

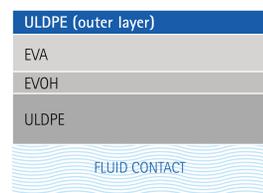
PureFlex™ Plus Single-use Process Container Film Extractables and Leachables Study

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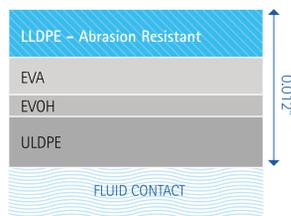
Introduction

- The newly developed PureFlex™ Plus film is built on a combination of low and ultra low density polyethylene, polyvinyl acetate and poly vinyl alcohol layers to provide enhanced mechanical strength, creep resistance and limited permeability to air and gases.
- This study addresses the bioprocess industry's need to evaluate and understand single-use process container extractables over prolonged periods of time.

PureFlex™ film



PureFlex™ Plus film



Film Construction Materials

- PureFlex™ and PureFlex™ Plus films** are constructed utilizing a high-purity, medical grade, composite film designed to provide strength, resistance to flex-cracking, gas barrier and inert contact.
- PureFlex™ Plus film** is constructed with a tough, linear low density polyethylene (LLDPE) outer layer that increases the film's resistance to leak formation through abrasion, puncture, stretching, and tearing.

Model Stream Approach and Analysis

- PureFlex™ and PureFlex™ Plus single-use 5 L process containers were evaluated in a model stream approach designed to simulate usage in worst-case conditions, including extremes of pH and the presence of organic solvents.
- Containers were Gamma treated at >45 kGy.
- In order to simulate worst-case conditions with respect to extractable levels, the containers were not rinsed prior to the static soak.
- This study was conducted at room temperature and at 45°C for over 120 days.

Model Solvents used for Extraction:

- Milli-Q® reagent-grade water
- 1N sodium hydroxide
- 1N hydrochloric acid
- 50% Ethyl Alcohol
- 10% Dimethyl sulfoxide

Analysis:

TOC (except ethanol and DMSO), RP-HPLC profiles at 214 and 254 nm UV, Ion Chromatography (water only), Purge and trap GC-MS for volatiles and ICP-OES for metals (water only)

Extraction Procedure

- Individual containers served as extraction vessels
- Containers were placed in stands designed to allow each container to expand, such that the surface area to volume ratio would be maintained at 2 sq. cm per mL
- Multiple sample aliquots were removed from each container without altering the surface area to volume ratio.
- The stands held four containers each with the containers separated by aluminum panels. Each stand contained three (3) gamma-treated containers and one (1) non-treated container.
- Each stand contained one film type, filled with one type of solution and was stored at room temperature or 45°C.

Extraction Test Stand



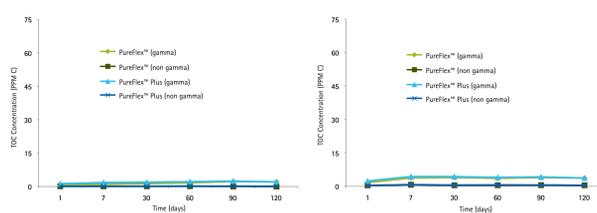
Learn more at

www.merckmillipore.com/PureFlexPlus

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FIGURE 1: TOC of water extracts at ambient and 45°C over extraction time (ppm C)

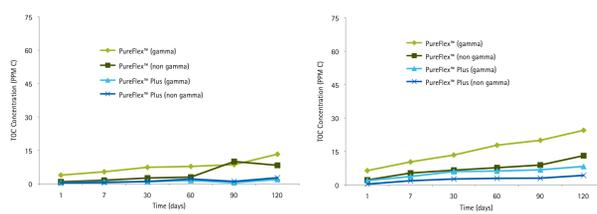


Graphical representation of TOC results in PPM of carbon in the Milli-Q® water extraction solution at ambient temperature

Graphical representation of TOC results in PPM of carbon in the Milli-Q® water extraction solution at 45°C

The PureFlex™ Plus single-use containers had slightly lower TOC in water than the PureFlex™ containers. This difference was not significant because the fluid contact layer for PureFlex™ and PureFlex™ Plus film is the same. TOC for single-use containers stored at 45°C was greater compared at room temperature due to higher rates of diffusion through the film.

FIGURE 2: TOC of 1N NaOH extracts at ambient and 45°C over extraction time.

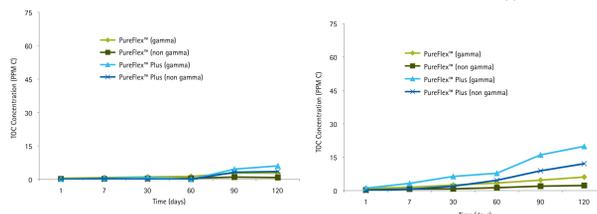


Graphical representation of TOC results in PPM of carbon in the 1N NaOH extraction solution at ambient temperature

Graphical representation of TOC results in PPM of carbon in the 1N NaOH extraction solution at 45°C

The PureFlex™ Plus single-use film containers had lower TOC in NaOH than the PureFlex™ containers. This difference was not significant because the fluid contact layer for PureFlex™ and PureFlex™ Plus film is the same. TOC for single-use containers stored at 45°C was significantly greater compared at room temperature due to higher rates of diffusion through the film.

FIGURE 3: TOC of 1N HCl extracts at ambient and 45°C over extraction time (ppm C)

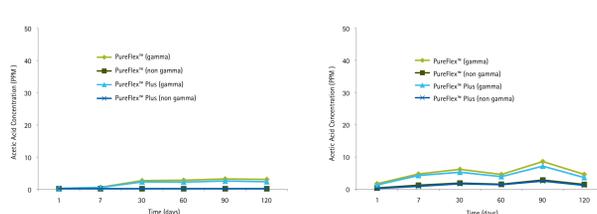


Graphical representation of TOC results in PPM of carbon in the 1N HCl extraction solution at ambient temperature

Graphical representation of TOC results in PPM of carbon in the 1N HCl extraction solution at 45°C

The TOC concentrations in 1N HCl increased as extraction time and temperature increased due to higher rate of diffusion rate through the film. PureFlex™ Plus film showed slightly higher TOC than PureFlex™ film.

FIGURE 4: Acetate ion in water extraction at ambient and at 45°C over extraction time (ppm C)

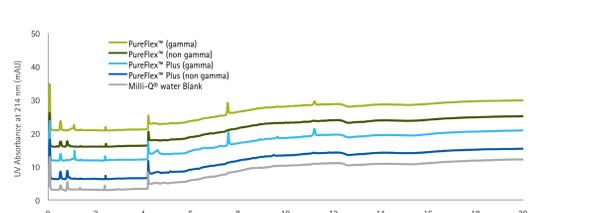


Acetic Acid Concentration in PPM versus time for containers extracted with Milli-Q® water at ambient temperature

Acetic Acid Concentration in PPM versus time for containers extracted with Milli-Q® water at 45°C

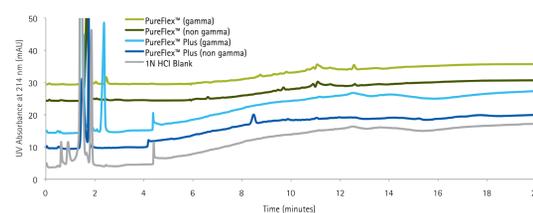
The organic acids detected by IC were: formic acid, acetic acid, propionic acid, and butyric acid formed as breakdown products of gamma irradiation and subsequent oxidation reactions. The IC data showed similar trends to TOC data. The PureFlex™ Plus single-use containers had lower concentrations than the PureFlex™ containers for acetic acid similar to TOC.

FIGURE 5: HPLC water profiles of 30 Days extraction at ambient temperature at 214 nm UV



RP-HPLC Chromatograms of 30 Days Milli-Q® water Extractables at ambient temperature

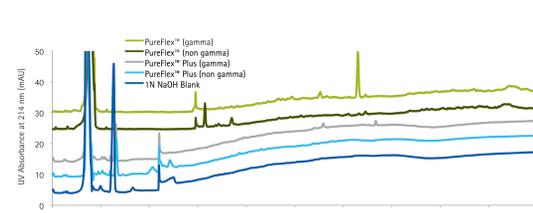
FIGURE 6: HPLC Profiles of 1N HCl extracts of 30 Days at ambient temperature at 214 nm UV



RP-HPLC Chromatograms of 30 Days 1N HCl Extractables at ambient temperature

RP-HPLC is not generally capable of detecting all the potential extractables from thermoplastics, but it can often provide a fingerprint-type pattern for a subset of the extractables. It gives a clear indication whether extractables have the potential to interfere with an active pharmaceutical ingredient (API) or other solute. HPLC profiles indicated no significant difference between PureFlex™ and PureFlex™ Plus films in all the model streams.

FIGURE 7: HPLC Profiles of 1N NaOH extracts of 30 Days at ambient Temperature at 214 nm UV



RP-HPLC Chromatograms of 30 Days 1N NaOH Extractables at ambient temperature

Overall, there were very few peaks observed in the HPLC chromatographs. Relatively more peaks were observed in the extractions from the gamma-treated container compared to the extractions from the non-gamma-treated container. The samples extracted at 45°C (data not shown) had more peaks of larger area than observed in the samples extracted at room temperature.

Conclusion

- TOC:** Several trends in TOC values were noticed. Generally TOC increased with increased in extraction time and with temperature.
- IC:** The organic acids detected by IC were: formic acid, acetic acid, propionic acid, and butyric acid formed as breakdown products of gamma irradiation and subsequent oxidation reactions. The PureFlex™ Plus single-use containers had slightly lower values than the PureFlex™ containers for acetic acid.
- Comparison of RP-HPLC chromatograms for PureFlex™ and PureFlex™ Plus films showed similar peak profiles.
- ICP-OES:** The water extractables of both PureFlex™ and PureFlex™ Plus films showed no detectable metals.
- GC-MS:** The non-gamma irradiated PureFlex™ and PureFlex™ Plus single-use containers showed significantly lower VOC and SVOC but similar levels of acetone and acetaldehyde. The gamma-treated containers had three classes of compounds present: ketones (e.g., acetone, 2-butanone), aldehydes (e.g., acetaldehyde, butanal, pentanal) and alcohols (e.g., tert-butyl alcohol). The three compounds most frequently detected were: acetone, 2-butanone and tert-butyl alcohol.

Summary

- Overall, both PureFlex™ and PureFlex™ Plus films showed similar levels of TOC, RP-HPLC profiles extractables, with some small variation depending on the extraction solution.
- In GC-MS, both PureFlex™ and PureFlex™ Plus films revealed similar volatile and semi-volatile organic compounds.
- PureFlex™ and PureFlex™ Plus films showed similar levels of anion extractables.
- No metals were detected by ICP-OES for both films.
- Based on the results of this set of studies, there is no observable, significant difference in the extractables profiles from the PureFlex™ and PureFlex™ Plus films.
- The data generated in this study should only be used as a general guide. Results presented here should not be interpreted as providing absolute levels of extractables for disposable process containers used under process conditions.

Footnotes:

¹ EMD Millipore, 2009, Extractables Testing Report: PureFlex™ and SureFlex™ Disposable Process Container Films