Entegris
PURE ADVANTAGE

FEBRUARY 27, 2018

Single-Use Bag Permeability Testing in Cryopreservation Conditions

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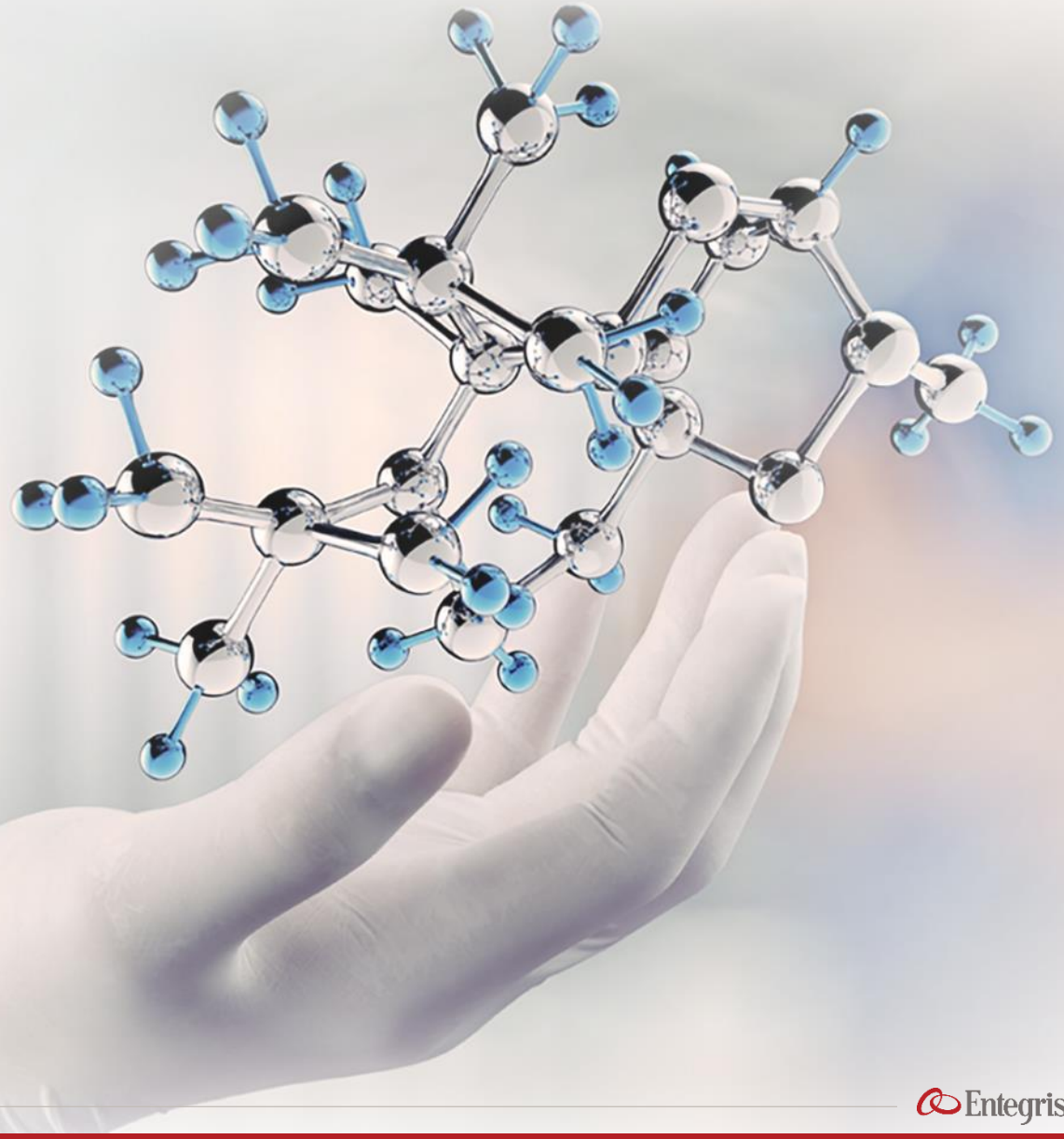
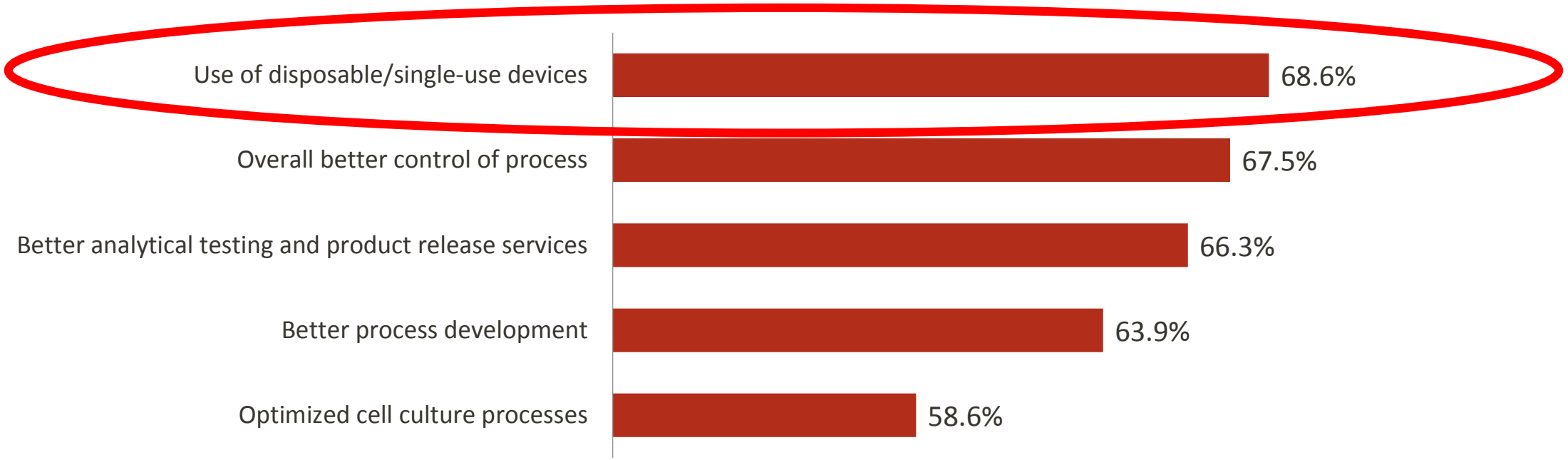


AGENDA

BIOPHARM PERFORMANCE IMPROVEMENTS

Improving Biomanufacturing Performance Factors Creating “Significant” or “Some” Improvements

How much have each improved biomanufacturing performance over the past 12 months? 2010–2015

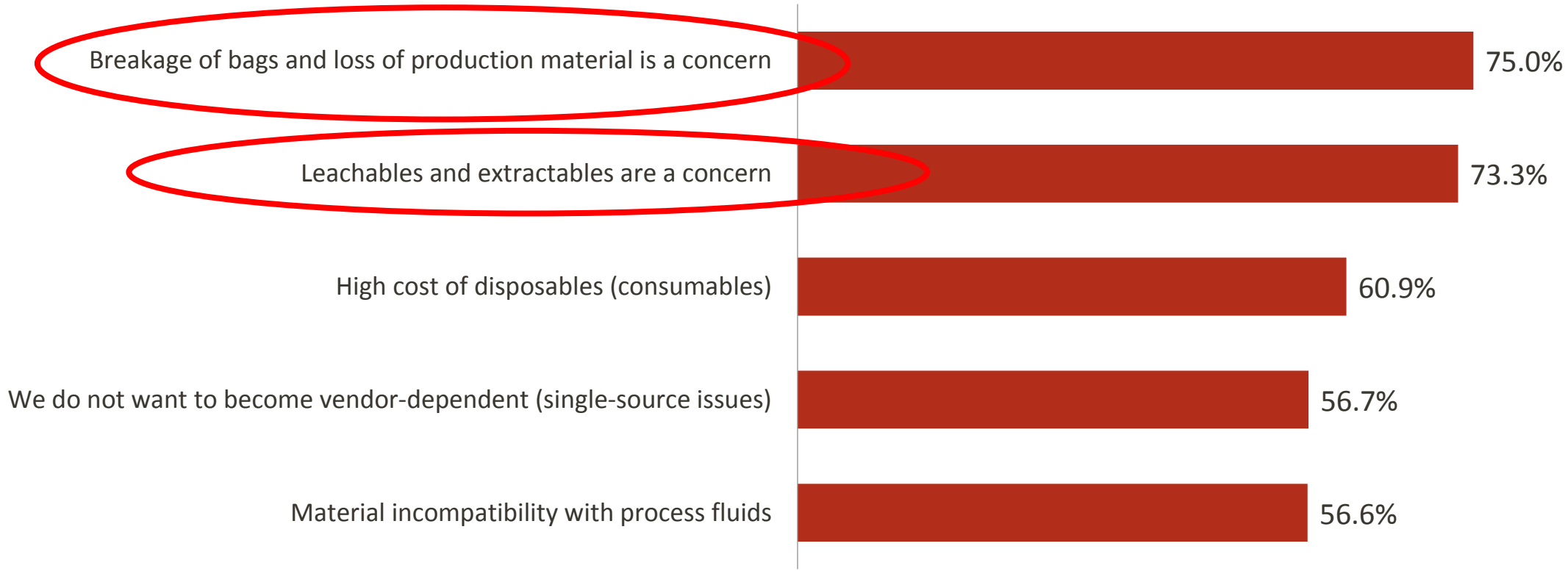


Source: “Twelfth Annual Report and Survey of Biopharmaceutical Manufacturing Capacity and Production,” BioPlan Associates, Inc., Rockville, MD, April 2015, pp. 109

SINGLE-USE CHALLENGES

Factors that may restrict use of disposables in biopharmaceutical manufacturing

Percent indicating "Strongly agree" or "Agree"



Source: "Twelfth Annual Report and Survey of Biopharmaceutical Manufacturing Capacity and Production," BioPlan Associates, Inc., Rockville, MD, April 2015, pp. 294

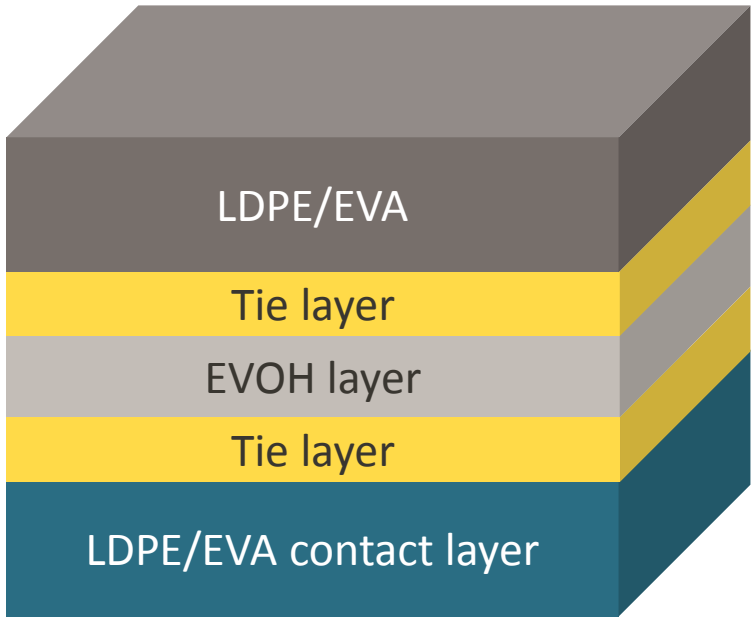
SINGLE-USE INNOVATION

- What is particularly needed is the development of new materials, particularly new and improved plastics and variations of current plastics that enable major design innovations. Possible innovations might include homogenous polymers (single polymer films/bags, no lamination of different plastic films with adhesives between layers) and unitary (single-piece) molded, solid structurally self-supporting, plastic bioreactors.

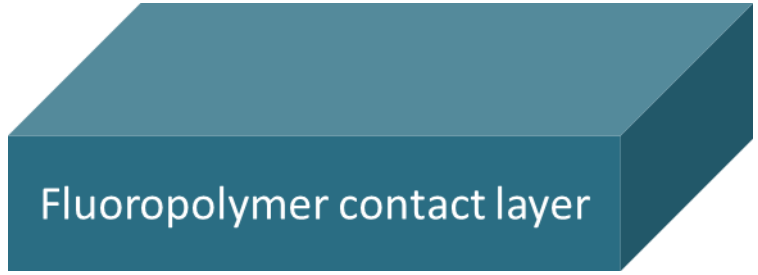
Source: “Twelfth Annual Report and Survey of Biopharmaceutical Manufacturing Capacity and Production,” BioPlan Associates, Inc., Rockville, MD, April 2015, pp. 101

SINGLE-USE INNOVATION

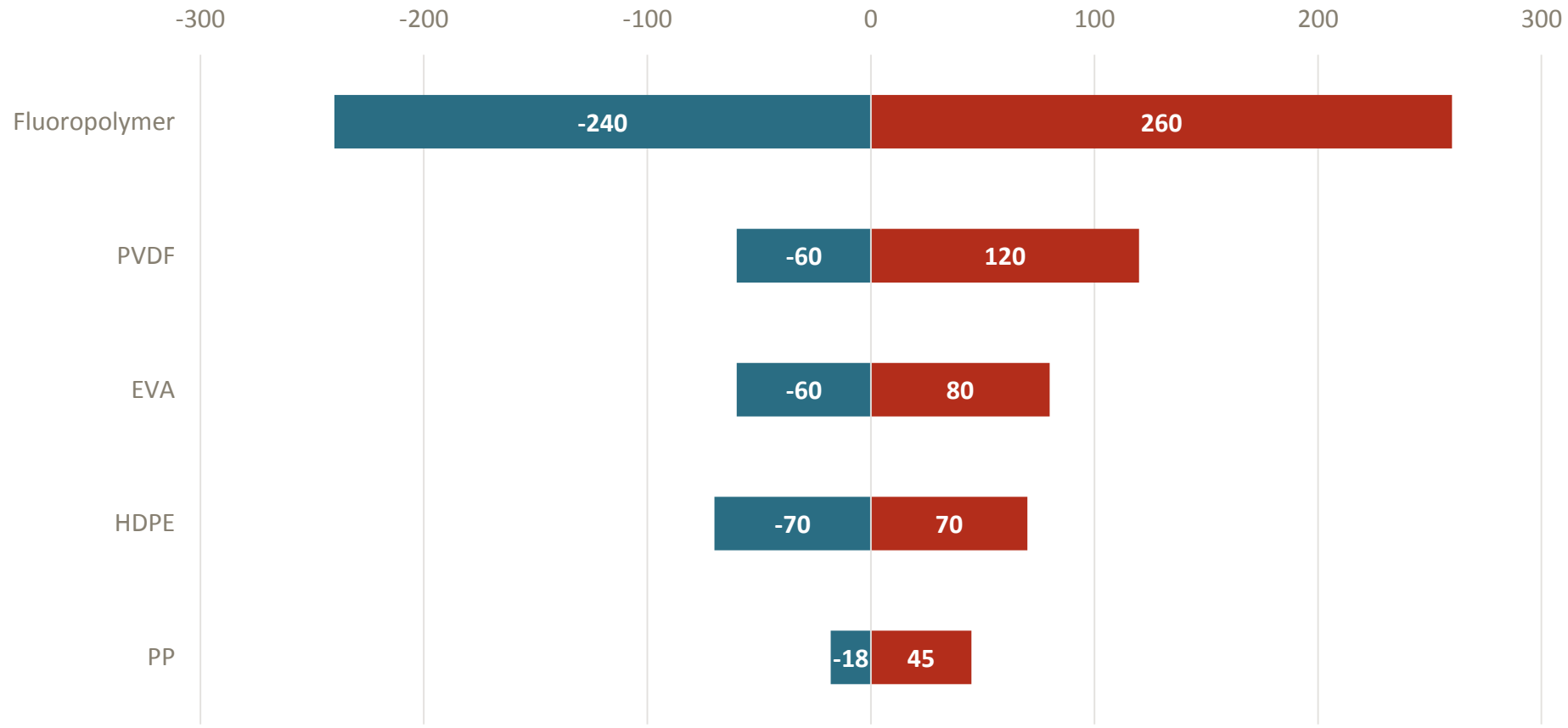
Standard film material



Advanced film material



RESIN TEMPERATURE USE RANGE (°C)



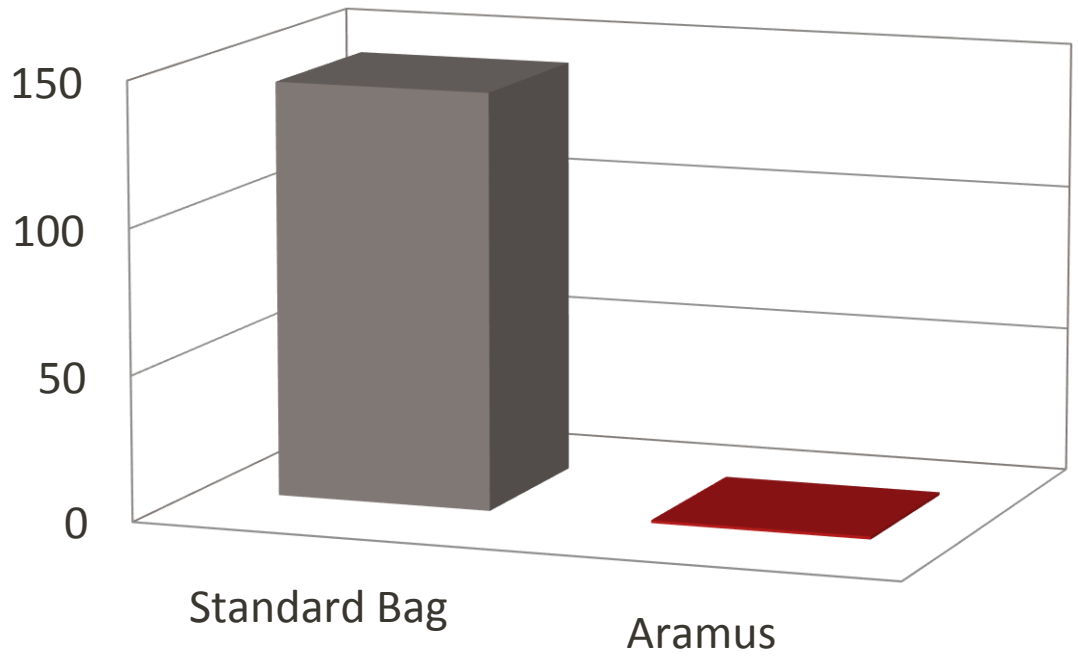
Source: Compass Publications Chemical Resistance Guide for Plastics, ©2000

REFLUX EXTRACTION EVALUATION: TOTAL EXTRACTABLES

Fluoropolymer single-use bag versus multi-layer single-use bag

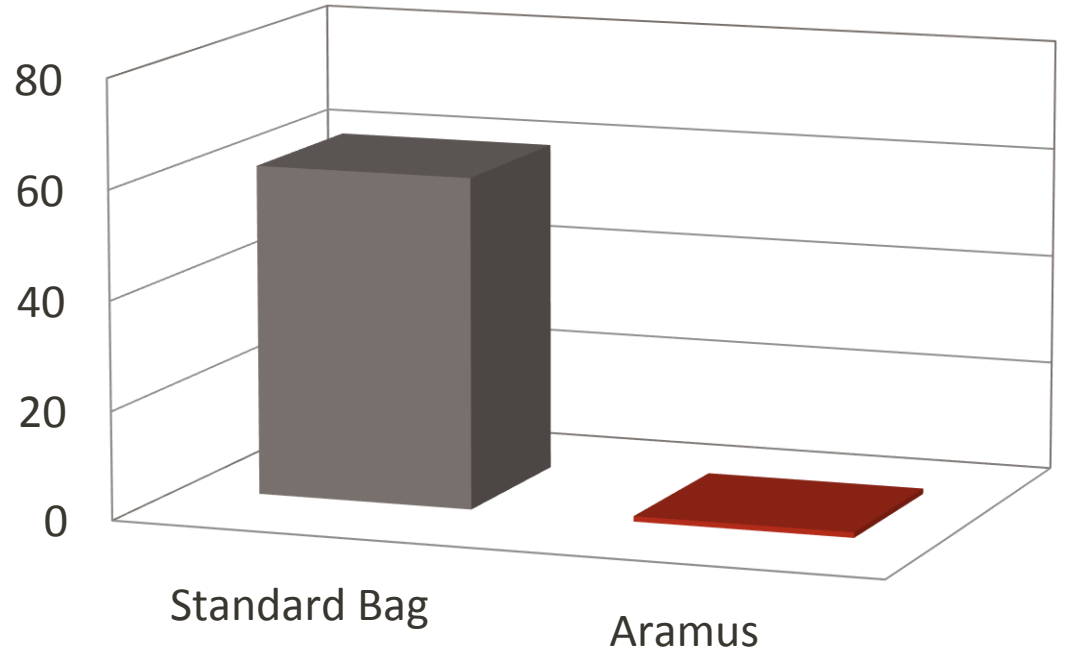
100% Ethanol

Standard bag: 144× greater TOC



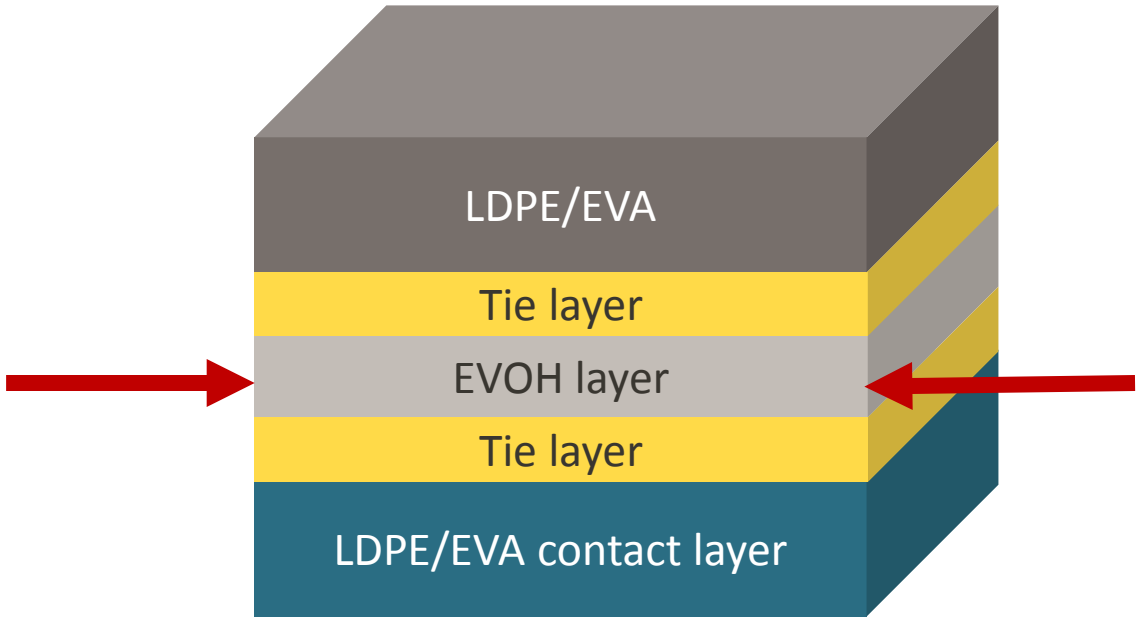
100% Isopropanol

Standard bag: 60× greater TOC



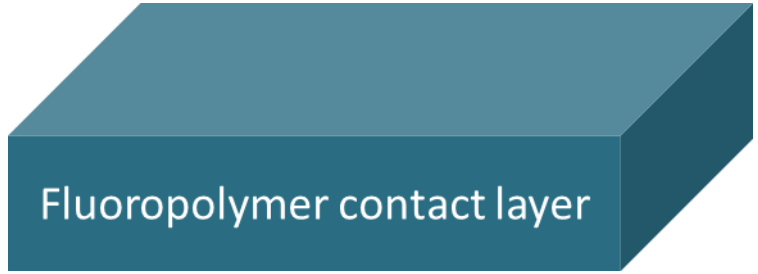
SINGLE-USE INNOVATION

Standard film material



The implementation of an EVOH layer increases the risk of detrimental extractable compounds from the material and/or adhesives used to manufacture the multi-layer bag

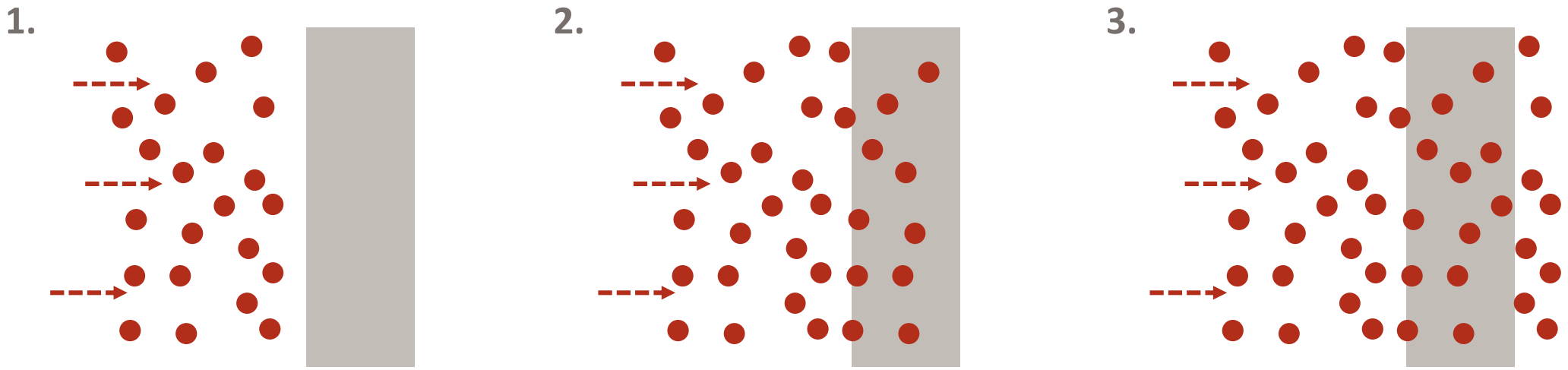
Advanced film material



The elimination of the EVOH layer provides a very low risk of extractable contamination

PERMEATION OVERVIEW

Permeation: To diffuse through or penetrate something



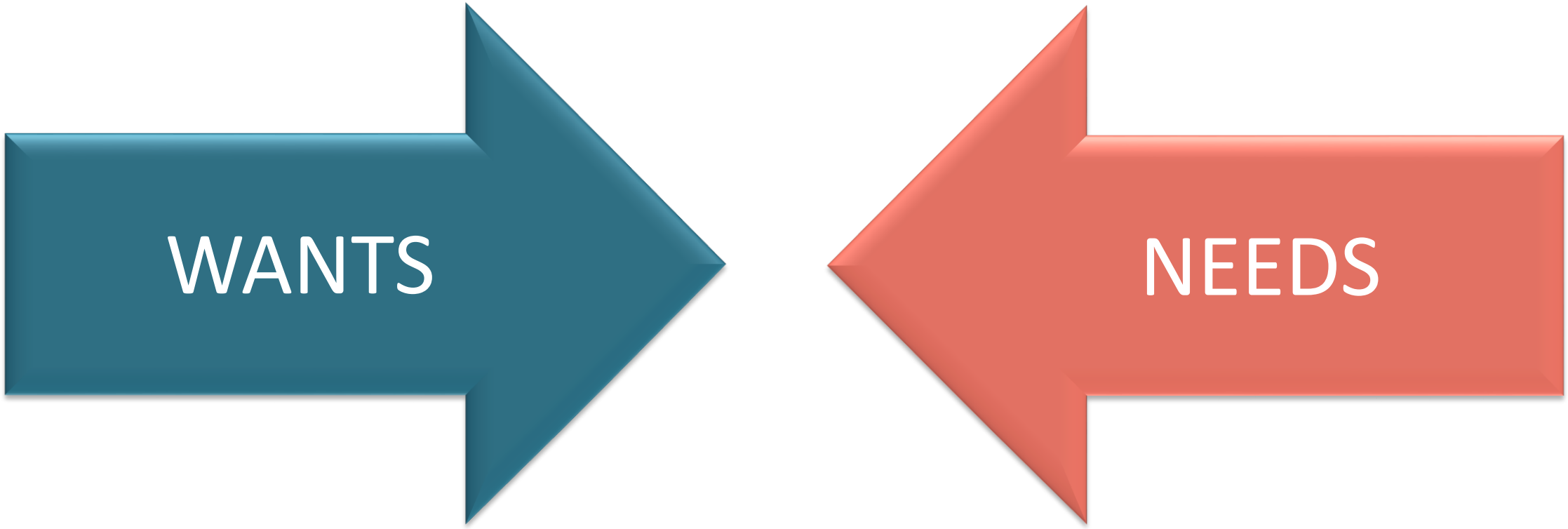
Permeation Factors: Molecule Type/Size, Temperature, Material Properties

PERMEATION OVERVIEW

- Different materials have different rates

Permeation Rate	EVOH	PFA	EVA	Silicone
Moisture ($g\ mm/m^2\ day$)	.01	.002	.93	X
Oxygen ($cm^3\ mm/m^2\ day\ atm$)	.02	335	196	40,700
Carbon Dioxide ($cm^3\ mm/m^2\ day\ atm$)	.03	700	1100	212,000

PERMEATION OVERVIEW



PERMEATION OVERVIEW

- Low O_2 permeation
- Low CO_2 permeation
- Low moisture permeation

PERMEATION OVERVIEW

- pH Stability
- Concentration Stability

ABSTRACT

- The stability of frozen solutions stored in single-use bags is a primary concern in bioprocessing.
- This presentation will share pH stability data from a frozen solution stored in single-use bags with different gas permeation rates that are subjected to CO_2 gas from sublimating dry ice.

TEST PROTOCOL

What was tested:

- Bags: 1 liter
  - Aramus™ single-layer bag
  - EVA/EVOH/EVA multi-layer bag
  - Aramus bag in Mylar® bag
  - EVA/EVOH/EVA bag in Mylar bag
- Media: 5 mM Sodium phosphate solution
- Test conditions:
  - Frozen at -85°C and stored in chamber with dry ice at -78°C

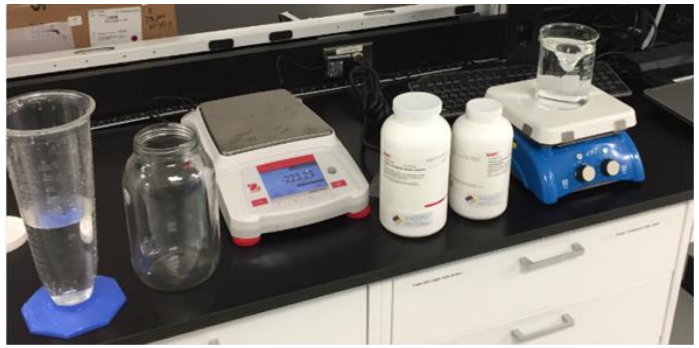
What was measured:

- pH: pH of solution post-thaw
  - Aramus single-layer bag
  - EVA/EVOH/EVA multi-layer bag
  - Aramus bag in Mylar bag
  - EVA/EVOH/EVA bag in Mylar bag
- Time points:
  - 0 days
  - 1 day
  - 3 days
  - 7 days
  - 21 days

TEST PROTOCOL

Procedure:

- Prepare 5 mM sodium phosphate solution
  - Target pH: 6.1
- Prepare bags
  - Weigh bags empty
  - Fill bags with 1 liter solution
  - Weigh bags with media
  - Place required quantity of primary bags in Mylar bags and vacuum seal as necessary



TEST PROTOCOL

Procedure:

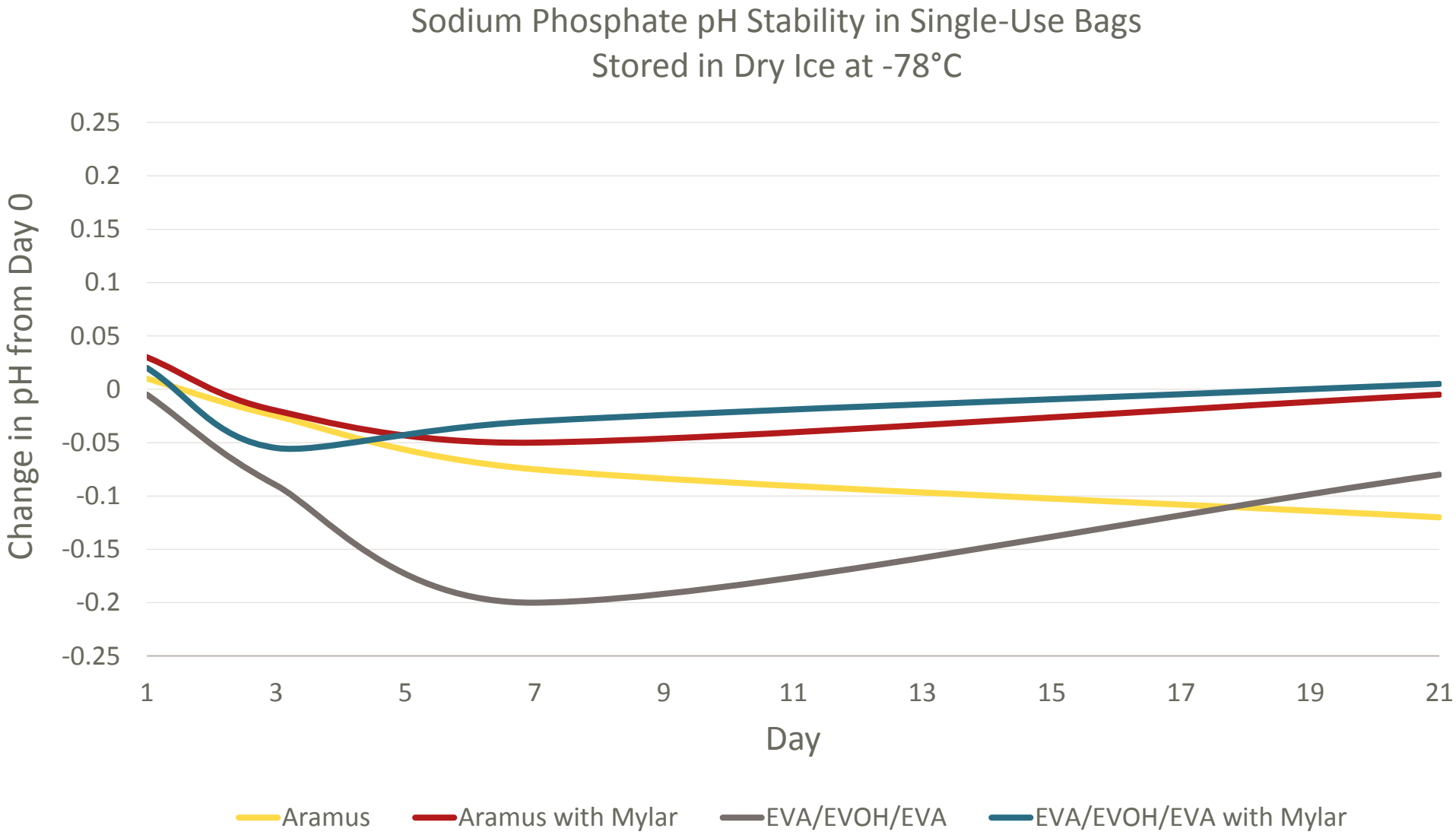
- Freeze and store bags
  - Place bags in -85°C freezer until frozen (2 hours)
  - Transfer frozen bags into insulated chamber with 200 pounds of dry ice

TEST PROTOCOL

Procedure:

- Thaw
  - Remove required samples at day 1, 3, 7 and 21
  - Place bags in 23.5°C water bath until solution is thawed and stable at 23.5°C
- Measure
  - Remove 30 mL from each bag
  - Measure and record pH

RESULTS


Sodium Phosphate pH Stability in Single-Use Bags
Stored in Dry Ice at -78°C
Change in pH from Day 0
0.25
0.2
0.15
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
-0.25
1
3
5
7
9
11
13
15
17
19
21
Day
Aramus
Aramus with Mylar
EVA/EVOH/EVA
EVA/EVOH/EVA with Mylar

TEST PROTOCOL ROUND 2

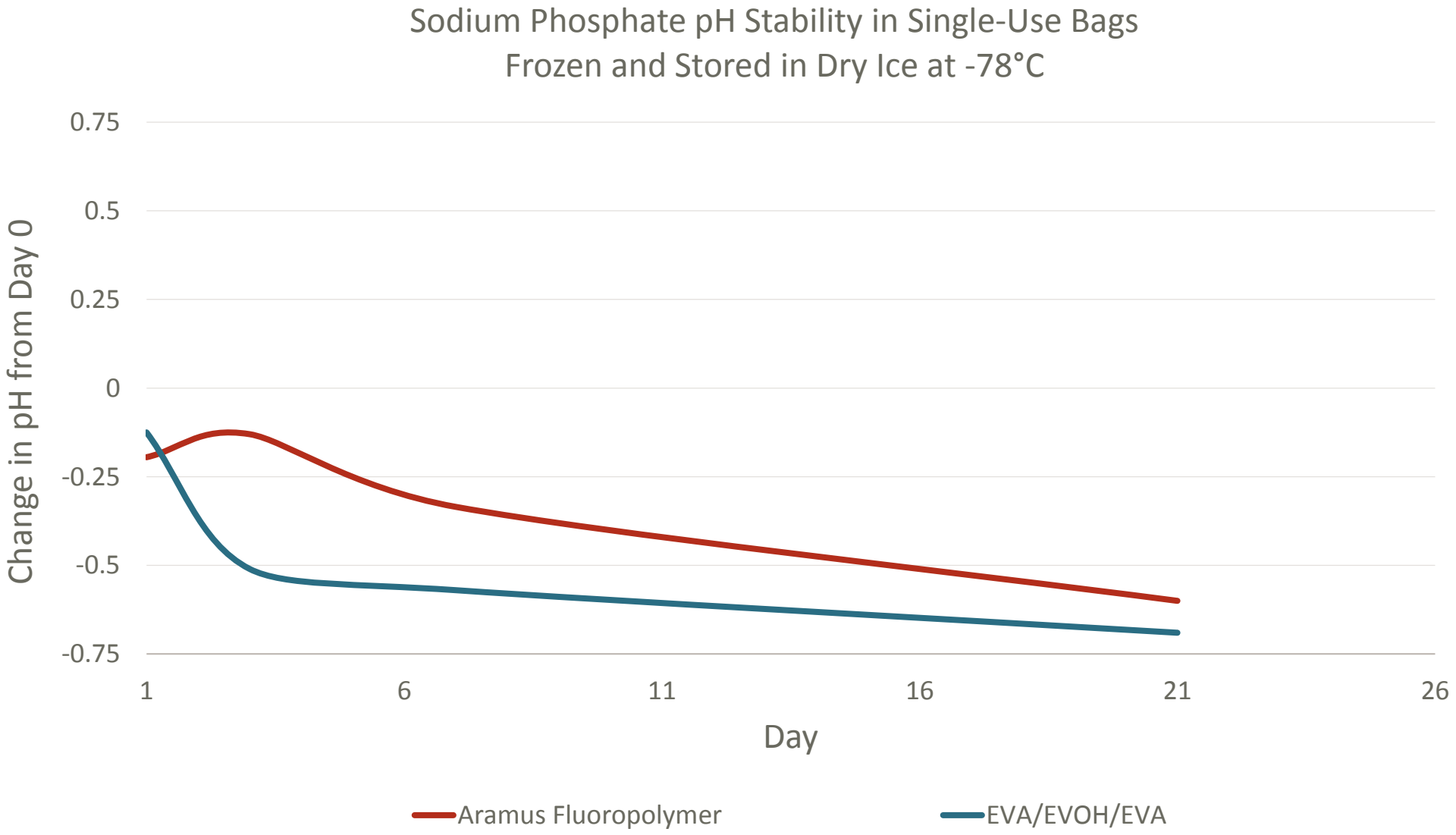
What was tested:

- Bags: 1 liter
  - Aramus single-layer bag
  - EVA/EVOH/EVA multi-layer bag
- Media: 5 mM Sodium phosphate solution
- Test conditions:
  - **Placed directly into the dry ice chambers in a liquid state and allowed to freeze via the dry ice**

What was measured:

- pH: pH of solution post-thaw
  - Aramus single-layer bag
  - EVA/EVOH/EVA multi-layer bag
- Time points:
  - 0 days
  - 1 day
  - 3 days
  - 7 days
  - 21 days

RESULTS


Sodium Phosphate pH Stability in Single-Use Bags
Frozen and Stored in Dry Ice at -78°C
Change in pH from Day 0
0.75
0.5
0.25
0
-0.25
-0.5
-0.75
1
6
11
16
21
26
Day
Aramus Fluoropolymer
EVA/EVOH/EVA

CONCLUSION

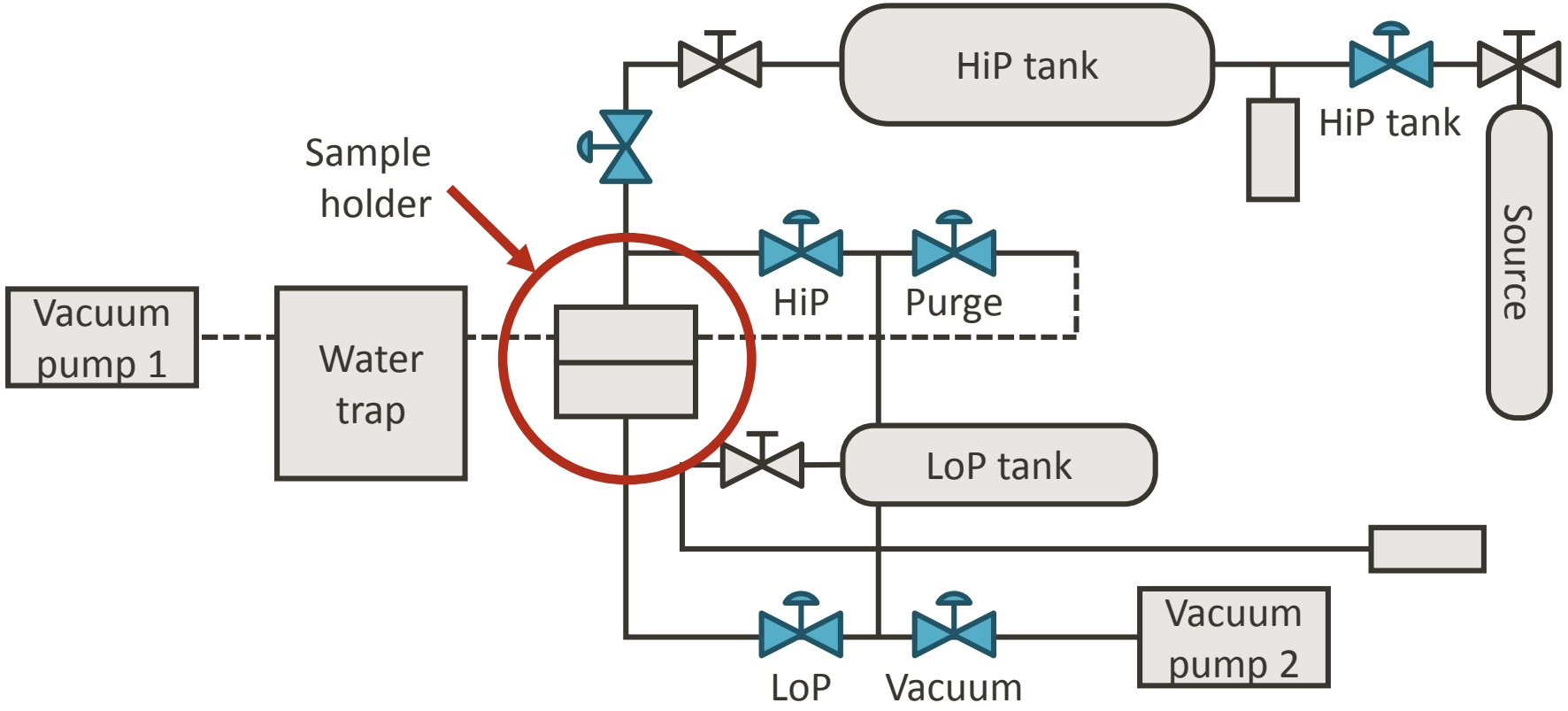
- Changes in pH of frozen sodium phosphate solutions contained in single-use bags, pre-frozen, and stored in dry ice chamber at -78°C, were similar in bags containing a gas barrier EVOH layer and the fluoropolymer bags that did not
- Encasing the single-use polymeric bags with a Mylar bag did reduce the amount of pH change slightly
- There was a greater change in pH when the storage bags were placed directly into the dry-ice chamber, though the changes in pH were similar between the bags containing a gas barrier EVOH layer and the fluoropolymer bags that did not
- Based on a 21-day exposure to CO_2 from sublimating dry ice, there does not appear to be a CO_2 gas permeation performance benefit from the EVOH layer on the pH stability of frozen sodium phosphate solution

THANK YOU

- For more information:
  - Mike.Johnson@Entegris.com
  - www.Entegris.com/lifesciences

GAS PERMEATION TESTING

- ASTM D1434: Gas Permeability of Plastic Film
- ASTM D3985: Oxygen Gas Transmission Rate Through Plastic Film



GAS PERMEATION TESTING

PERMEATION OVERVIEW

- What is the actual requirement from end-users?
  - Product stability
    - pH
    - Concentration
- How do different single-use bag materials with different gas transmission rates impact the actual user requirements?