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Application Note

# Ensuring Long Term Stability for Buffers and Cell Culture Media with Single-Use Assemblies Made with Ultimus® Film

## Introduction

The biopharmaceutical manufacturing industry is increasingly adopting single-use technologies as an alternative to stainless-steel systems to enhance operational efficiency and flexibility. Before implementing these technologies into their workflow end-users must thoroughly evaluate the components, including the bioprocessing film, to ensure they are fit for their intended purpose and do not pose a risk to patient safety. This evaluation typically begins with a risk assessment to analyze how the materials in the film may interact with the fluid solution. Analysis is based on extractables data that can be used to determine toxicity based on maximum dosage of potential leachables.

- Extractables are compounds that can be extracted from the elastomeric or plastic components of single-use assemblies when exposed to certain solvents under extended time periods and exaggerated temperatures.
- Leachables are chemical components that migrate from a contact surface into a process fluid under normal conditions or storage[1].

Ultimus® film is a bioprocessing film designed for challenging, high-value, single-use bioprocessing liquid applications and provides durability and leak resistance. Extractables data for Ultimus® film can be found in the Emprove® Program Operational Excellence Dossiers, as well as in a summary document titled “Understanding the Impact of Extractables and Leachables from Single-Use Films”[2]. This extractables data can be used to assess the product or process risk to patient safety and, if a safety risk is identified, a leachables study should be conducted to assess the safety risk in the context of mitigation steps.

In many instances, single-use containers are used for storing and transporting fluids that are used for processing, such as buffers or cell culture media. For single-use films in particular, there is a concern that the release of potentially toxic or inhibitory substances from the plastic materials, especially following gamma irradiation could interact with buffers or cell culture media over long periods of time.

For cell culture media stored in single-use containers, the release of substances from film to stored cell culture media could adversely affect the growth and viability of production cells, impacting product titer and quality. The white paper titled "Single-Use Upstream Processing: Ultimus® Film Delivers Comparable Cell Growth Performance to Glass" summarizes cell growth, product titer, and product quality using Ultimus® film under worst-case conditions, directly after exposure to gamma irradiation[3]. Buffer stability can be impacted by evaporation and dissolution of oxygen. Plastics with high evaporation rates can result in fluid loss and change physical attributes, such as conductivity. Likewise, high oxygen dissolution through the plastic film can alter buffer characteristics. For stability, low gas permeation is desirable to maintain buffer pH and concentration.

This application note explores the impact of extended storage (>60 days) of process buffers and cell culture media under typical process conditions in single-use bags made with Ultimus® film, to assess their stability, integrity, and suitability for biopharmaceutical applications.



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## Study 1: Buffer Stability

The objective of this study was to evaluate the long-term stability of buffers stored in single-use bags made with Ultimus® film. Buffer stability in 2D single-use bags made with Ultimus® film was compared to storage in glass bottles over a 60 day period and focused on pH stability, conductivity, and changes in composition due to evaporation or oxygen dissolution. Through these comparisons we aim to assess the impact of temperature and material properties on buffer performance over time.

### Materials and Methods

Five buffers were held in 2D bags made with Ultimus® film and glass bottles for more than 60 days at two temperatures, for a total of 14 conditions, shown in **Table 1.** The 2D bags were selected as a worst-case scenario due to their high surface area to volume ratio as compared to 3D bags. Before testing, bags were gamma irradiated at 25-40 kGy and glass bottles were autoclaved. Buffers were sterile filtered into each container within 48 hours of sterilization. Fill volume was 2 L, resulting in 1.8 mL/cm$^2$ of Ultimus® film; filled containers were stored at 4 °C or at Room Temperature (RT), measured to be 20.7±0.6 °C for the 63 days of the study.

To track buffer stability and oxygen dissolution, samples were collected for pH and conductivity measurement. The pH and conductivity of the buffers were measured by a SevenGO Duo™ pH/Cond meter (Mettler Toledo). Evaporation for two buffers was tracked by checking the change in weight of the filled containers. For comparison, bags made from another commercially available film, Film A, were assessed in parallel.

**Table 1. Buffer, temperature, and vessel material for each condition tested.**

| Condition | Buffer | Temperature | Vessel Material |
|---|---|---|---|
| 1 | 50 mM Tris, pH 7 | 4 °C | Ultimus® Film |
| 2 | 50 mM Tris, pH 7 | RT | Ultimus® Film |
| 3 | 50 mM Acetic Acid, pH 3 | 4 °C | Ultimus® Film |
| 4 | 50 mM Acetic Acid, pH 3 | RT | Ultimus® Film |
| 5 | 50 mM Tris, pH 7 | 4 °C | Glass |
| 6 | 50 mM Tris, pH 7 | RT | Glass |
| 7 | 50 mM Acetic Acid, pH 3 | 4 °C | Glass |
| 8 | 50 mM Acetic Acid, pH 3 | RT | Glass |
| 9 | 0.5 m Tris, pH 7 | RT | Ultimus® Film |
| 10 | 0.5 m Tris, pH 7 | RT | Glass |
| 11 | 0.1 m Tris Base, pH 10-11 | RT | Ultimus® Film |
| 12 | 0.1 m Tris Base, pH 10-11 | RT | Glass |
| 13 | 50 mM Tris + 1 m NaCl, pH 7 | RT | Ultimus® Film |
| 14 | 50 mM Tris + 1 m NaCl, pH 7 | RT | Glass |

## Results and Discussion

**Figure 1** shows pH and conductivity of the buffers in 2D single-use bags made from Ultimus® film and glass bottles over 63-day duration at both room temperature and 4 °C. Buffer stability in 2D bags made from Ultimus® film was consistent over time and similar to that of buffer stored in glass bottles. Additionally, no precipitation was observed in the high salt buffer (50 mM Tris, 1 M NaCl, pH 7) at the end of the storage period.

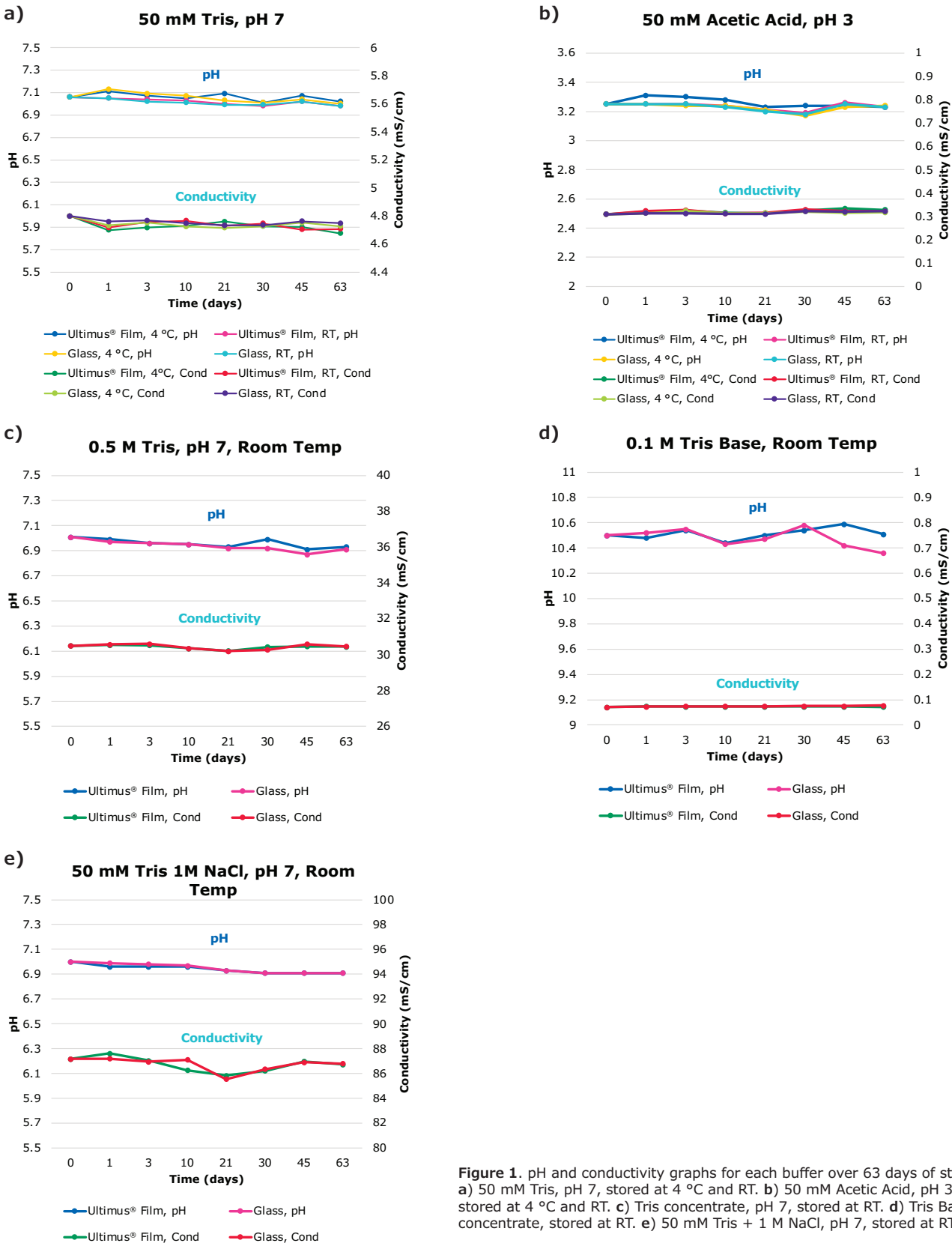


**Figure 1**. pH and conductivity graphs for each buffer over 63 days of storage. **a**) 50 mM Tris, pH 7, stored at 4 °C and RT. **b**) 50 mM Acetic Acid, pH 3, stored at 4 °C and RT. **c**) Tris concentrate, pH 7, stored at RT. **d**) Tris Base concentrate, stored at RT. **e**) 50 mM Tris + 1 M NaCl, pH 7, stored at RT.

To assess evaporation, containers were weighed throughout the 63 days. In addition to 2D bags made with Ultimus® film, buffer was stored in bags made from a different commercially available film, designated Film A, and glass containers. As shown in **Figure 2a**, 50 mM Tris pH 7 buffer stored in bags made from Ultimus® film or Film A lost only 0.14% of volume due to evaporation after 63 days of storage at room temperature. At 4 °C, losses were even lower: volume loss in Ultimus® film containers was 0.03% which was comparable to the 0.05% volume loss in glass at the same temperature. Similar results were observed with 50 mM Acetic Acid pH 3 buffer, with 0.13% of volume loss after 63 days at room temperature, and minimal losses in the 4 °C storage conditions. In summary, evaporation was shown to be negligible after two months of storage, indicating 2D bags made from Ultimus® film provide stable buffer storage for extended durations.

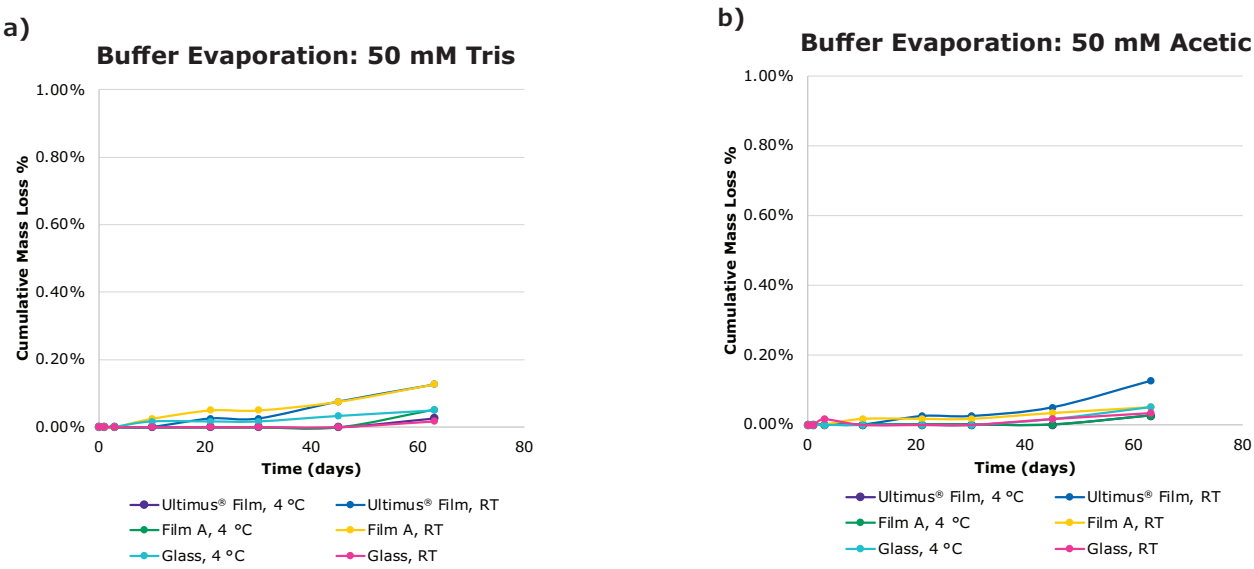


**Figure 2.** Cumulative loss of buffer volume due to evaporation during 63 days of buffer storage. **a**) 50 mM Tris buffer. **b**) 50 mM Acetic Acid buffer.

## Study 2: Water Hold to Evaluate Consistent Media Quality for Cell Growth Performance

This study assessed whether leachables from gamma-irradiated bags made with Ultimus® film negatively impacted the quality of cell culture media. To ensure our results are applicable to other media and cell types, a standardized cell culture test was implemented. This test follows a previously published protocol by DECHEMA Temporary Group on "Single-Use Technology in Biopharmaceutical Manufacturing"[4]. Bags made with Ultimus® film and glass bottles were filled with water, stored for at least 60 days, and then used to prepare cell culture media. The prepared media were then used to assess performance of two cell lines.

### Materials and Methods

Two cell lines that express different monoclonal antibodies with their respective cell culture medias were tested. Single-use bags made with Ultimus® film were gamma irradiated at 25-40kGy then filled with Milli-Q® water within 48 hours; glass bottles were autoclaved. The fill volume was 2 L, resulting in a surface to volume ratio of 1.8 mL/cm$^2$ for Ultimus® film. The stored water was used to make media after 0, 28, and 63 days then used for cell culture. The cells lines were grown in their respective media for 7 days and performance was assessed throughout the duration of culture.

**Table 2. Cell lines and cell culture media tested for cell growth performance.**

| Cell Line Number | Cell Line | Cell Culture Medium |
|---|---|---|
| Cell Line 1 | CHOZN® GS | Cellvento® 4CHO COMP |
| Cell Line 2 | CHOZN® GS | EX-CELL® Advanced CHO Fed-batch Medium |

## Results and Discussion

**Figures 3** and **4** show the average Viable Cell Density (VCD) and the average cell viability of the two cell lines during 7 days of cell culture. Ultimus® film data are the average of six replicate cultures, while glass and Day 0 conditions are the average of three replicate cultures each. **Figure 3** depicts the data of cells cultured in media made with water stored for 28 days while **Figure 4** shows similar data for water that had been stored for 63 days. As an additional control, data from cells cultured with media made with water that had not been stored is shown as Day 0 in **Figure 3**.

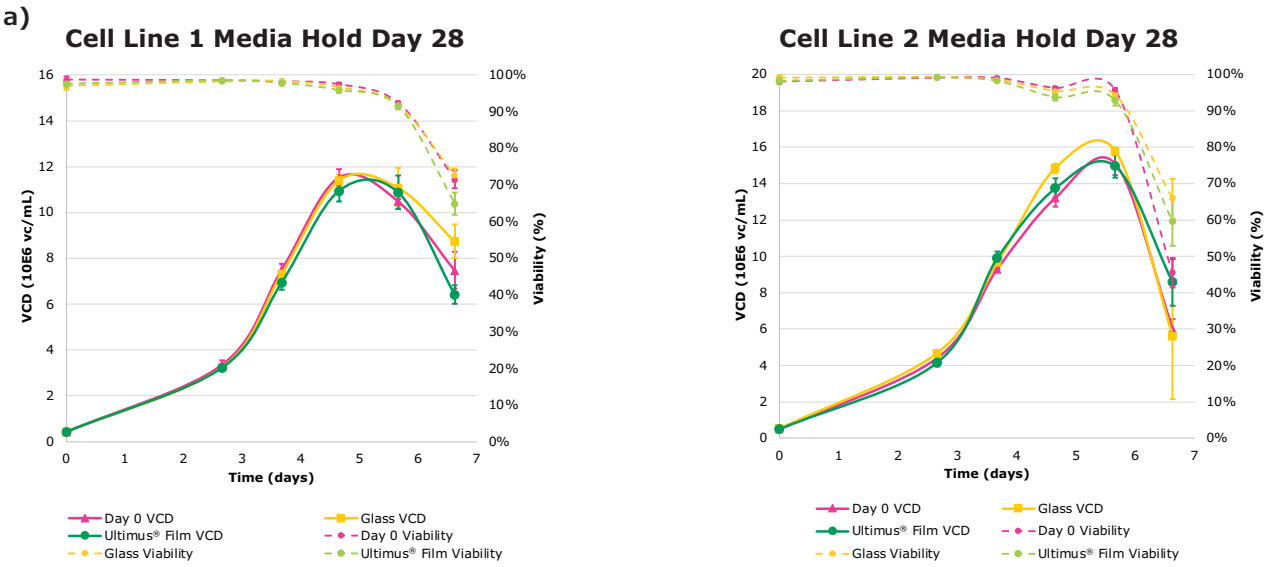


**Figure 3.** Viable Cell Density and Viability during 7 days of culture for cells grown in media made with water stored for 28 days, and cells grown in media made with fresh water as an added control.

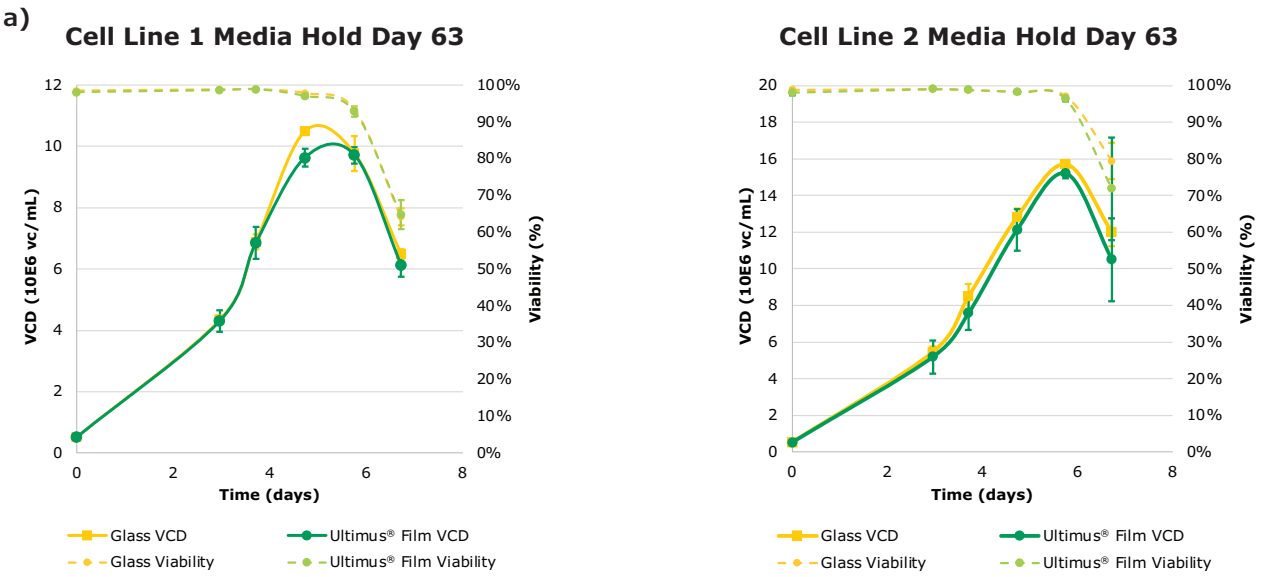


**Figure 4.** Viable Cell Density and Viability during 7 days of culture for cells grown in media made with water stored for 63 days.

Minitab® software was used to compare cell culture performance between the different water storage conditions. A two-sample t-test was ran with a 95% confidence interval for the mean difference. The data points chosen for analysis are VCD (10E6 vc/mL) and cell viability (%) for both cell lines on day 4 of culture. Day 4 was selected for analysis as it allowed sufficient time for cell growth before the decline of cell viability due to nutrient depletion.

**Table 3** shows the mean, standard deviation, T-value, and p-value after running a two-sample T-test. For every test the p-value is greater than the significance level, indicating no significant difference between the mean, VCD, and cell viability of each condition. Growth of each cell line was identical irrespective of whether the water used to make media was stored in Ultimus® film bags or glass bottles, indicating that leachables from single-use bags did not impact cell culture performance and that these bags are suitable for storage of water for critical cell culture applications.

**Table 3. Two-Sample T-Test of Viable Cell Density and Viability for Cell Lines on Day 4 of Culture.**

| Storage Time | Cell Line | Variable | Condition | N | Mean | StDev | SE Mean | T-Value | DF | P-Value | P-Value > 0.05 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 28 days | Cell Line 1 | VCD | Glass | 3 | 7.32 | 0.13 | 0.07 | 1.8 | 7 | 0.115 | Yes |
| | | | Ultimus® Film | 6 | 6.94 | 0.35 | 0.14 | | | | |
| | | Viability | Glass | 3 | 0.984 | 0.003 | 0.002 | 2.01 | 7 | 0.087 | Yes |
| | | | Ultimus® Film | 6 | 0.978 | 0.004 | 0.002 | | | | |
| | Cell Line 2 | VCD | Glass | 3 | 9.66 | 0.30 | 0.17 | -0.94 | 7 | 0.376 | Yes |
| | | | Ultimus® Film | 6 | 9.91 | 0.40 | 0.16 | | | | |
| | | Viability | Glass | 3 | 0.985 | 0.004 | 0.002 | 1.37 | 7 | 0.212 | Yes |
| | | | Ultimus® Film | 6 | 0.982 | 0.002 | 0.001 | | | | |
| 63 days | Cell Line 1 | VCD | Glass | 3 | 6.89 | 0.29 | 0.17 | 0.11 | 7 | 0.913 | Yes |
| | | | Ultimus® Film | 6 | 6.84 | 0.57 | 0.23 | | | | |
| | | Viability | Glass | 3 | 0.988 | 0.000 | 0.000 | -0.4 | 7 | 0.704 | Yes |
| | | | Ultimus® Film | 6 | 0.989 | 0.003 | 0.001 | | | | |
| | Cell Line 2 | VCD | Glass | 3 | 8.50 | 0.84 | 0.49 | 0.78 | 4 | 0.481 | Yes |
| | | | Ultimus® Film | 3 | 8.00 | 0.70 | 0.40 | | | | |
| | | Viability | Glass | 3 | 0.988 | 0.001 | 0.001 | -0.19 | 4 | 0.862 | Yes |
| | | | Ultimus® Film | 3 | 0.988 | 0.002 | 0.001 | | | | |

## Study 3: Media Hold for Sterility

This study evaluated whether bags made with Ultimus® film can maintain sterility of contents over a 60 day period. Cell culture media-filled periodically throughout the extended incubation period bags were tested for pH, osmolality, and bioburden to assess whether any microbial growth occurred, indicating compromised microbial integrity.

### Materials and Methods

2D bags made with Ultimus® film were gamma irradiated at 25 to 40 kGy, filled with 2 L of EX-CELL® Advanced CHO Fed-batch Medium and stored for over 60 days. Samples were collected at 0, 4, 31, 35, 60, and 64 days to measure pH and osmolality. The pH of the media was measured by a SevenGO Duo™ pH meter (Mettler Toledo), and osmolality was measured by a BioProfile® FLEX2™ (Nova Biomedical®). At the end of the hold time, the presence of bioburden was assessed via membrane filtration using 0.45 µm mixed esters of cellulose membrane in which 40% of the total volume was plated on Tryptic Soy Agar (TSA) and incubated at 30-35 °C and 40% of the total volume was plated on Sabouraud Dextrose Agar (SDA) and incubated at 20-25 °C. Plates were assessed for colonies following 3 and 7 days incubation. Growth promotion was performed on the remaining media to show the cell culture medium could support microbial growth using *Geobacillus stearothermophilus*, *Aspergillus brasiliensis*, and *Candida albicans*.

### Results and Discussion

**Figure 5** shows the pH and osmolality of cell culture medium stored in bags made with Ultimus® film for over 60 days. The pH remained stable at 7.4 with minimal fluctuations, and the osmolality of the cell culture medium remained stable within specifications for the study duration.

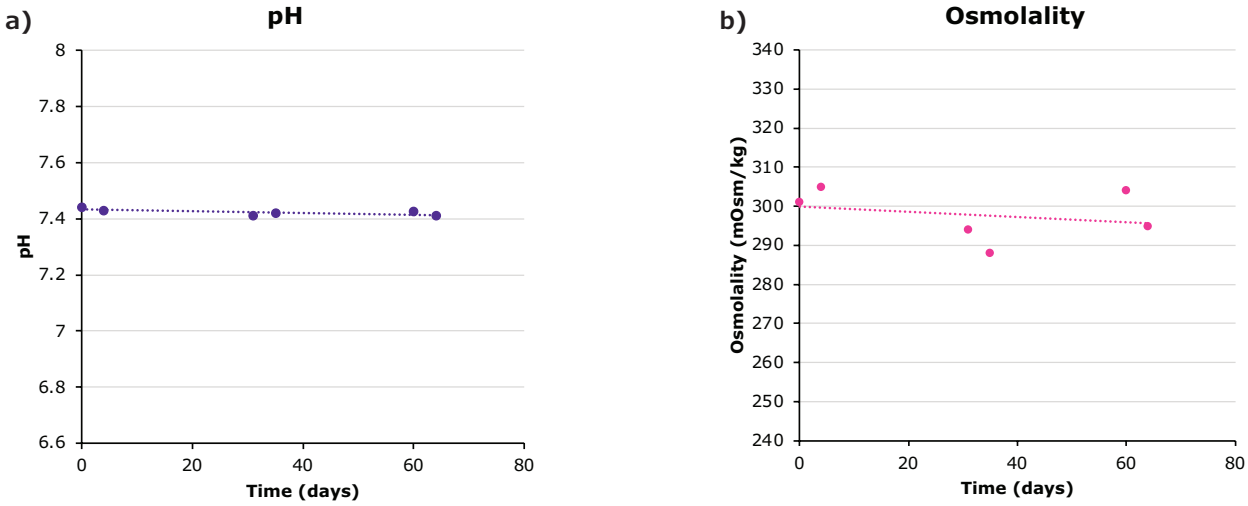


**Figure 5**. Cell culture media properties during 64 days of storage. **a**) pH of media. **b**) Osmolality of media, measured in mOsm/kg.

**Table 4** shows the bioburden test results from cell culture medium stored in bags made with Ultimus® film for over 60 days. No microorganisms were detected after 3 and 7 days of growing in TSA and SDA, confirming the medium remained sterile during extended storage. Growth promotion results demonstrated the cell culture media could support microbial growth.

**Table 4. Bioburden measurements, number of microorganisms (CFU) found in the medium after 3 and 7 days incubation on TSA and SDA.**

| Sample | Day 3 TSA | Day 3 SDA | Day 7 TSA | Day 7 SDA |
|---|---|---|---|---|
| Sample 1 60 days | <3 | <3 | <3 | <3 |
| Sample 2 60 days | <3 | <3 | <3 | <3 |
| Sample 3 64 days | <3 | <3 | <3 | <3 |
| Sample 4 64 days | <3 | <3 | <3 | <3 |

## Conclusion

Process buffers and cell culture media were stored in bags made from Ultimus® film for over 60 days to compare their stability, integrity, and suitability for biopharmaceutical applications when compared to traditional glass bottles. Three studies were conducted and results confirmed:

- In the Buffer Stability study the results showed that various buffers, at different concentrations and pH levels, maintained stable pH and conductivity when stored in bags made from Ultimus® film.
- In the Water Hold to Evaluate Consistent Media Quality study, results confirmed that media quality using water that had been stored in bags made from Ultimus® film delivered equivalent cell growth to media that used water stored in glass bottles.
- In the Media Hold for bioburden, no microorganisms were detected following the extended storage period.

These studies clearly demonstrate that leachables from Ultimus® film do not pose any additional risk for long-term storage of process buffers and media, and provide a useful data set to support implementation of bags and containers made from Ultimus® film into the bioprocessing workflow.

## Emprove® Program: Your Technical, Regulatory and Supply Information

The Emprove® Program (**SigmaAldrich.com/emprove**) provides convenient online access to comprehensive documentation needed for implementation of single-use systems in pharmaceutical manufacturing. Detailed product information contained within the Emprove® Dossiers reduces the time and investment typically needed to gather and/or prepare documentation and enables more agile, risk-based decisions. The Emprove® Program includes single-use components, filters, connectors, and films, as well as a wide range of other products used in bioprocessing such as chromatography resins, cell culture media and chemicals. The description for the filter and single-use dossiers are below:

- The Emprove® Material Qualification Dossiers (MQD) include product test specifications, release criteria, and regulatory information for the components as well as materials used for component qualification.

- The Emprove® Quality Management Dossiers (QMD) includes quality and validation information including site qualification, irradiation sterilization process and packaging validations and site qualification.
- The Emprove® Operational Excellence Dossier (OED) includes extractables data following BioPhorum and USP <665> guidance to support patient safety evaluation. OED's are available for individual filters.
- The Emprove® Component Extractable Reports includes extractables data following USP <665> to support patient safety evaluation. The reports are for single-use components made by third-party suppliers.

**Dossier specifically for single-use assemblies** The Emprove® Advanced Qualification Dossier (AQD) was developed to provide biomanufacturers with the information they need to support risk assessments on single-use assemblies containing multiple components. This first-of-its-kind dossier supports custom and configurable assemblies and includes:

All available component qualification information along with extensive USP <665> extractables data for all the components in the assembly.

Information on extractables, bill of materials, regulatory statements, component surface area information, original manufacturer information, shelf-life statements, and customer-approved drawings of the assemblies.

The data needed for end users to conduct risk assessments and patient safety evaluations of their assemblies.

**References**


1. Shukla, Abhinav A., and Uwe Gottschalk. "Single-use disposable technologies for biopharmaceutical manufacturing." Trends in biotechnology 31.3 (2013): 147-154.
2. Understanding the Impact of Extractables and Leachables from Single-Use Films (Lit. # AN14138EN).
3. Single-Use Upstream Processing: Ultimus® Film Delivers Comparable Cell Growth Performance To Glass (Lit. # AN9607EN).
4. Eibl, R., et al. "Standardized cell culture test for the early identification of critical films for CHO cell lines and chemically defined culture media."DECHEMA, Frankfurt aM ISBN (2014): 978-3.


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