

# Quantifying and Qualifying Extractable Substances from Components of Disposable Assemblies

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**B**iopharmaceutical companies are using disposable assemblies at a rapidly increasing rate. Single-use technologies are becoming available for a range of applications and process steps. Disposable assemblies encompass both stand-alone devices and multicomponent systems.

The purpose of our study was to provide extractables data relevant to the components of disposable filtration assemblies. For each disposable component, we quantified extractables using total organic carbon (TOC). In addition, for the process containers, we quantified extractables with nonvolatile residue (NVR) analysis and qualified them using reverse-phase high-performance liquid chromatography (RP-HPLC) and Fourier-transform infrared spectroscopy (FTIR). Among the components we tested were three types of filter devices, two types of tubing, and two kinds of 250-mL process containers. Table 1 lists the specific components tested, as well as their materials of construction.

## TEST SUMMARY

**Disposable Components:** We evaluated disposable assembly components for TOC analysis using model solvent streams designed to simulate a drug product formulation. To simulate worst-case conditions for extractables, components were not



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flushed before static soaking (see below). The test steps were as follows:

- 1) All components were received gamma-radiation sterilized. No further sterilization was performed.
- 2) Components, tested in triplicate, were filled with Milli-Q water, hydrochloric acid solution (HCl) at pH 2.0, or sodium hydroxide solution (NaOH) at pH 10.0, then static soaked for 24 or 336 hours. All extractions were performed at controlled room temperature. We used additional soaks without the system components as controls.
- 3) Extractables in the soak solutions were quantified by TOC analysis.

**Process containers:** SureFlex process containers are manufactured using SureFlex process container film, which is a high-purity, medical-grade composite film. The fluid-contact material is ethylene vinyl acetate (EVA). The gas-barrier layer is made of polyethylene vinyl alcohol copolymers (EVOH). And the outer layer material is linear low-density

polyethylene (LLDPE). PureFlex process containers are manufactured using PureFlex process container film, which is a high-purity, medical-grade, monolayer, coextruded film. Its fluid-contact material is ultralow-density polyethylene (ULDPE). The gas-barrier layer is made of EVOH. And the outer layer materials are EVA and ULDPE.

We evaluated the disposable process containers using model solvent streams designed to simulate a drug product formulation. To simulate worst-case conditions for extractables, the process containers were not flushed before static-soaking. The test steps here were as follows:

- 1) SureFlex and PureFlex 250-mL process containers were received gamma-radiation sterilized (>35 kGy, <45 kGy). No further sterilization was performed.
- 2) Process containers, tested in triplicate, were filled with Milli-Q water, HCl at pH 2.0, or NaOH at pH 10.0, then static-soaked for 336 hours. All extractions were performed at controlled room temperature. We used additional soaks without the system components as controls.
- 3) Extraction solutions were quantified by NVR analysis and qualified by RP-HPLC and FTIR.

## ANALYTICAL METHODS

**Total Organic Carbon Analysis (Shimadzu TOC-5000):** The filter

devices, tubing, and disposable process containers that make up disposable assemblies are organic in nature. The organic components in their respective extraction solutions are oxidized to produce carbon dioxide in TOC analysis. The amount of CO<sub>2</sub> present is used to quantify the oxidizable carbon in a sample.

#### Nonvolatile Residue Analysis (Mettler-Toledo AT201 and PM4000):

Gravimetric analysis consists of evaporating the solvent from an extraction solution sample to dryness and then weighing the residue. This method quantifies the amount of nonvolatile residue extracted from process containers. Most extractables are nonvolatile.

**Fourier-Transform Infrared Spectroscopy (Thermo-Electron Nexus 470 Spectrophotometer and Continuum Microscope):** FTIR is used to characterize nonvolatile residues extracted from process containers. It reveals structural information relevant to the observed extractable substances. FTIR is the best analytical method for examining polymeric and oligomeric solutes. Functional groups — such as esters, amides, ethers, and aliphatic groups — can be detected using this method.

**Reverse-Phase High-Performance Liquid Chromatography (Waters HPLC/UV System):** RP-HPLC is used to analyze extractables solutions for low levels of organic solutes. This method is sensitive to most low molecular weight solutes such as solvents, monomers, and antioxidant additives, but it does not generally detect polymeric or oligomeric solutes.

**Acceptance Criteria:** TOC amounts were quantified and the results reported. Then NVR amounts were quantified and the results reported. FTIR and RP-HPLC analysis of the extractable solutions must identify extractables as consistent with the materials of construction for SureFlex and PureFlex process container components.

## EXPERIMENTAL PROCEDURES

**System Components:** We tested each type of system component in triplicate by TOC (filter device, tubing, and

**Table 1:** Disposable assembly components

Component	Catalog Number/Description	Materials of Construction
Filter	KGEP S03 HH3: Opticap XL capsule (0.22 µm, PES, 3-in. sterile)	Hydrophilic polyethersulfone (PES) filter media; polysulfone core; gamma-stable rigid polypropylene capsule, cage, sleeve, supports, and end caps; silicone O-rings
Filter	KVGL S04 HH3: Opticap XL capsule (0.22 µm, Durapore, 4-in. sterile)	Hydrophilic polyvinylidene fluoride (PVDF) filter media; polyester pleat supports upstream and downstream; gamma-stable rigid polypropylene capsule, cage, core, and end caps; silicone O-rings
Filter	MPGL 2GC A3: Millipak 200 Gamma Gold (0.22 µm sterile)	Hydrophilic PVDF membrane; polycarbonate support disks and housing
Tubing	SF Medical SFM3-5650-200: Pharmatube silicone tubing (3/8-in., 9.5-mm ID; 5/8-in., 15.9-mm OD; 1/8-in., 3.4-mm wall)	Thermal-set rubber; siloxane polymers and amorphous silica
Tubing	Consolidated Polymer Technology 082-375-4: C-Flex 082 (3/8-in., 9.5-mm ID; 5/8-in., 15.9-mm OD; 1/8-in., 3.4-mm wall)	Thermoplastic elastomer; styrene-ethylene-butylene modified block copolymer
Bioprocess container	PureFlex bioprocess container (250-mL, end-ported)	Multilayer coextruded polyolefin film; polyethylene vinyl alcohol copolymers (gas barrier); ultralow-density polyethylene (fluid contact); ultralow-density polyethylene outer layer
Bioprocess container	SureFlex bioprocess container (250-mL, end-ported)	Multilayer composite polyolefin film; polyethylene vinyl alcohol copolymers (gas barrier); ethylene vinyl acetate (fluid contact); ultralow-density polyethylene outer layer

process container). We also tested the SureFlex and PureFlex process containers at 335 hours by NVR, RP-HPLC, and FTIR.

**Sterilization:** Filters, tubing, and process containers came gamma-radiation sterilized (>35 kGy, <45 kGy). We performed no further sterilization.

**Reagents and Materials:** Reagents included Milli-Q water, HCl at pH 2.0 (Milli-Q water adjusted to a pH of 2.0 using ACS-grade HCl), and NaOH at pH 10.0 (Milli-Q water adjusted to a pH of 10.0 using ACS-grade NaOH).

**Extraction:** For the filter devices, filter capsules with PTFE stoppers served as extraction vessels. Each capsule was completely filled with a solvent, and similar volumes of solvent were used to fill the remaining filters for each soak solution. A PTFE vessel was filled with a test solution using the highest soak-solvent volume as a control. Each capsule or control vessel was sealed to prevent evaporation and extracted for either 24 or 336 hours at a controlled room temperature (20–25 °C).

For the tubing, 150-cm lengths of each type sealed with PTFE stoppers served as extraction vessels. Each length of tubing was entirely filled with solvent, and similar volumes were used to fill the remaining tubing in each soak solution. A PTFE vessel was filled with the control solution. Each length of tubing or control vessel was sealed to prevent evaporation and extracted for either 24 or 336 hours at a controlled room temperature (20–25 °C).

For the disposable process containers, individual 250-mL containers served as extraction vessels. Each disposable container was entirely filled with solvent, and similar volumes were used to fill the remaining containers in each soak solution. A PTFE vessel was filled with the control solution. The ports of each process container and control vessel were sealed to prevent evaporation and extracted for either 24 or 336 hours at a controlled room temperature (20–25 °C).

**Analysis:** Extraction samples were analyzed for TOC. Additionally, the process containers were further

**Table 2:** Sampling schedule

Sample ID#	Solvent	Extraction Conditions	Analysis	
			All Components	Bioprocess Containers
H <sub>2</sub> O-Control	Water	24 hours @ CRT	TOC	
H <sub>2</sub> O-1	Water	24 hours @ CRT	TOC	
H <sub>2</sub> O-2	Water	24 hours @ CRT	TOC	
H <sub>2</sub> O-3	Water	24 hours @ CRT	TOC	
NaOH-Control	NaOH pH 10.0	24 hours @ CRT	TOC	
NaOH-1	NaOH pH 10.0	24 hours @ CRT	TOC	
NaOH-2	NaOH pH 10.0	24 hours @ CRT	TOC	
NaOH-3	NaOH pH 10.0	24 hours @ CRT	TOC	
HCl-Control	HCl pH 2.0	24 hours @ CRT	TOC	
HCl-1	HCl pH 2.0	24 hours @ CRT	TOC	
HCl-2	HCl pH 2.0	24 hours @ CRT	TOC	
HCl-3	HCl pH 2.0	24 hours @ CRT	TOC	
H <sub>2</sub> O-Control	Water	336 hours @ CRT	TOC	NVR, HPLC, FTIR
H <sub>2</sub> O-1	Water	336 hours @ CRT	TOC	NVR, HPLC, FTIR
H <sub>2</sub> O-2	Water	336 hours @ CRT	TOC	NVR, HPLC, FTIR
H <sub>2</sub> O-3	Water	336 hours @ CRT	TOC	NVR, HPLC, FTIR
NaOH-Control	NaOH pH 10.0	336 hours @ CRT	TOC	NVR, HPLC, FTIR
NaOH-1	NaOH pH 10.0	336 hours @ CRT	TOC	NVR, HPLC, FTIR
NaOH-2	NaOH pH 10.0	336 hours @ CRT	TOC	NVR, HPLC, FTIR
NaOH-3	NaOH pH 10.0	336 hours @ CRT	TOC	NVR, HPLC, FTIR
HCl-Control	HCl pH 2.0	336 hours @ CRT	TOC	NVR, HPLC, FTIR
HCl-1	HCl pH 2.0	336 hours @ CRT	TOC	NVR, HPLC, FTIR
HCl-2	HCl pH 2.0	336 hours @ CRT	TOC	NVR, HPLC, FTIR
HCl-3	HCl pH 2.0	336 hours @ CRT	TOC	NVR, HPLC, FTIR

analyzed by NVR, RP-HPLC, and FTIR according to Table 2.

## RESULTS

**TOC Results:** Table 3 summarizes the results of our TOC analysis of the extraction solutions for both 24-hour and 336-hour extractions of the tested components. Data presented should help determine the contribution to

total extractables of individual assembly components for three aqueous solvents under two static extraction conditions. As expected, the filtration devices represent the largest single contributor (having the largest surface areas), whereas the contribution of the disposable process containers is minimal.

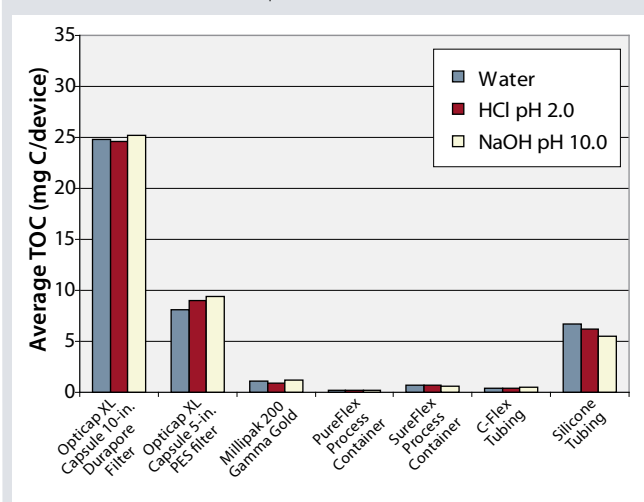
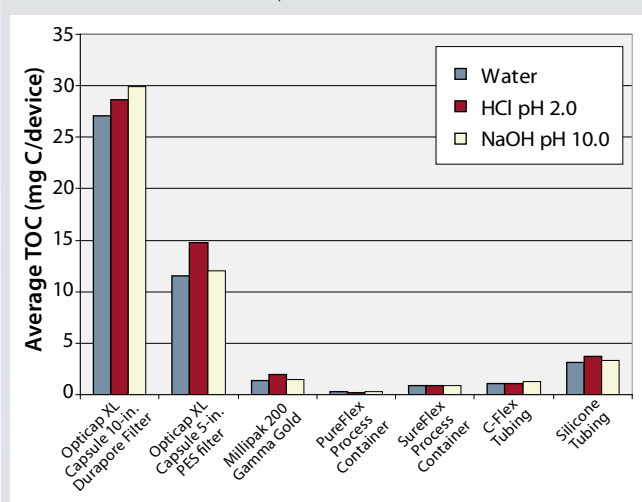
Under our experimental extraction conditions, we saw no essential difference in the level of extractables among the three aqueous solvents (Figures 1 and 2), which demonstrates wide pH compatibilities of the materials within the range tested. In addition, the levels increase only slightly for each component between 24 and 336 hours of static extraction — except in the case of the silicone tubing, for which the 336-hour extraction solution showed lower TOC levels. Some volatile extractable material, possibly a byproduct of gamma irradiation, may permeate the silicone tubing over that extended extraction time.

The surface-area normalized data in Table 3 also could aid in determining the level of extractables expected from disposable assembly components of different dimensions (those differing in extractable surface area). As the data suggest, silicone tubing generates the largest amount of extractables per unit of surface area.

Figures 1 and 2 graphically compare results of the 24-hour and 336-hour disposable assembly component extractions for all three extraction solutions.

**NVR Results:** All results for the NVR are below the limit of quantitation (LOQ). This type of analysis may not be sensitive enough for testing process containers.

**RP-HPLC Results:** Chromatographic analysis of the water, HCl pH 2.0, and NaOH pH 10.0 extraction solutions

**Figure 1a:** Average TOC (mg/device) for filters and tubing following 24-hour extraction in three aqueous solvents**Figure 1b:** Average TOC (mg/device) for filters and tubing following 336-hour extraction in three aqueous solvents

indicated no presence of significant extractable peaks when compared with negative controls (data not shown).

**FTIR Results (Water Extraction):**

(Editor's Note: All FTIR spectra can be found online at [www.bioprocessintl.com/bpiextra](http://www.bioprocessintl.com/bpiextra).) In Figure 3, the infrared spectra generated by the PureFlex and SureFlex process container extraction solutions are compared with a typical Milli-Q water residue spectrum. Those spectra show similarities to the Milli-Q water residue. However, some bands suggest the presence of an organic acid salt (attributed to the EVA used in manufacturing process containers) and possibly a carboxylic acid salt, both of which are substances that may be used in the manufacture of plastics. Additionally, the SureFlex extraction residue spectrum (Figure 4) suggests traces of a nonionic surfactant based on low molecular weight polyethylene glycol (PEG). A PEG-based processing aid is known to be used in the manufacture of polymer films.

**FTIR Results (Hydrochloric Acid pH**

**2.0 Extraction):** (Editor's Note: All FTIR spectra can be found online at [www.bioprocessintl.com/bpiextra](http://www.bioprocessintl.com/bpiextra).) Figure 5 shows infrared spectra obtained from the residues of PureFlex and SureFlex process container HCl pH 2.0 extracts, along with the spectrum of that obtained from the HCl pH 2.0 control solution. The control solution residue indicates

**Table 3:** TOC results normalized for component extractable surface area; results for mg C/m<sup>2</sup> are based on published or calculated component surface areas (Opticap XL/Durapore 10-in = 0.72 m<sup>2</sup>, Opticap XL/PES 5-in. = 0.31 m<sup>2</sup>, Millipak 200 = 0.10 m<sup>2</sup>, BPC = 0.020 m<sup>2</sup>, tubing = 447.68 cm<sup>2</sup> or 0.045 m<sup>2</sup>)

Assembly Component	Extraction Solvent	Average TOC (24-hour)	mg C/m <sup>2</sup>	Average TOC (336-hour)	mg C/m <sup>2</sup>
Opticap XL capsule (0.22 µm, Durapore, 4-in. sterile)	Water	24.8	34.4	27.1	37.6
	HCl	24.6	34.1	28.6	39.7
	NaOH	25.2	35.0	29.9	41.5
Opticap XL capsule (0.22 µm, Durapore, 4-in. sterile)	Water	8.1	26.1	11.5	37.1
	HCl	9.0	29.0	14.8	47.7
	NaOH	9.4	30.3	12.0	38.7
Millipak 200 Gamma Gold (0.22 µm sterile)	Water	1.1	11.0	1.4	14.0
	HCl	0.9	9.0	2.0	20.0
	NaOH	1.2	12.0	1.5	15.0
Pureflex BPC (monolayer, coextruded film, 250 mL)	Water	0.2	8.2	0.3	12.9
	HCl	0.2	7.9	0.2	12.1
	NaOH	0.2	8.7	0.3	15.7
Sureflex BPC (composite, 250 mL)	Water	0.7	34.2	0.9	46.8
	HCl	0.7	33.0	0.9	43.8
	NaOH	0.6	31.8	0.9	44.8
C-Flex 082 tubing (9.5 mm ID × 15.9 mm OD × 150 cm L)	Water	0.4	8.9	1.1	24.6
	HCl	0.4	8.9	1.1	24.6
	NaOH	0.5	11.2	1.3	29.0
Pharmatube silicone tubing (9.5 mm ID × 15.9 mm OD × 150 cm L)	Water	6.7	149.6	3.1	69.2
	HCl	6.2	140.6	3.7	82.6
	NaOH	5.5	122.8	3.3	73.7

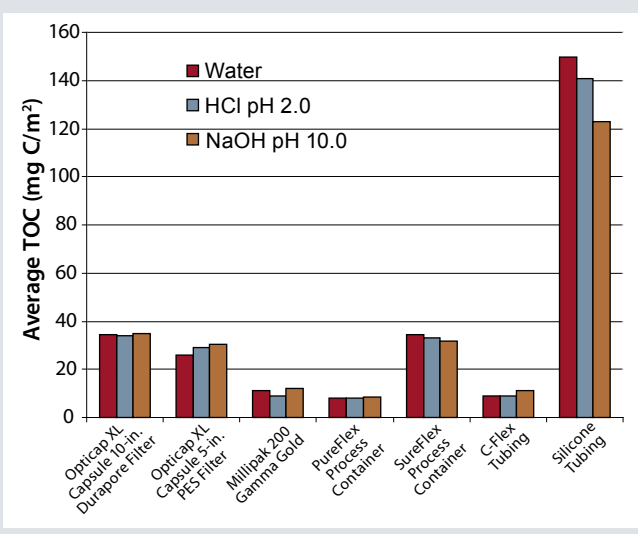
mainly inorganic substances (chloride salts), as does the PureFlex extraction residue in Figure 6. Those salts are inherent in the HCl solution and can overshadow trace-level extractable substances from test articles. The spectrum generated by the SureFlex process container extraction solution also shows traces of chloride salts, which can be removed by subtracting the control spectrum from the sample

spectrum. The resulting spectrum in Figure 7 indicates a nonionic surfactant based on low molecular weight PEG.

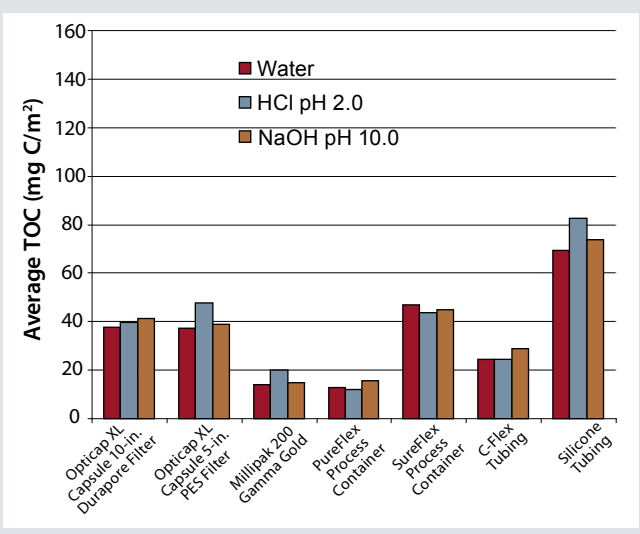
**FTIR Results (Sodium Hydroxide pH**

**10.0 Extraction):** (Editor's Note: All FTIR spectra can be found online at [www.bioprocessintl.com/bpiextra](http://www.bioprocessintl.com/bpiextra).) Figure 8 shows infrared spectra obtained from the residues of the PureFlex and SureFlex process

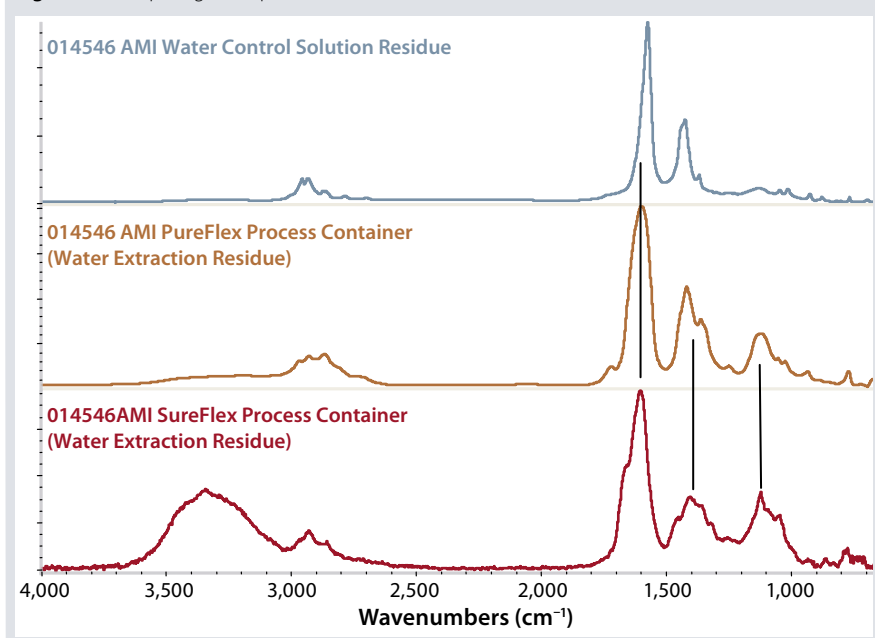
**Figure 2a:** Average TOC normalized for extractable surface area (mg C/m<sup>2</sup>) for filters and tubing following 24-hour extraction in three aqueous solvents



**Figure 2b:** Average TOC normalized for extractable surface area (mg C/m<sup>2</sup>) for filters and tubing following 336-hour extraction in three aqueous solvents



**Figure 3:** Comparing FTIR spectra of PureFlex, SureFlex, and Milli-Q water residues



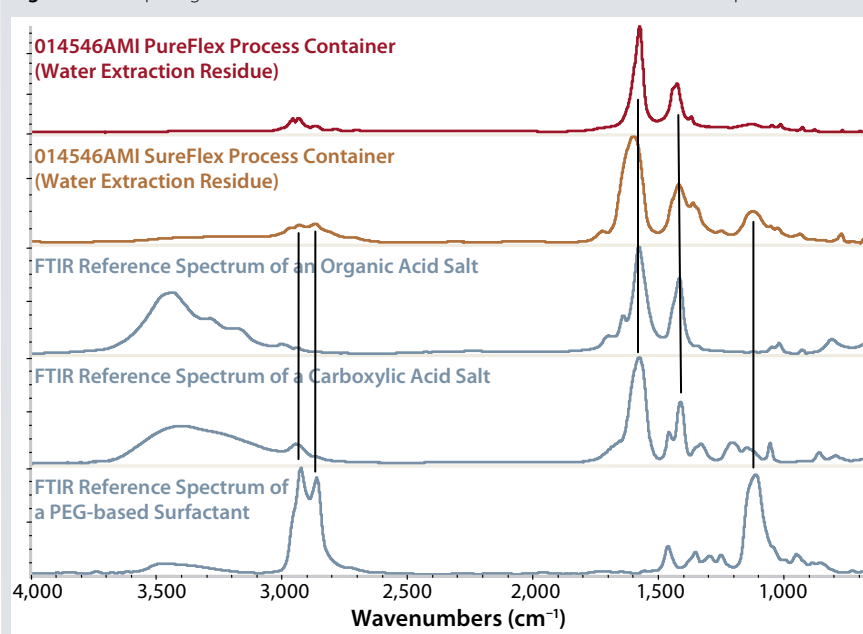
container NaOH pH 10.0 extracts, along with the spectrum of that obtained from a NaOH pH 10.0 control solution. The spectrum generated by the PureFlex process container extraction indicates mainly those inorganic carbonates and sodium hydroxide inherent to the control solution. In addition, these data may show traces of the organic acid salt (Figure 9). The spectrum generated by the SureFlex process container residue suggests the presence of the organic acid salt and/or possibly a carboxylic acid salt (Figure 10).

### A GOOD PLACE TO START

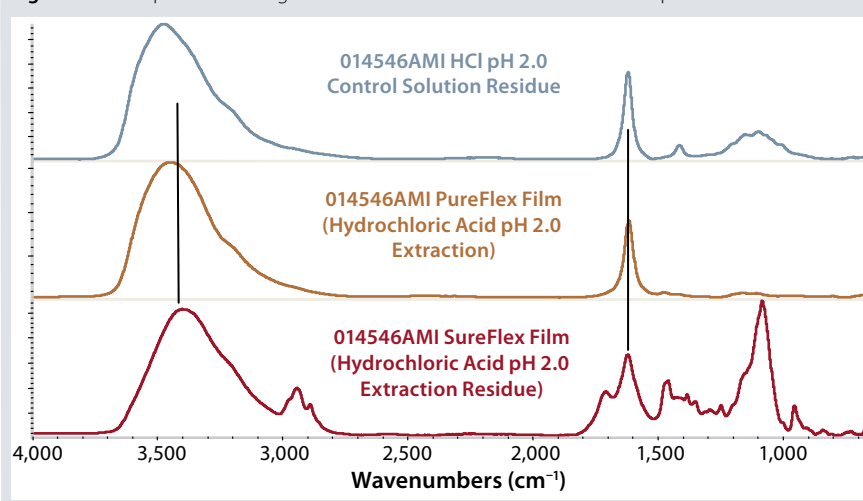
The data generated by our study should be used only as a general guide. Results presented here should not be interpreted as providing absolute levels of extractables for disposable assemblies used under dynamic conditions. ☒

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**Figure 4:** Comparing PureFlex and SureFlex water extractions with reference FTIR spectra

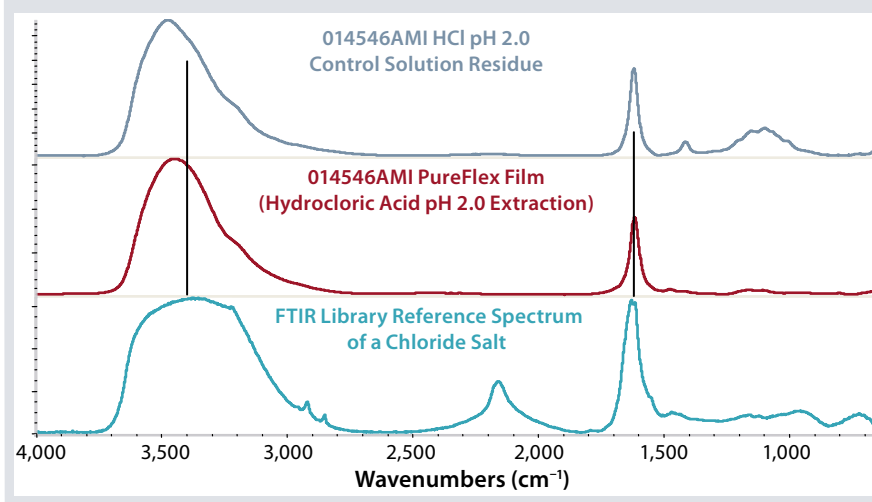


**Figure 5:** FTIR spectra showing residues of PureFlex and SureFlex BPC HCl pH 2.0 extracts

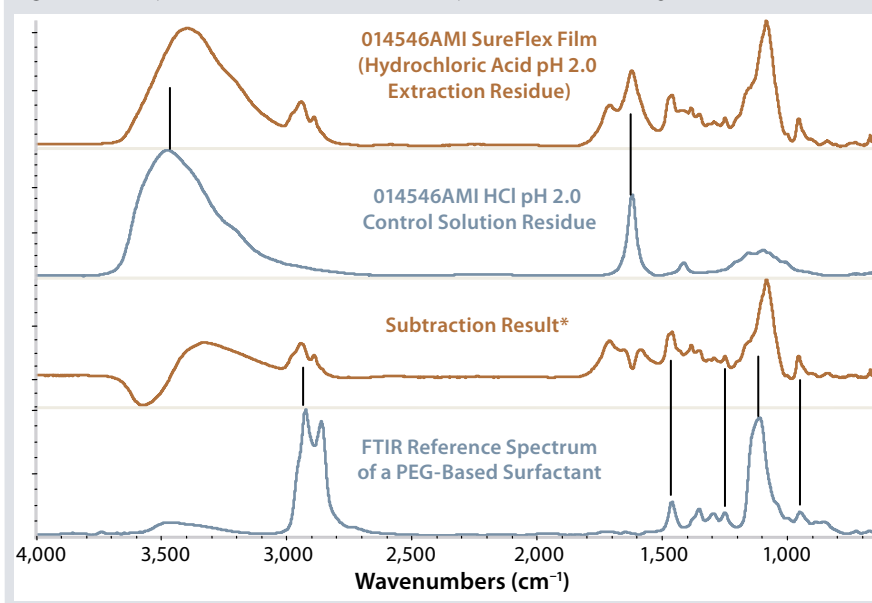




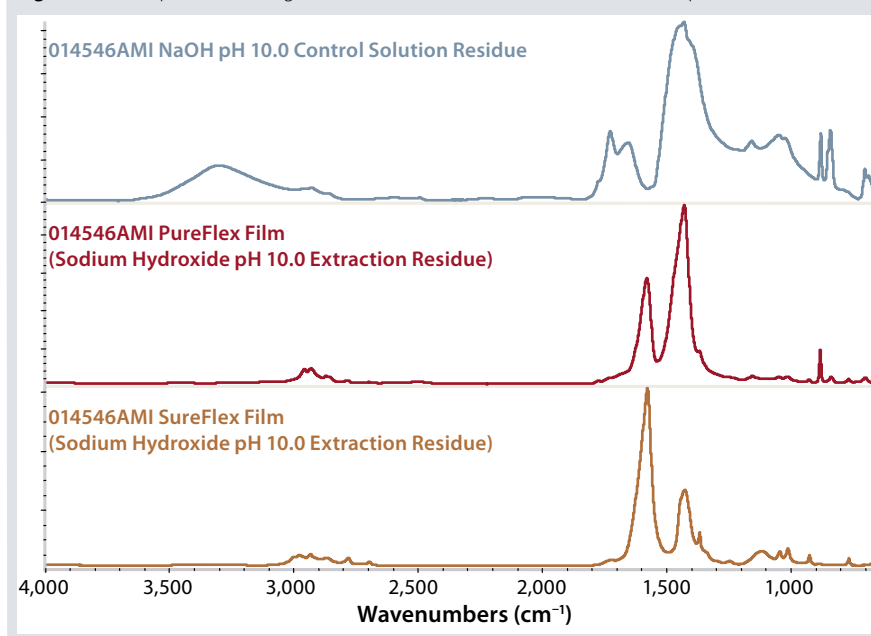
**Figure 6:** HCl pH 2.0 control and library spectra



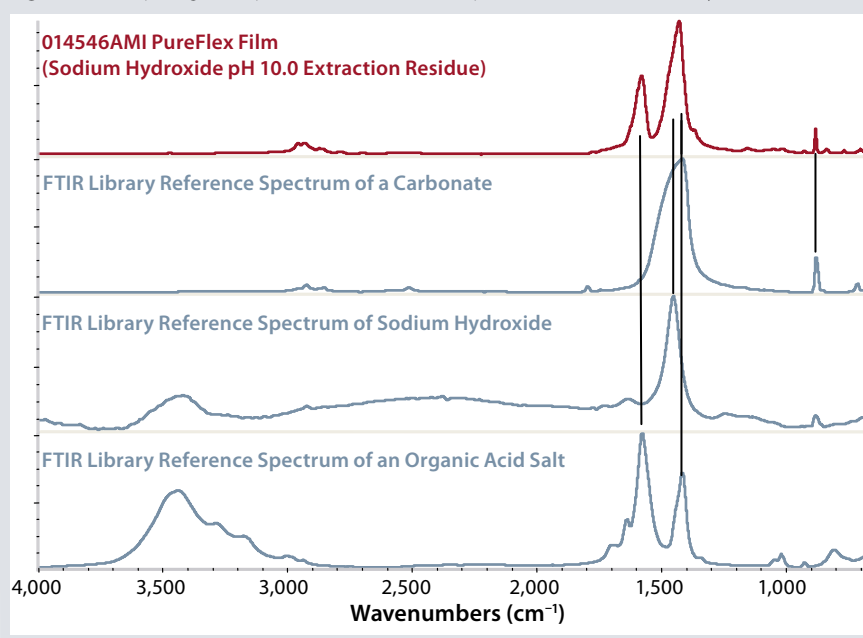
**Figure 7:** FTIR spectra of SureFlex and control HCl pH 2.0 residues showing subtraction results



**Figure 8:** FTIR spectra showing residues of PureFlex and SureFlex BPC NaOH pH 10.0 extracts



**Figure 9:** Comparing FTIR spectra of PureFlex NaOH pH 10.0 residues with library references



**Figure 10:** Comparing FTIR spectra of SureFlex NaOH pH 10.0 residues with reference spectra

