancer cells

Example: Introduction to CAR-T cell Therapy

Cell culture media storage

Traditionnally, Sartorius supplied cell culture media in PET (polyethylene terephthalate) bottles and bags

→ Chemical stability, low gas permeability, and long-term storage
→ Easy to handle and store
→ Compatible with a wide range of media formulations





Example: Introduction to CAR-T cell Therapy

Cell culture media storage



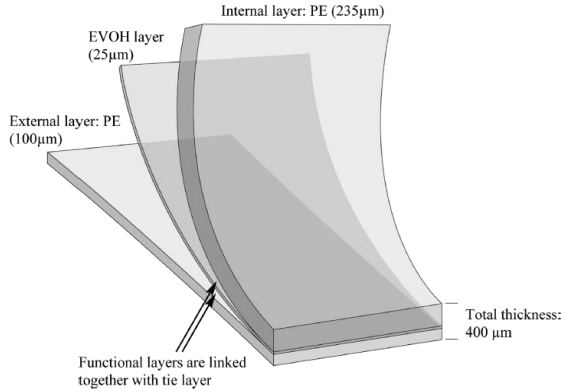
Media can be supplied in sterile flexible bags to bioreactor workflows

→ to facilitate direct transfer of media into bioreactors **while in closed system**

→ reducing handling steps and contamination risks

Flexible bags are made from multilayer films to balance:

✓ gas barrier properties
✓ mechanical strength
✓ compatibility with irradiation sterilization



PE/EVOH/PE or EVA/EVOH/EVA:

- **PE (Polyethylene) or EVA (Ethylene Vinyl Acetate)** →flexibility, weldability
- **EVOH (Ethylene Vinyl Alcohol)**→gas barrier

Thickness: 300-400 µm

Example: Introduction to CAR-T cell Therapy

Cell culture media storage

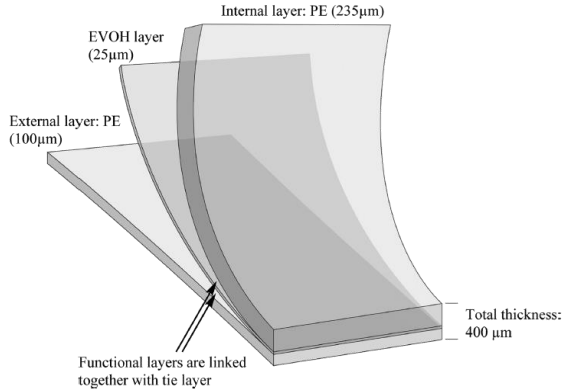


Advantages of using flexible bags

1. Sterilized (X ray or gamma irradiation)
   → aseptic transfer of media into bioreactors in **CLOSED SYSTEM**

2. Reduces handling steps

3. Flexible bags are scalable
   → ideal for different cell production scales



PE/EVOH/PE or EVA/EVOH/EVA:

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PET bottles vs flexible bags

	PET bottles	VS	Flexible bags
Plastic Waste & Circularity	▪ 50–100 g of plastic per liter of media ▪ Technically recyclable		▪ About 10–30 g of plastic per liter ▪ Multilayer films can be separated and recycled ▪ PE/EVOH/PE can be provided with ISCC+ certification (upon request)
Carbon footprint	▪ **PET bottle**: slightly lower than PCF of films* ▪ Transport: heavier than flexible bags → higher fuel consumption per unit volume		▪ **PE/EVOH/PE or EVA/EVOH/EVA:** PCF per kg of film is around 10% more than the PCF per kg of PET bottles* ▪ Flat-packed bags reduce shipping volume by 60%

CF = Carbon Footprint
PCF = Product Carbon Footprint

ISCC+ = International Sustainability and Carbon Certification

** According to estimations made in Sphera LCA for Experts software. The values are also in the range of the uncertainty, so it is not possible to state that the CF of bottles is clearly and effectively lower than the one for films.*

Regulatory and industry trends

Single-Use (SU) systems

- Align with BioPhorum guidelines for sustainability in biopharma
- FDA/EMA encourage SU for reduced contamination risks and scalability

FDA/EMA Guidelines on ATMPs (2022/2023)

FDA = Food and Drug Admnistration
EMA = European Medecine Agency

BioPhorum is an international collaboration network that brings together major biopharmaceutical manufacturers, suppliers, and industry experts to align on best practices, accelerate innovation, and harmonize standards across the biopharma industry

Sartorius' Commitment

- SBTi validated targets for 2030 (mid-term)
- Current initiatives: closed-loop recycling programs with biopharma partners

SBTi= Science Based Targets initiative

https://www.sartorius.com/en/company/sustainability

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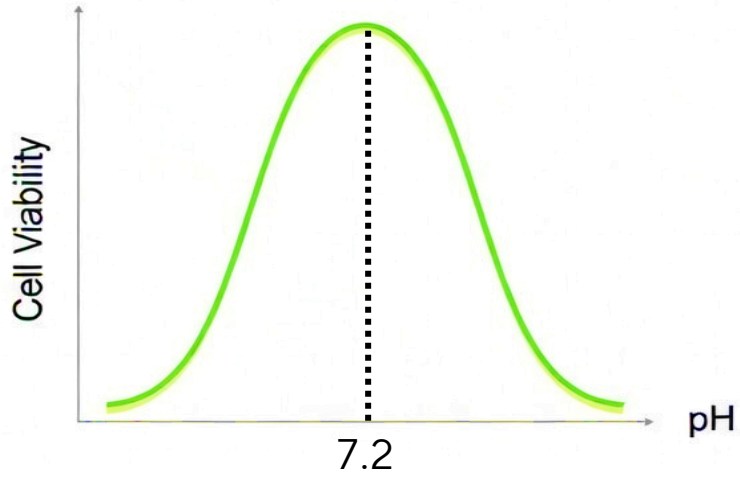
CO_2 diffusion

Media and CO_2 buffer

Cell culture media depends on optimal conditions, particularly a stable pH

T-cells require stable pH for viability and functionality:

- **pH < 7.2:** acidic conditions → **cellular stress and metabolic dysfunction**
- **pH > 7.4:** alkaline conditions → **disrupted enzyme activity, membrane transport, and receptor**



Buffer system
Bicarbonate (HCO_3^-/CO_2): pKa = 6.1 → sensitive to CO_2 fluctuations
→ maintain the pH within the physiological range (7.2–7.4) by neutralizing acids

Even minor deviations (±0.2 pH) can compromise CAR-T cell therapy

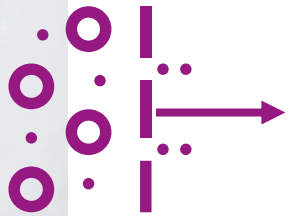
CO_2 diffusion

Phenol Red is a dye used in cell culture media → **changes color according to [H^+]**





pH	Color	Interpretation
< 6.8	Yellow	Acidic medium
7.2 – 7.4	Red	Optimal physiological pH
> 7.6	Purple	Alkaline drift



Purple zones indicate pH increase
→ **CO_2 diffusion out of the media**

Root cause → Material CO_2 permeability

Transition from PET bottles to flexible bags, while reducing contamination risks

→ need configuration optimization for pH stability

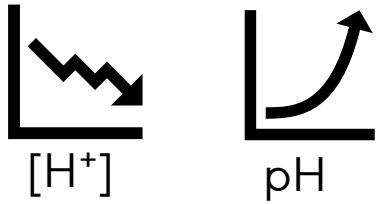
CO_2 diffusion

T-cell culture media generally rely on CO_2 equilibrium:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

Concentration of dissolved CO_2 determines pH stability ⟶ $pH = -\log_{10}[H^+]$

If CO_2 diffuses out of the media:



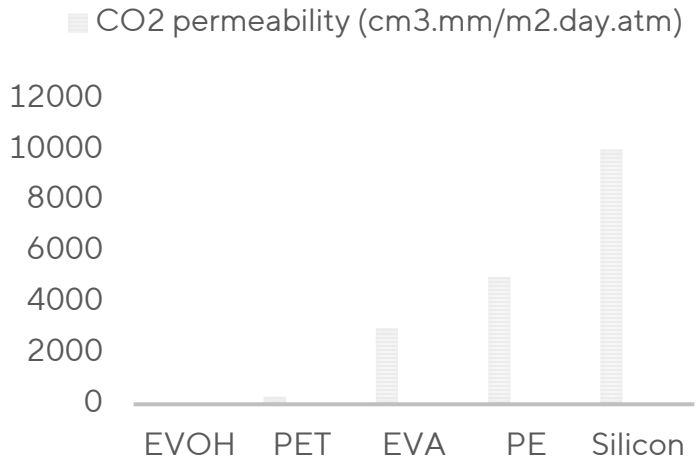
⟶ medium more basic

Maintaining adequate CO_2 level is critical to preserve buffer stability

CO_2 diffusion

CO_2 permeability of materials

- Thicker films reduce CO_2 loss but increase material use



Material	Recyclability	Relative Cost
EVOH	Limited	High
PET	Excellent	Low-Medium
EVA	Limited	Medium
PE (LDPE/HDPE)	Good	Low
Silicon	Poor	High

CO_2 permeability varies significantly across materials

- EVOH provides a strong CO_2 barrier, ensuring stable pH
- PET has low permeability, ensuring stable pH but is not sustainable for single-use applications
- EVA and PE offer moderate permeability and are widely used
- Silicone, while highly permeable to CO_2 and thus risking pH drift

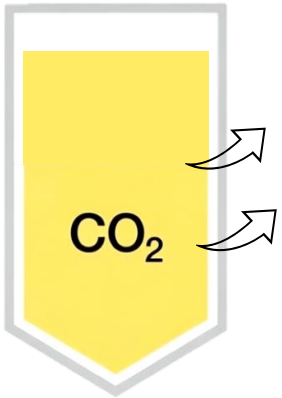
pH stability is therefore time dependant

McKeen, L. W. (2016). Barrier Properties of Plastics and Elastomers (4th ed.). Plastics Design Library

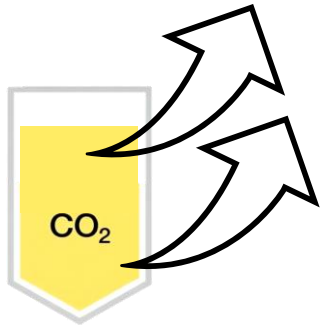
Surface to Volume ratio

The physicochemical mechanisms behind pH drift are rooted in the **CO_2/HCO_3^- equilibrium**

- When CO_2 diffuses out of the media through the bag material equilibrium shifts → HCO_3^- → increasing the pH
- **This process is influenced by the surface-to-volume ratio of the bag**:
  smaller bags lose more CO_2 due to a higher surface-to-volume ratio



- Big Volume → S/V<<1
- CO_2 loss a small fraction of total CO_2
- pH remains stable over time



- Small volume → S/V>>1
- CO_2 loss is **SIGNIFICANT**
- pH drift occurs

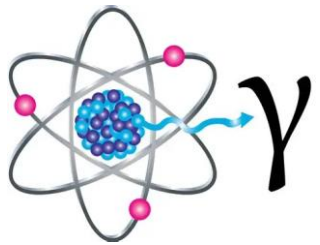
High S/V → more sensitive to CO_2 loss over time → need careful design optimization

Sterilization



Sterilization of bags with Gamma or X ray irradiation

- ✓ To prevent microbial contamination during cell culture
- ✓ For patient safety
- ✓ **Sterility Assurance Level (SAL) of 10^{-6}**



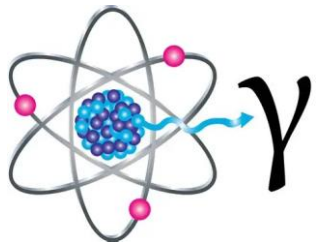


Sterilization



Impact of irradiation on polymer bags and cell culture media

- Polymer chain scission, radiation breaks the covalent bonds in the polymer material
  - → Compromised mechanical properties
  - → Increased brittlenesss
- Cross-linking polymer chains bond together
  - → Increased rigidity and potential loss of flexibility
  - → **Reduced gas permeability**
- Release of extractables
  - → Acetic acid, formic acid, and other degradation products from polymers like EVA or PE
  - → May migrate into the cell culture media





Samuel Dorey, *Industrial & Engineering Chemistry Research* **2019** *58* (31), 14115-14123

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Objectives

Goal	18 month with stable pH	▪ No bag dimension or S/V ratio modification ▪ No media formulation change allowed ▪ **Use well known and characterized materials**
Solution	Conduct a feasibility study	▪ Determine optimal material, design combination
Benefits	Supports reliable use of SU bags for CO_2-buffered media in cell culture	

Overview of material combinations

Each polymer has a diffrent function

Tubings	Packaging	Bag multilayers films
Silicone	PP	PE/EVOH/PE -1
TPE – 1		PE/EVOH/PE -2
TPE – 2	PA/PE	EVA/EVOH/EVA
TPE - 3		

Tests
pH stability
Conductivity
Osmolality

Osmolality = how many particles are dissolved in a liquid

To maintain media stability in small bags
→ Identify the most effective bag × tubing × packaging combination
→ Need material characteristics comprehension

Define long-term strategy based on study results

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Material Challenges

A trade-off between contamination risk and pH stability



PET bottles have low CO_2 permeability

- but not in closed system

Flexible bags

- Reduce contamination risk
- More sustainable
- **Need optimized configuration**



pH stability contributing factors

1. **Material thickness** → PET bottles thickness >> flexible bags → limiting CO_2 diffusion
2. **Material CO_2 permeability**. A strong permeability (Silicon) → CO_2 diffusion → pH increase
3. **Bag design and surface to volume ratio**: more surface in contact with liquid = more CO_2 diffusion
4. **Material interaction with sterilization process.** Extractables or cross linking have a impact



Material knowledge and design are important for pH stability and thus cell culture efficiency



Thank you.

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