

White Paper

PureFlex™ and PureFlex™ Plus Disposable process container films extractables evaluation

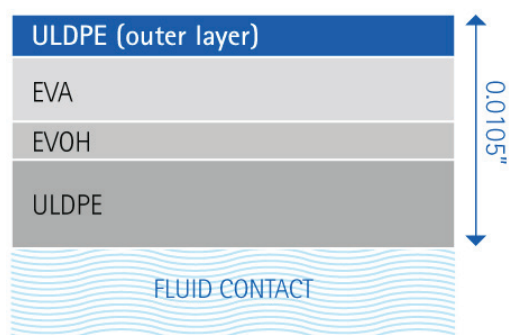
Biopharmaceutical companies are using disposable assemblies at an ever increasing rate, as single-use technologies are gaining acceptance for an increasing range of applications. Our single-use flexible process containers are designed specifically for bioprocess operations, storage, and transport of biopharmaceutical fluids under sterile conditions. They are free of animal derived components.

The purpose of this report is to provide extractable data relevant to disposable process containers assembled with our PureFlex™ or PureFlex™ Plus films and to demonstrate the comparability of the extractables profile from the two films. This report is a compilation of three studies performed using PureFlex™ and PureFlex™ Plus film.

PureFlex™ disposable process containers are manufactured utilizing a high-purity, medical grade, composite film designed to provide strength, flexibility, excellent gas barrier performance, and an inert contact layer. The fluid contact material is made of ultra low-density polyethylene (ULDPE). The gas barrier is made of a polyethylene vinyl alcohol copolymer (EVOH). The outer layers are made of polyethylene vinyl acetate (EVA) and ULDPE.

PureFlex™ Plus film is constructed with a tough, linear low density polyethylene (LLDPE) outer layer. This rugged outer layer increases the film's resistance to leak formation through abrasion, puncture, stretching, and flex-cracking. The inner layers of PureFlex™ Plus film, including the product contact layer, are identical to PureFlex™ film.

PureFlex™ film



PureFlex™ Plus film

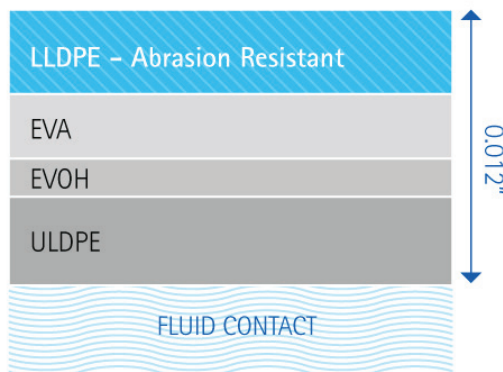


Table 1.
Disposable Process Containers –
Materials of Construction

Test procedure summaries

The PureFlex™ and PureFlex™ Plus disposable process containers (described in Table 1) were evaluated in model solvent streams designed to simulate possible usage in worst-case conditions, including extremes of pH and the presence of organic solvents.^{1,2} The containers were not rinsed prior to the static soak in order to simulate worst-case conditions with respect to extractable levels. Testing was conducted under the guidance of our Standard Operating Procedure #0065342SO. The three studies were conducted at different times, so there were some differences between the methods.

1. Study #1 was a 120-day study with sampling occurring throughout the study. The study consisted of five different extraction solutions: Milli-Q® reagent grade water, 1N sodium hydroxide, 1N hydrochloric acid, 50% ethanol, and 10% dimethylsulfoxide (DMSO). The analyses included: total organic carbon (TOC), ion chromatography (IC), and high performance liquid chromatography (HPLC). This study included containers made of PureFlex™ or PureFlex™ Plus films for water and PureFlex™ Plus only for other solutions.

2. Study #2 was a 90-day study with sampling occurring at the end of the study. The extraction solution was Milli-Q® reagent grade water. The analyses included inductively-coupled plasma – optical emission spectrometer (ICP-OES) for metals. This study included containers made of PureFlex™ or PureFlex™ Plus films.

3. Study #3 was a 30-day study with sampling occurring at the end of the study. The extraction solutions consisted of Milli-Q® water, 1N sodium hydroxide, 1N hydrochloric acid and 50 % ethanol. The analyses included: GC-MS for volatile organic compounds (VOC) and for semi-volatile organic compounds (SVOC). This study included containers made of PureFlex™ Plus film.

Study #1: Test steps

Step 1. PureFlex™ and PureFlex™ Plus containers (5 L volume) were received untreated by gamma irradiation. A majority of the containers were sent for gamma sterilization (>45 kGy), while the rest were left untreated for use as controls. The PureFlex™ and PureFlex™ Plus containers came equipped with tubing and connectors (1 port per container). The tubing at each port was cut to a length of approximately 3", removing the connectors. All container ports were closed using acetal screw clamps on the tubing, and the clamps never contacted the extraction solutions.

Step 2. The 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:1 mL. The stands allowed for multiple sample aliquots to be 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 gamma-treated containers and one non-treated container. Each stand contained one film type, filled with one type of solution and was stored at room temperature or 45°C.

Step 3. Disposable process containers were extracted with one of the following solutions:

- Water: 18.2 MΩ·cm ultrapure water from a Milli-Q® Lab Water System (Milli-Q® reagent grade water)
- 1 N sodium hydroxide solution pH 14 (NaOH), EMD Chemicals, Catalogue No. SX0597-1
- 1 N hydrochloric acid solution pH 0 (HCl), EMD Chemicals, Catalogue No. HX0603F-6
- 50% denatured ethanol 3A (EtOH), OmniSolv® HPLC Grade, Catalogue No. MAX0445
- 10% dimethylsulfoxide (DMSO), Alfa Aesar HPLC Grade, Catalogue No. AA22914

Sample aliquots were removed from the containers on days: 1, 7, 30, 60, 90 and 120. Aliquots of the initial solutions were used as controls.

Step 4. Extractables in the soak solutions were quantified using total organic carbon (TOC) and ion chromatography (IC), and qualitatively examined using reverse phase high performance liquid chromatography (RP-HPLC).

In addition, further testing was conducted and results are available on request for the following:

Extraction solvents: WFI at pH 10, WFI at pH 3, and 5M NaCl

Extracted at 45°C

Sampling at Day 1, 7, 30, 60, 90, and 120

Study #2: Test steps

Step 1. PureFlex™ and PureFlex™ Plus disposable process containers (5 L volume) were received gamma-sterilized (>45 kGy). No further sterilization was performed.

Step 2. Disposable process containers were extracted with 18.2 MOhm-cm ultrapure water from a Milli-Q® Lab Water System (Milli-Q® reagent grade water). The disposable process containers were exposed to water and placed in a lab oven at 45°C for 90 days.

Step 3. Metal analysis was performed using inductively coupled plasma optical emission spectrometry (ICP-OES).

Study #3: Test steps

Step 1. PureFlex™ Plus disposable process containers (5 L volume) were gamma sterilized (>45 kGy), except for one set used for a portion of the GC-MS analysis that was not irradiated. No further sterilization was performed.

Step 2. Disposable process containers were extracted with one of the following solutions:

- Water: 18.2 MOhm-cm ultrapure water from a Milli-Q® Lab Water System (Milli-Q® reagent grade water)
- 1 N hydrochloric acid solution pH 0 (HCl)
- 1 N sodium hydroxide solution pH 14 (NaOH)
- 50% denatured ethanol (EtOH)

Disposable process containers were exposed to each of the above solutions at 45°C for 30 days.

Step 3. Volatile organic compounds (VOC Purge and Trap) and semi-volatile organic compounds (SVOC) were performed using Gas Chromatography-Mass Spectrometry (GC-MS).

Acceptance criteria

The TOC results were quantified using internal standards. A standard check was analyzed on the same day as the samples to verify that the internal calibration was valid. The IC results were quantified using standards analyzed on the same day. The ICP-OES metals concentrations were quantified using standards and the results reported. GC-MS results were quantified and the results were reported.

The RP-HPLC analyses of the extraction solutions indicate whether or not significant peaks could be introduced into a customer stream by the disposable process containers. Such information can be useful to the customer because any unknown HPLC peak observed in an active pharmaceutical ingredient (> 0.1% of the active pharmaceutical ingredient (API)) must be investigated and its toxicology understood. HPLC peaks from disposable process containers would not be considered unknowns, even if their molecular structure is not known, because their source is known (the container) and the toxicology of the containers has been well documented.

Experimental procedure

Components: In Study #1, four 5 L containers were obtained for each model stream to be tested, for each extraction time (e.g., 4 PureFlex™ or PureFlex™ Plus containers x 5 extraction solvents x 2 temperature conditions = 40 PureFlex™ and PureFlex™ Plus containers required). In Study #2, the same 5 L container was used for each model stream to be tested. In Study #3, the same 5 L container was used for each model stream to be tested.

Sterilization: The containers were received untreated by gamma irradiation. The majority of the containers were sent for gamma irradiation (>45 kGy). No further sterilization was performed. In Study #1 and Study #3, a portion of the containers were left untreated to be used as controls.

Reagents:

- Milli-Q® reagent grade water
- 1N NaOH pH 14: Milli-Q® water adjusted to 1N with ACS grade NaOH
- 1N HCl pH 0: Milli-Q® water adjusted to 1N with ACS grade concentrated HCl
- HPLC grade denatured ethanol or equivalent, diluted to 50% with water
- DMSO: HPLC grade, diluted to 10% with water

Materials – TOC Analyses: 40 mL disposable glass vials with PTFE-lined lids were used to collect the samples.

Materials – GC-MS VOC and SVOC:

- 4 mL glass vials with PTFE-lined lids were used for most of the HPLC analyses
- 2 mL glass vials with PTFE-lined lids were used for the IC analyses
- Glass or polyethylene pipettes were used to transfer the samples from the 40 mL vials to the smaller sample vials
- Perkin Elmer polyethylene disposable test tubes were used for sampling prior to the ICP-OES metals analysis.

Analytical methods

Total Organic Carbon (TOC)

The organic components in the respective extraction solutions were acidified and oxidized to carbon dioxide in the TOC analyzer. The net amount of carbon dioxide present was used to quantify the oxidizable carbon in the sample.

Reverse phase high performance liquid chromatography (RP-HPLC)

RP-HPLC was used to analyze extractables solutions for low levels of organic solutes. RP-HPLC is sensitive to most low-molecular weight solutes such as solvents, monomers, or polymer additives, but does not generally detect polymeric or oligomeric solutes. The HPLC method used a C18 column and a gradient flow from 100% Milli-Q® reagent grade water to 100% acetonitrile. Detection was performed using UV detectors at 214 nm and 254 nm.

Ion Chromatography (IC)

IC was used to identify and quantify small organic acids present in the extracts. The IC method used an IonPac® column and a gradient flow from 3 mM to 50 mM sodium hydroxide. Detection was performed using a conductivity detector.

Inductively coupled plasma optical emission spectrometry (ICP-OES)

ICP-OES is a type of spectrometer that is highly sensitive and capable of the determination of a range of metals and several non-metals in liquid solutions. The ICP-OES allows determination of elements by measuring the intensity of characteristic optical emission lines and capable of providing detection limits ranging from 100 ppm to 10 ppb (100 µg/mL to 0.010 µg/mL),

Gas Chromatography – Mass Spectrometry (GC-MS)

GC-MS is a technique that combines the separation feature of gas-liquid chromatography and the identification capability of mass spectrometry. The GC separates chemicals based on their volatility. The separated molecules are then ionized and fragmented in a quadrupole mass spectrometer. The fragmented ions are then detected based on their mass to charge ratios. There are two separate GC-MS methods utilized, purge and trap for VOC and solvent extraction for SVOC. These methods fundamentally differ in their sample preparation prior to the GC-MS analysis. The GC-MS VOC and SVOC testing of the extractables were performed in labs at EMD Millipore in Bedford and Billerica, Massachusetts.

Extraction

Study #1

- a) Individual containers served as extraction vessels.
- b) Each 5 L disposable container was filled with 800 mL of solution. The stands were made such that a 2:1 surface area to volume ratio (cm^2/mL) was always maintained, even as volume was removed for sample analyses.
- c) The tubing ports of each disposable process container were clamped shut.
- d) The containers sat undisturbed until the time of sampling. At each sampling interval the stands were placed in a holder which would allow the stands to tilt. Samples would be collected from the port by filling a 40 mL vial. (In the case of the NaOH and HCl solutions, the vials were pretreated with neutralizing solutions). After mixing, 2 mL and 4 mL aliquots from the 40 mL vial were then transferred to smaller vials for the IC and HPLC analyses. The remainder of the solution in the 40 mL vial was used for the TOC analysis.

Study #2

- a) As in Study #1, 5 L disposable process containers served as extraction vessels. Wide-mouth PTFE bottles or glass bottles served as control vessels. For metal analysis, fresh controls in Perkin Elmer test tubes were also generated at the time of container sampling.
- b) To avoid microbial growth that could interfere with the analytical tests, water was sterilized by filtration with a 0.22 μm sterile Millipak® 100 filter unit (catalog number MPGL1GCA3), prior to filling the process containers. The filters were initially flushed with 500 mL of extraction solution, and then the process containers were filled.
- c) The ports of each disposable process container were clamped; the PTFE control vessels were sealed to prevent evaporation.
- d) Samples from the disposable process containers were collected after 90 days of extraction at 45°C. Following the extraction procedure, the solutions and controls were transferred to sample vials and containers for analysis, and the remaining solutions were retained in storage bottles.

Study #3

- a) As in study #1, 5 L disposable process containers served as extraction vessels. Wide-mouth PTFE bottles or glass bottles served as control vessels.
- b) Each disposable container was filled with solvent to obtain a 2:1 surface area to volume ratio (cm^2/mL). The solvent/surface area calculations were recorded on the appropriate worksheet. The filled disposable process containers were oriented such that all of the interior surface area was contacted by the extraction solvent. A PTFE or glass vessel was filled with the appropriate control solution.

- c) To avoid microbial growth that could interfere with the analytical tests, water was sterilized by filtration with a 0.22 µm sterile Millipak® 100 filter unit (Catalogue No. MPGL1GCA3) prior to filling the process containers. The filters were initially flushed with 500 mL of extraction solution, and then the process containers were filled. The other solutions were not filtered; they were not expected to sustain microbial growth.
- d) The ports of each disposable process container were clamped; the PTFE control vessels were sealed to prevent evaporation.
- e) Samples from the disposable process containers were collected after 30 days at 45°C. Following the extraction procedure, the soak solutions were transferred to 40 mL TOC sample vials for analysis.

Extraction samples were analyzed according to the following sample schedule shown in Tables 2 through 4.

Table 2.
Sample Schedule
for Study # 1.

Film	Solvent	# Bags – Sterilization	Temperature	Extraction times	Analyses
Pureflex™	Milli-Q® water	3 - treated 1 - untreated	Room temperature	1, 7, 30, 60, 90 and 120 days	TOC, IC, RP - HPLC
Pureflex™	Milli-Q® water	3 - treated 1 - untreated	45°C	1, 7, 30, 60, 90 and 120 days	TOC, IC, RP - HPLC
PureFlex™ Plus	Milli-Q® water	3 - treated 1 - untreated	Room temperature	1, 7, 30, 60, 90 and 120 days	TOC, IC, RP - HPLC
PureFlex™ Plus	Milli-Q® water	3 - treated 1 - untreated	45°C	1, 7, 30, 60, 90 and 120 days	TOC, IC, RP - HPLC
PureFlex™ Plus	1N NaOH	3 - treated 1 - untreated	Room temperature	1, 7, 30, 60, 90 and 120 days	TOC, RP - HPLC
PureFlex™ Plus	1N NaOH	3 - treated 1 - untreated	45°C	1, 7, 30, 60, 90 and 120 days	TOC, RP - HPLC
PureFlex™ Plus	1N HCl	3 - treated 1 - untreated	Room temperature	1, 7, 30, 60, 90 and 120 days	TOC, RP - HPLC
PureFlex™ Plus	1N HCl	3 - treated 1 - untreated	45°C	1, 7, 30, 60, 90 and 120 days	TOC, RP - HPLC
PureFlex™ Plus	50% Ethanol	3 - treated 1 - untreated	Room temperature	1, 7, 30, 60, 90 and 120 days	RP - HPLC
PureFlex™ Plus	50% Ethanol	3 - treated 1 - untreated	45°C	1, 7, 30, 60, 90 and 120 days	RP - HPLC
PureFlex™ Plus	10% DMSO	3 - treated 1 - untreated	Room temperature	1, 7, 30, 60, 90 and 120 days	IC, RP - HPLC
PureFlex™ Plus	10% DMSO	3 - treated 1 - untreated	45°C	1, 7, 30, 60, 90 and 120 days	IC, RP - HPLC

Table 3.
Sample Schedule
for Study # 2.

Film	Solvent	# Bags – Sterilization	Temperature	Extraction times	Analyses
Pureflex™	Milli-Q® water	3 - treated 1 - untreated	45°C	90 days	ICP
PureFlex™ Plus	Milli-Q® water	3 - treated 1 - untreated	45°C	90 days	ICP

Film	Solvent	# Bags – Sterilization	Temperature	Extraction times	Analyses
Pureflex™	Milli-Q® water	3 - treated 1 - untreated	45°C	1, 7, 30 days	GC - PT GC - HS GC - DI
PureFlex™ Plus	Milli-Q® water	3 - treated 1 - untreated	45°C	1, 7, 30 days	GC - PT GC - HS GC - DI
PureFlex™ Plus	1N NaOH	3 - treated 1 - untreated	45°C	30 days	GC - PT GC - DI
PureFlex™ Plus	1N HCl	3 - treated 1 - untreated	45°C	30 days	GC - PT GC - DI
PureFlex™ Plus	50% Ethanol	3 - treated 1 - untreated	45°C	1, 7, 30 days	GC - PT GC - HS GC - DI

Table 4.
Sample Schedule
for Study # 3.

GC-PT: gas chromatography purge and trap

GC-HS: gas chromatography head space

GC-DI: gas chromatography direct injection

Analysis results

Study #1. TOC analyses of the extraction solutions results are summarized in Tables 5 through 10.

The alkaline (1N NaOH) solutions were acidified to < pH 3, with acid at the time of sampling. The acidic

(1N HCl) solutions were adjusted to pH 3, with a sodium hydroxide solution at the time of sampling.

Results in blue are from previous extraction studies for PureFlex™ film which are summarized in the

white paper, "Extractables Testing Report- PureFlex™ and SureFlex™ Disposable Process Container Films".

Comparisons of the TOC levels from the PureFlex™ and PureFlex™ Plus film extractions are shown for each solution in Figures 1 through 6.

Temp	Film	Sample	Day 1	Day 7	Day 30	Day 60	Day 90	Day 120
Room Temp	PureFlex™	Average of 3 γ -irradiated containers	0.60	1.00	1.32	1.71	2.23	2.06
		Non- γ irradiated container	0.11	0.10	0.11	0.17	0.05	0.05
	PureFlex™ Plus	Average of 3 γ -irradiated containers	1.25	1.75	1.93	2.12	2.41	2.07
		Non- γ irradiated container	0.13	0.10	0.13	0.14	0.15	0.10
45 °C	PureFlex™	Average of 3 γ -irradiated containers	1.66	3.73	3.89	3.61	3.89	3.69
		Non- γ irradiated container	0.42	0.69	0.44	0.45	0.53	0.44
	PureFlex™ Plus	Average of 3 γ -irradiated containers	2.36	4.37	4.33	3.98	4.21	3.68
		Non- γ irradiated container	0.33	0.70	0.55	0.61	0.58	0.37

Table 5.
TOC Concentrations
(PPM C) for
Extractables in
Milli-Q® reagent
grade water

Temp	Film	Sample	Day 1	Day 7	Day 30	Day 60	Day 90	Day 120
Room Temp	PureFlex™	Average of 3 γ -irradiated containers	0.30	0.47	0.59	0.72	0.89	0.77
		Non- γ irradiated container	0.05	0.05	0.05	0.07	0.02	0.02
	PureFlex™ Plus	Average of 3 γ -irradiated containers	0.62	0.83	0.86	0.90	0.96	0.77
		Non- γ irradiated container	0.07	0.05	0.06	0.06	0.06	0.04
45 °C	PureFlex™	Average of 3 γ -irradiated containers	0.82	1.57	1.34	0.98	0.95	0.72
		Non- γ irradiated container	0.21	0.29	0.15	0.12	0.13	0.09
	PureFlex™ Plus	Average of 3 γ -irradiated containers	1.17	1.84	1.50	1.08	1.04	0.72
		Non- γ irradiated container	0.16	0.30	0.19	0.17	0.14	0.07

Table 6.
TOC Concentrations
($\mu\text{g C/cm}^2$) for
Extractables in
Milli-Q® reagent
grade water

Table 7.
TOC Concentrations
(PPM C) for
Extractables in
1N NaOH

Temp	Film	Sample	Day 1	Day 7	Day 30	Day 60	Day 90	Day 120
Room Temp	PureFlex™	Average of 3 γ -irradiated containers	3.90	5.40	7.40	7.80	8.60	13.3
		Non- γ irradiated container	0.90	1.60	2.60	3.00	10.0	8.30
	PureFlex™ Plus	Average of 3 γ -irradiated containers	0.51	0.86	0.92	1.49	0.49	1.95
		Non- γ irradiated container	0.42	0.58	1.05	2.15	1.07	2.73
45 °C	PureFlex™	Average of 3 γ -irradiated containers	6.40	10.3	13.4	17.8	20.0	24.5
		Non- γ irradiated container	2.20	5.30	6.60	7.70	8.90	13.1
	PureFlex™ Plus	Average of 3 γ -irradiated containers	1.93	3.75	5.98	6.21	6.75	8.28
		Non- γ irradiated container	0.35	1.84	2.63	2.93	3.00	4.25

(Data in blue was obtained from previous white paper published in 2010)

Table 8.
TOC Concentrations
($\mu\text{g C/cm}^2$) for
Extractables in
1N NaOH

Temp	Film	Sample	Day 1	Day 7	Day 30	Day 60	Day 90	Day 120
Room Temp	PureFlex™	Average of 3 γ -irradiated containers	1.97	2.70	3.70	3.89	4.29	6.66
		Non- γ irradiated container	0.43	0.82	1.29	1.49	4.98	4.15
	PureFlex™ Plus	Average of 3 γ -irradiated containers	0.27	0.43	0.44	0.68	0.21	0.78
		Non- γ irradiated container	0.22	0.29	0.50	0.97	0.46	1.10
45 °C	PureFlex™	Average of 3 γ -irradiated containers	3.20	5.95	6.70	8.88	10.02	12.3
		Non- γ irradiated container	1.10	3.28	3.31	3.87	4.43	6.57
	PureFlex™ Plus	Average of 3 γ -irradiated containers	1.01	1.87	2.84	2.49	2.53	2.90
		Non- γ irradiated container	0.18	0.93	1.27	1.19	1.14	1.51

(Data in blue was obtained from previous white paper published in 2010)

Table 9.
TOC Concentrations
(PPM C) for
Extractables in
1N HCl

Temp	Film	Sample	Day 1	Day 7	Day 30	Day 60	Day 90	Day 120
Room Temp	PureFlex™	Average of 3 γ -irradiated containers	0.50	0.80	1.10	1.30	2.80	2.80
		Non- γ irradiated container	0.20	0.20	0.40	0.50	1.00	0.80
	PureFlex™ Plus	Average of 3 γ -irradiated containers	0.12	0.46	0.88	0.01	0.87	1.95
		Non- γ irradiated container	0.01	0.25	0.01	0.01	3.18	3.51
45 °C	PureFlex™	Average of 3 γ -irradiated containers	1.00	1.50	2.50	3.40	4.70	6.10
		Non- γ irradiated container	0.30	0.70	0.80	1.30	2.00	2.30
	PureFlex™ Plus	Average of 3 γ -irradiated containers	1.10	3.24	6.37	7.79	12.5	16.8
		Non- γ irradiated container	0.01	0.61	1.97	4.62	8.89	12.1

(Data in blue was obtained from previous white paper published in 2010)

Table 10.
TOC Concentrations
($\mu\text{g C/cm}^2$) for
Extractables in
1N HCl

Temp	Film	Sample	Day 1	Day 7	Day 30	Day 60	Day 90	Day 120
Room Temp	PureFlex™	Average of 3 γ -irradiated containers	0.27	0.38	0.55	0.66	1.40	1.40
		Non- γ irradiated container	0.08	0.1	0.21	0.25	0.48	0.40
	PureFlex™ Plus	Average of 3 γ -irradiated containers	0.06	0.22	0.40	0.01	1.83	2.26
		Non- γ irradiated container	0.01	0.12	0.01	0.01	1.28	1.32
45 °C	PureFlex™	Average of 3 γ -irradiated containers	0.52	0.74	1.25	1.72	2.35	3.06
		Non- γ irradiated container	0.14	0.36	0.41	0.67	0.98	1.15
	PureFlex™ Plus	Average of 3 γ -irradiated containers	0.55	1.56	2.89	2.96	5.68	6.55
		Non- γ irradiated container	0.01	0.29	0.90	1.77	3.17	4.01

(Data in blue was obtained from previous white paper published in 2010)

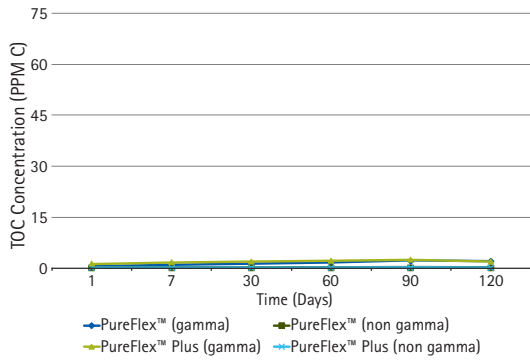


Figure 1.
Graphical representation of TOC results in PPM of Carbon in the Milli-Q® water extraction solution at ambient temperature.

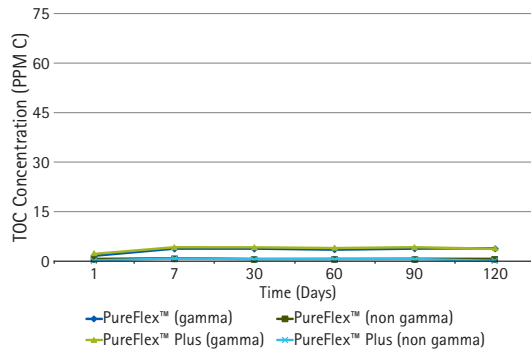


Figure 2.
Graphical representation of TOC results in PPM of carbon in the Milli-Q® water extraction solution at 45°C.

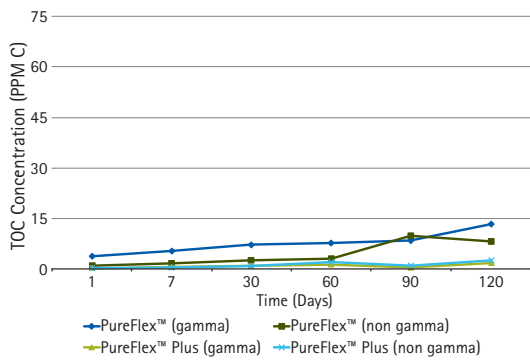


Figure 3.
Graphical representation of TOC results in PPM of Carbon in the 1N NaOH extraction solution at ambient temperature.

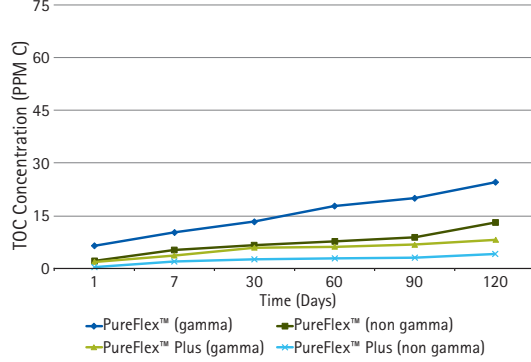


Figure 4.
Graphical representation of TOC results in PPM of Carbon in the 1N NaOH extraction solution at 45°C.

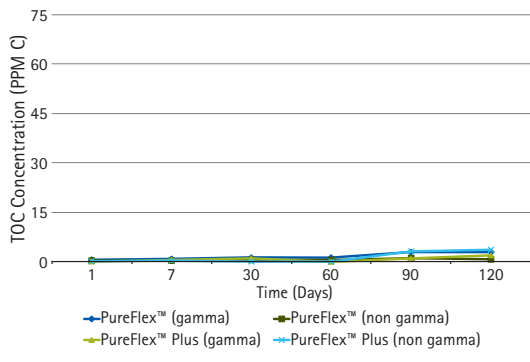


Figure 5.
Graphical representation of TOC results in PPM of Carbon in the 1N HCl extraction solution at ambient temperature.

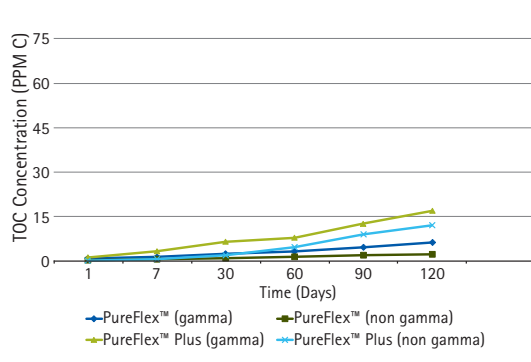


Figure 6.
Graphical representation of TOC results in PPM of Carbon in the 1N HCl extraction solution at 45°C.

Results of the IC analysis of the extractions in Milli-Q® reagent grade water are summarized in Table 11. The IC results are the average of the three gamma-irradiated containers. Results of the IC analysis of the extractions in DMSO are not presented, but are similar to the Milli-Q® reagent

grade water results. (Matrix interferences prevented IC analysis of the NaOH, HCl and EtOH extracts.)

Figures 7 and 8 show a comparison of acetic acid levels of the Milli-Q® solution in the PureFlex™ and PureFlex™ Plus containers over the course of the of extraction study.

Table 11.
IC Results in (PPM)
for Gamma irradiation
containers extractables in
Milli-Q® reagent grade water

Temp	Film	Sample	Day 1	Day 7	Day 30	Day 60	Day 90	Day 120		
Room Temp	PureFlex™	Acetic Acid	0.28	0.65	2.71	2.84	3.23	3.09		
		Propionic Acid	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2		
		Formic Acid	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2		
		Butyric Acid	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2		
	PureFlex™ Plus	Acetic Acid	0.30	0.57	2.31	2.26	2.61	2.36		
		Propionic Acid	<0.2	<0.2	0.33	<0.2	<0.2	0.28		
		Formic Acid	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2		
		Butyric Acid	<0.2	<0.2	<0.2	<0.2	<0.2	<0.2		
		45 °C	PureFlex™	Acetic Acid	1.70	4.73	6.17	4.58	8.59	4.60
				Propionic Acid	<0.2	0.35	0.44	0.42	0.83	0.38
Formic Acid	<0.2			0.31	0.72	0.67	1.42	0.95		
PureFlex™ Plus	Acetic Acid		1.32	4.26	5.27	3.91	7.16	3.61		
	Propionic Acid		<0.2	0.37	0.35	0.36	0.64	0.33		
	Formic Acid		<0.2	0.51	1.03	0.76	1.63	1.03		
		Butyric Acid	<0.2	<0.2	0.30	<0.2	0.38	0.21		

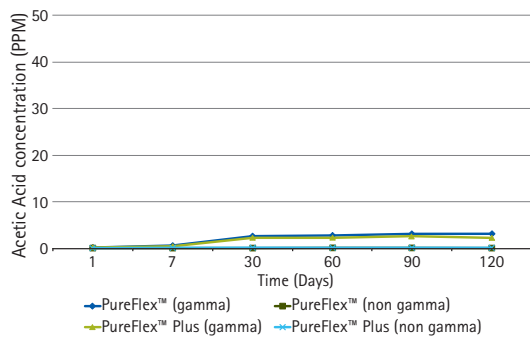


Figure 7.
Acetic Acid Concentration in PPM versus time for containers extracted with Milli-Q® water at ambient temperature.

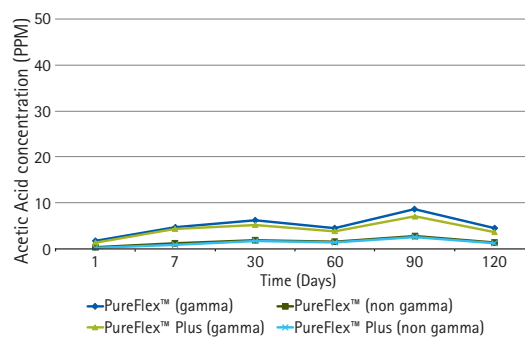


Figure 8.
Acetic Acid Concentration in PPM versus time for containers extracted with Milli-Q® water at 45°C.

Discussion

TOC: The TOC analyses provided the best quantitative data of extractables for all the aqueous-based extractions. Several trends were evident from the TOC data, as shown in Figures 1-6. The greatest TOC extractables were observed with the containers filled with the 1N NaOH solution, and the least with containers filled with the 1N HCl solution for PureFlex™ film (2010 white paper). PureFlex™ Plus film showed higher TOC values for 1N HCl extract than 1N NaOH (2014). The TOC concentrations for containers stored at 45°C were significantly greater compared to the containers stored at room temperature. This was not surprising since the aqueous solubility of most organic compounds tends to increase with an increase in temperature, increasing the diffusion rate of molecules from inside the film to the surface. The gamma-treated containers had higher TOC concentrations than the untreated containers. The PureFlex™ Plus containers had slightly lower TOC concentrations in water than the PureFlex™ containers but were not significantly different. This is because the fluid contact layer for PureFlex™ film is the same as the fluid contact layer for PureFlex™ Plus film.

IC: The four most prominent organic acids detected by IC were formic acid, acetic acid, propionic acid, and butyric acid. They correlate to C1 through C4 organic acids. These organic acids formed as breakdown products from the gamma irradiation and subsequent oxidation reactions. The IC data had similar trends to those observed with the TOC data. The samples stored at 45°C had relatively greater concentrations of organic acids when compared to those stored at room temperature. The gamma-treated containers had higher concentrations than the untreated containers. The PureFlex™ Plus containers had lower concentrations than the PureFlex™ containers (especially for acetic acid). The IC results also were similar to the TOC results in the shape of the curves. In both plots there was an initial rapid increase in concentration, followed by a slow and gradual increase with time.

RP-HPLC: Reverse phase high pressure liquid chromatography (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. Additionally, such chromatograms can give a clear indication whether extractables have the potential to interfere with an active pharmaceutical ingredient (API) or other solute analysis by RP-HPLC, a commonly employed practice.

Overall, there were very few peaks observed in the HPLC chromatographs. Relatively more peaks were observed in the extracts from the gamma-treated container compared to the extracts from the non-gamma-treated container (see Figures 9-13). Overall, the samples extracted at 45°C (data not shown) had more peaks of larger area than observed in the samples extracted at room temperature. The solution that extracted the most compounds was the 50% ethanol (Figure 13). The chromatographs for this solution had the largest peaks, as well as late eluting peaks (hydrophobic compounds). In addition to these late-eluting peaks, intermediate eluting peaks (medium polar) were present in the extract from the container that was treated with gamma irradiation, as seen in Figure 13. This indicated that these intermediate eluting peaks are likely related to an antioxidant additive which has been degraded by the gamma irradiation or has been oxidized. The non-gamma treated container showed only the late eluting peaks. The next strongest extraction solution was the 10% DMSO solution (Figure 12). The NaOH solution (Figure 10) had the largest peaks of the three aqueous solutions, whereas the Milli-Q® water (Figure 9) and HCl solution (Figure 11) had no significant peaks.

Overall the extracts from the PureFlex™ and PureFlex™ Plus containers had similar profiles with a few early-eluting peaks, as well as some late-eluting peaks. In all cases, the peaks observed were relatively small.

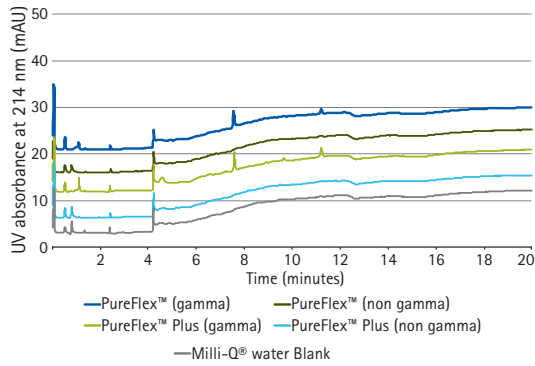


Figure 9.
RP-HPLC Chromatograms of 30 Days Milli-Q® water Extractables at ambient temperature.

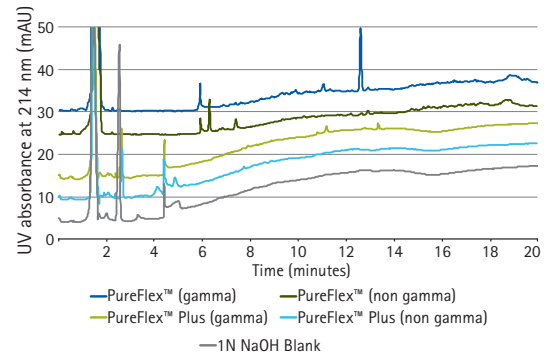


Figure 10.
RP-HPLC Chromatograms of 30 Days 1N NaOH Extractables at ambient temperature.

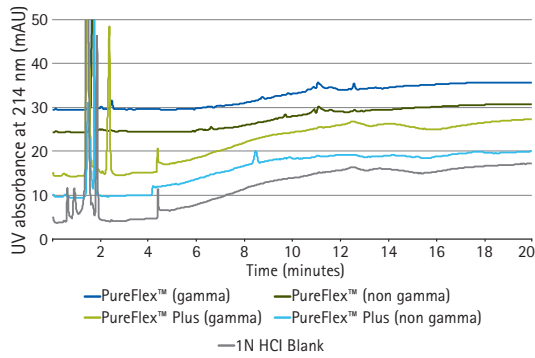


Figure 11.
RP-HPLC Chromatograms of 30 Days 1N HCl Extractables at ambient temperature.

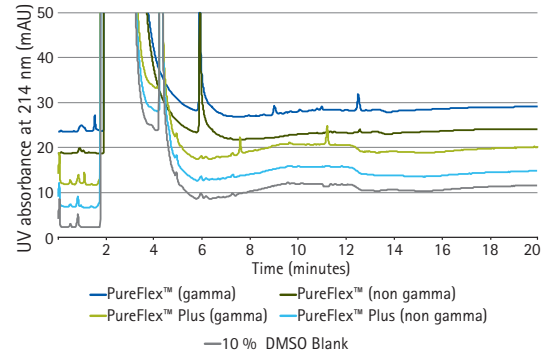


Figure 12.
RP-HPLC Chromatograms of 30 Days 10% DMSO Extractables at ambient temperature.

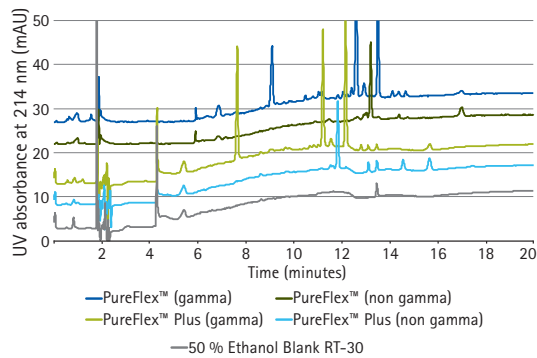


Figure 13.
RP-HPLC Chromatograms of 30 Days 50% Ethanol Extractables at ambient temperature.

ICP-OES: The water extractables of both PureFlex™ and PureFlex™ Plus disposable process containers contained no detectable metals.

Element	Symbol	Instrument	Control	PureFlex™ Plus film	PureFlex™ Plus film	PureFlex™ film	PureFlex™ film
				Non-Gamma	Gamma	Non-Gamma	Gamma
		Detection		45°C - 90 Days	45°C - 90 Days	45°C - 90 Days	45°C - 90 Days
		Limit (ppm)	45°C - 90 Days (ppm)	45°C - 90 Days (ppm)	45°C - 90 Days (ppm)	45°C - 90 Days (ppm)	45°C - 90 Days (ppm)
Silver	Ag	0.02	n.d	n.d	n.d	n.d	n.d
Aluminum	Al	0.02	n.d	n.d	n.d	n.d	n.d
Arsenic	As	0.02	n.d	n.d	n.d	n.d	n.d
Barium	Ba	0.02	n.d	n.d	n.d	n.d	n.d
Beryllium	Be	0.02	n.d	n.d	n.d	n.d	n.d
Bismuth	Bi	0.02	n.d	n.d	n.d	n.d	n.d
Calcium	Ca	0.02	n.d	n.d	n.d	n.d	n.d
Cadmium	Cd	0.02	n.d	n.d	n.d	n.d	n.d
Cobalt	Co	0.02	n.d	n.d	n.d	n.d	n.d
Chromium	Cr	0.02	n.d	n.d	n.d	n.d	n.d
Copper	Cu	0.02	n.d	n.d	n.d	n.d	n.d
Iron	Fe	0.02	n.d	n.d	n.d	n.d	n.d
Gallium	Ga	0.02	n.d	n.d	n.d	n.d	n.d
Indium	In	0.02	n.d	n.d	n.d	n.d	n.d
Potassium	K	0.05	n.d	n.d	n.d	n.d	n.d
Lithium	Li	0.02	n.d	n.d	n.d	n.d	n.d
Magnesium	Mg	0.02	n.d	n.d	n.d	n.d	n.d
Manganese	Mn	0.02	n.d	n.d	n.d	n.d	n.d
Sodium	Na	0.05	n.d	n.d	n.d	n.d	n.d
Nickel	Ni	0.02	n.d	n.d	n.d	n.d	n.d
Lead	Pb	0.02	n.d	n.d	n.d	n.d	n.d
Selenium	Se	0.02	n.d	n.d	n.d	n.d	n.d
Strontium	Sr	0.02	n.d	n.d	n.d	n.d	n.d
Tallium	Tl	0.02	n.d	n.d	n.d	n.d	n.d
Uranium	U	0.02	n.d	n.d	n.d	n.d	n.d
Vanadium	V	0.02	n.d	n.d	n.d	n.d	n.d
Zinc	Zn	0.02	n.d	n.d	n.d	n.d	n.d

Table 12. Metals Analysis by ICP-OES of water extractables from the PureFlex™ Plus and PureFlex™ containers extracted at 45°C for 90 Days

GC-MS: The PureFlex™ and PureFlex™ Plus containers that were not gamma irradiated showed similarly low levels of acetone and acetaldehyde as VOC and SVOC extractables, as can be seen in Tables 13 and 14. 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.

In the PureFlex™ film VOC extracts, the highest concentration of extractables was in the HCl solution followed closely by the NaOH solution (2010 White Paper), then the Milli-Q® water solution. (The ethanol extracts for both films had to be diluted due to matrix interferences, resulting in no identified compounds). In the PureFlex™ Plus film VOC extracts, the highest concentration of extractables was the NaOH solution, followed by the HCl, then the Milli-Q® water. In the PureFlex™ and PureFlex™ Plus film SVOC extracts, water and NaOH showed one compound in the non-gamma irradiated bags. Only one compound was detected

in the NaOH and water solution, and several in the ethanol solutions for the gamma irradiated bags. The PureFlex™ and PureFlex™ Plus containers had similar types of SVOC extractables.

The results reported are corrected for baseline value associated with the control. The identity and concentrations are tentatively identified compounds (TIC) that are determined by the best fit to an internal standard of different molecules. Therefore TIC values are provided for informational purposes only.

The results of analyses for SVOC are presented in Table 14. The results are corrected for baseline values associated with the control. The identity and concentrations are tentatively identified compounds (TIC) that are determined by the best fit to an internal standard of different molecules. Therefore TIC values are provided for informational purposes only.

Table 13.

VOC target compounds and tentatively identified compounds (TIC) from extracts by GC-MS

Film	Solvent	Gamma (yes/no)	(common name)	Compound (CAS#)	(µg/L)
PureFlex™	water	no	acetone	67-64-1	10.0
			Acetaldehyde (TIC)	75-07-0	51
	water	yes	acetone	67-64-1	75
			tert-butyl alcohol	75-65-0	64
			2-butanone	78-93-3	29
			acetaldehyde (TIC)	75-07-0	188
			Butanal (TIC)	123-72-8	58
			Pentanal(TIC)	111-62-3	147
			2-butanol	78-92-2	20
			3,3,-dimethy-2-butanone (TIC)	75-97-8	13
			Hexanal(TIC)	66-25-1	146
			1N HCl	no	none
	1N HCl	yes	acetone	67-64-1	115
			tert-butyl alcohol	75-65-0	333.5
			2-butanone	78-93-3	17.9
			Butanal (TIC)	123-72-8	10.7
			Pentanal(TIC)	110-62-3	12.1
	1N NaOH	yes	Hexanal(TIC)	66-25-1	12.3
			acetone	67-64-1	129.8
			tert-butyl alcohol	75-65-0	133.9
2-butanone			78-93-3	39.5	
2-hexanone			591-78-6	19.6	
2 methyl 1-propene(TIC)			115-11-7	32.6	
20% EtOH	yes	3,3,-dimethy-2-butanone (TIC)	75-97-8	70.2	
		2-octanone (TIC)	111-13-7	10.7	
			none	N/A	N/A

Film	Solvent	Gamma (yes/no)	(common name)	Compound (CAS#)	(µg/L)
PureFlex™ Plus	water	no	acetone	67-64-1	14
			Acetaldehyde(TIC)	75-07-0	31
			acetone	67-64-1	93
			tert-butyl alcohol	75-65-0	91
			Acetaldehyde (TIC)	75-07-0	143
	water	yes	2-butanone	78-93-3	12
			Butanal (TIC)	123-72-8	128
			pentanal (TIC)	110-62-3	245
			hexanal (TIC)	66-25-1	306
			3,3,-dimethyl-2-butanone(TIC)	75-97-8	31
	1N HCl	no	2-pentanone	107-87-9	17
			acetone	67-64-1	22
			Acetaldehyde (TIC)	75-7-0	60
			Tert-butyl alcohol	75-65-0	18
			acetone	67-64-1	50
	1N HCl	yes	tert-butyl alcohol	75-65-0	98
			Acetaldehyde (TIC)	75-07-0	43
			2-butanol	78-92-2	11
			Butanal (TIC)	123-72-8	45
			Pentanal (TIC)	110-62-3	105
	1N NaOH	No	Hexanal (TIC)	66-25-1	92
			3,3,-dimethyl-2-butanone (TIC)	75-97-8	14
			acetone	67-64-1	120
			1-propene, 2-methyl (TIC)	115-11-7	141
			2,4-Di-tert-butylphenol	96-76-4	101
	1N NaOH	yes	acetone	67-64-1	127
tert-butyl alcohol			75-65-0	74	
2-methyl-1-propene(TIC)			115-11-7	247	
3,3-dimethyl 2-butanone(TIC)			75-97-8	1260	
Pentanal (TIC)			110-62-3	39	
50% EtOH	yes	2-pentanone	107-87-9	81	
		2,4-Di-tert-butylphenol	96-76-4	1110	
			none	N/A	N/A

Data in blue was obtained from previous white paper published in 2010.

Note: Ethanol concentration used in 2010 white paper was 20%. This study used 50% Ethanol.

Table 13.

VOC target compounds and tentatively identified compounds (TIC) from extracts by GC-MS

Table 14.
SVOC target
compounds and
tentatively identified
compounds (TIC)
from extracts by
GC-MS

Film	Solvent	Gamma (yes/no)	(common name)	Compound (CAS#)	(µg/L)
PureFlex™	water	No	none	none	none
	water	Yes	none	none	none
	1N HCl	no	none	none	none
	1N HCl	yes	none	none	none
	1N NaOH	no	none	none	none
	1N NaOH	yes	Unknown (TIC)	N/A	25.3
	20% EtOH	yes	1,1-diethoxy ethane (TIC)	105-57-7	13.9
			Unknown (TIC)	N/A	36.8
			4-hydroxy-4-methyl-2-pentanone (TIC)	123-42-2	12.3
			2-4-di-tertiary butyl phenol (TIC)	96-76-4	15.3
PureFlex™ Plus	water	no	Adipic acid ester (TIC)	NIST 324711	20
	water	yes	Adipic acid ester (TIC)	NIST 324711	38
			2,4-Di-tert-butyl phenol	96-76-4	19.7
	1N HCl	no	none	none	none
	1N HCl	yes	none	none	none
	1N NaOH	no	none	none	none
	1N NaOH	yes	none	none	none
	50% EtOH	no	2,4-Di-tert-butyl phenol	96-76-4	1020
50% EtOH	yes	2,4-Di-tert-butyl phenol	96-76-4	890	

Data in blue was obtained from previous white paper published in 2010.

Note: Ethanol concentration used in 2010 white paper was 20%. This paper used 50% Ethanol.

This study was conducted using a surface area (cm²) to volume (mL) ratio of 2:1; whereas in practice, the ratio would be much lower. Since the concentrations of the extractables are due to contact with the surface of the container, as the volume increases the concentration of extractables

will decrease. Presented below in Table 15 are the expected concentrations in containers completely filled with Milli-Q® reagent grade water after 30 days at room temperature. As the size of the containers increases, the concentrations decrease, as seen in the table.

Table 15.
Expected concentration
of Total TOC (PPM C)
in a gamma irradiated
container filled to its
rated volume with
Milli-Q® water

Bag Type	Bag Size	Dimensions	PureFlex™ film (ppm)	PureFlex™ Plus film (ppm)
2-D Pillow	5 L	12.5" X 12.0"	3.89	4.33
	20 L	16.5" X 23.0"	2.44	2.75
	50 L	22.5" X 29.8"	1.74	1.94
3-D Tote	200 L	29.2" X 21.5" X 22.0"	1.12	1.25
	500 L	45.5" X 29.5" X 26.0"	0.85	0.96
	2000 L	45.5" X 36.5" X 82.0"	0.55	0.61

Summary of results

Overall, both PureFlex™ and PureFlex™ Plus films showed similar levels of TOC extractables, with small variation depending on the extraction solution. Comparison of RP-HPLC chromatograms for PureFlex™ and PureFlex™ Plus showed similar peak profiles. In GC-MS, both PureFlex™ and PureFlex™ Plus films revealed similar organic compounds. Volatile and semi-volatile organic compound profiles from the two films were also similar. Both PureFlex™ and PureFlex™ Plus films showed similar levels of anion extractables by ion chromatography. No significant levels of metals were detected in extractions from either film.

Based on the results of this set of studies, there was no observable, significant difference in the extractables profiles from the PureFlex™ and PureFlex™ Plus films.

Conclusion

Overall, very little was extracted from the films, especially in aqueous solutions. The PureFlex™ and PureFlex™ Plus films, with their ULDPE fluid contact layer, had similar concentrations of TOC and small organic acids and volatile gamma-degradation products of polyethylene.

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.



References

1. T. Stone, et al., "Methodology for Analysis of Filter Extractables: A Model Stream Approach," Pharm. Technol. 18 (1994), pp. 116–130.
2. T. Stone, et al., "The Model Stream Approach: Defining the Worst-Case Conditions," Pharm. Technol. 20 (1996), pp. 34–51.

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