



AN EVALUATION OF THE IMPACT OF STERILIZATION PROCESSES ON ELASTOMERIC CONTAINER CLOSURE COMPONENTS USED TO STORE AND ADMINISTER PARENTERAL DRUG PRODUCTS

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OBJECTIVES

- To evaluate the impact of various sterilization processes on the chemical and physical properties of elastomeric components.
- To compare the extractable/leachable profile of the elastomer following steam and gamma sterilization.
- To examine the functionality of components used in prefilled syringe systems after steam and gamma sterilization.

The ideal sterilization process destroys all microorganisms rapidly with minimal adverse impact on the chemical and physical properties of the elastomeric closure. Developing and validating an acceptable sterilization process is critical to the drug product quality. This study serves to help understand the potential deterioration of physical and chemical properties, the possible impact to functionality and the potential changes to the extractable/leachable profile as a result of sterilization.

METHODOLOGY

Compendia Standards

- European Pharmacopoeia, Section 3.2.9 Rubber Closures for Containers for Aqueous Parenteral Preparations for Powders and For Freeze-Dried Powders
- United States Pharmacopoeia, Section <381>, Elastomeric Closures For Injections
- Japanese Pharmacopoeia XV, Section 7.03 Test for Rubber Closures for Aqueous Infusions

Experimental Test Methods

- Detection and Quantitation of Rubber Extractables by RP-HPLC Utilizing UV Detection
- Qualitative Headspace Analysis of Elastomeric Formulations by Gas Chromatography/ Mass Spectroscopy (GC/MS)
- Qualitative Screening of Extracts by GC with Mass Selective Detector using an Agilent 6890 Gas Chromatograph with a 5973 Mass Selective Detector
- Detection and Quantitation of Trace Metals by Inductively Coupled Plasma Spectroscopy
- Quantitation of Anions in Aqueous Solution by Ion Chromatography
- Determination of Breakloose and Extrusion Force using Instron Model 3345 Single Column

EXPERIMENT DESIGN AND RESULTS

To evaluate the impact of steam sterilization on the chemical, physical and biological properties of elastomeric closures, a combined testing protocol following the major compendia standards was performed on stoppers exposed to a steam sterilization process of 121°C for 60 minutes and subsequently stored in controlled room temperature conditions (68°F -77°F, less than 60% relative humidity). The sample population consisted of three (3) lots each of a modern halobutyl rubber formulation in both 13mm and 20mm serum and lyophilizer style elastomeric closures all having a fluoropolymer laminate. Samples were tested over a two-year period to gauge the impact of steam sterilization over time. Results for all samples met the specifications for Type 1 closures as defined by the compendia standards for the two year period. Table 1A illustrates data for USP and EP. Table 1B illustrates the results for JP over the two-year period.

TAKE AWAY # 1:

- How did the steam sterilization process impact the chemical, physical and biological properties of elastomeric closures?
- RESULT: All results met the specifications set forth in USP, EP and JP (Type 1 compliant) over a two-year period.

TABLE 1A: AN ASSESSMENT OF USP/Ph.Eur. RESULTS AFTER STEAM STERILIZATION TO UNDERSTAND THE IMPACT ON CHEMICAL, PHYSICAL AND BIOLOGICAL ELASTOMERIC PROPERTIES OVER TIME

Test	Specification (Type 1)	Results						
		Time 0	3 Month	6 Month	12 Month	18 Month	24 Month	
Reducing Substance (mL)	Type I ≤ 3.0mL	0.0 to 0.2	0.0 to 0.2	0.0 to 0.1	0.1 to 0.2	0.0 to 0.5	0.1 to 0.6	
Absorbance (Abs.)	Type I ≤ 0.2 Abs.	0.0	0.0	0.0	0.0	0.0	0.0	
Residue on Evaporation (mg)	Type I ≤ 2.0mg/50mL of Solution S	0.0	0.0 to 0.2	0.2 to 0.5	0.0	0.1 to 0.3	0.0 to 0.4	
Appearance of Solution	Color ≤ GY5 Opalescence: ≤ 6 NTU	Color ≤ GY5 0 NTU	Color ≤ GY5 0 NTU	Color ≤ GY5 0 NTU	Color ≤ GY5 0 NTU	Color ≤ GY5 0 NTU	Color ≤ GY5 0 NTU	
Extractable Heavy Metals	Brown color of samples solution is ≤ the intensity of the standard	Pass	Pass	Pass	Pass	Pass	Pass	
Extractable Zinc (ppm)	≤ 5 ppm Zn ²⁺	None Detected	None Detected	None Detected	None Detected	None Detected to 0.0 ppm	0.0 to 0.1	
Ammonium	The color of the Solution S is ≤ the yellow color of the 2 ppm standard	Pass	Pass	Pass	Pass	Pass	Pass	
Acidity or Alkalinity (mL)	≤ 0.3mL of 0.01M NaOH or ≤ 0.8mL of 0.01M HCl	0.1 mL NaOH	0.1 mL NaOH	0.1 mL NaOH	0.1 mL NaOH	0.0 to 0.1 mL NaOH	0.1 mL NaOH	
Volatile Sulphides	Black stain on acetate paper is ≤ the reference	Pass	Pass	Pass	Pass	Pass	Pass	
Penetrability	< 10 (Newtons)	4 to 7	4 to 6	4 to 6	4 to 7	4 to 6	4 to 6	
Fragmentation	< 5 (Fragments)	0 to 1	0	0 to 3	0 to 1	0 to 2	0	
Self Sealing	None contain any trace of blue solution	Pass	Pass	Pass	Pass	Pass	Pass	
USP <87> Elution Toxicity	Not greater than grade 2 (mildly reactive)	0	0	0	0	0	0	
USP <88> Acute Systemic Toxicity	No animals treated show greater biological reactivity than the animals treated with the blank	Pass	Pass	Pass	Pass	Not Tested*	Not Tested*	
USP <88> Intracutaneous Reactivity Toxicity	Difference between sample and blank mean score is 1.0 or less	Negative Value	Negative Value	Negative Value	Negative Value to 0.1	Not Tested*	Not Tested*	

*The USP states that USP <88> Acute Systemic Toxicity and Intracutaneous Reactivity Toxicity is not required if the sample meets the requirements of USP <87> Elution Toxicity. For this reason samples that passed the USP <87> requirements did not undergo further USP <88> testing. This practice was affected for the 18 and 24 month timepoints.

TABLE 1B: AN ASSESSMENT OF JP RESULTS AFTER STEAM STERILIZATION TO UNDERSTAND THE IMPACT ON CHEMICAL ELASTOMERIC PROPERTIES OVER TIME

Test	Specification	Results						
		Time 0	3 Month	6 Month	12 Month	18 Month	24 Month	
Foam Test	Foam almost disappears, 3 min	Pass	Pass	Pass	Pass	Pass	Pass	
UV Spectrum (AU)	Type I ≤ 0.2 Abs.	0.00 to 0.01	0.00	0.00 to 0.01	0.00 to 0.01	0.00 to 0.01	0.01 to 0.03	
Description (%)	% Transmission may not fall below 99.0% at both wavelengths	99.6 to 100.0 at both	99.8 to 100.0 at both	99.6 to 99.9 at both	99.7 to 100.0 at both	99.5 to 100.0 at both	99.5 to 99.9 at both	
Reducing Substance (mL)	KMnO4 may not exceed 2.0 mL	0.8 to 1.8	0.8 to 1.1	0.0	0.2 to 0.7	0.3 to 0.8	0.7 to 0.8	
pH	Range of pH difference between -1.0 to + 1.0	0.1 to 0.2	0.2 to 0.3	0.1	0.1 to 0.5	0.5 to 0.6	0.4 to 0.5	
Residue on Evaporation (mg)	May not exceed 2mg/100mL	0.0 to 0.3	0.1 to 0.4	0.0	0.0 to 0.9	0.0 to 0.2	0.0	
Zinc	Absorbance of the sample not more than the standard (≤ 0.5 ppm Zn)	Pass	Pass	Pass	Pass	Pass	Pass	

To examine how chemical properties are impacted by the gamma irradiation sterilization process a combined testing protocol following the major compendia standards was performed on a sample population consisting of one (1) lot each of two different modern halobutyl rubber formulations in both 1mL standard and 1 mL long style pistons, both styles having a fluoropolymer laminate. Samples that had been gamma irradiated at both 20 kGy and 40 kGy energy levels were compared to non-irradiated samples. This study evaluated the results at time zero. Results for all samples met the specifications for Type 1 closures as defined by the USP and Ph.Eur. at time zero. Samples did not meet all JP specifications. Table 2 illustrates the data. The results for pH trended high as irradiation level increased and samples irradiated at 40 kGy failed to meet the specification at time zero. The pH test measures the concentration of hydrogen ion in solution which shows the degree of acidity or alkalinity of the water extract. This test can provide insight to the potential for polymer degradation which can cause pH shift of a parenteral drug product when packaged long term. The results for UV Spectrum % absorbance showed an increase as irradiation level increased. This test provides baseline information indicating the presence of UV active substances.

TAKE AWAY #2:

What was the impact to chemical properties of pistons at time zero following gamma irradiation at 20 kGy and 40 kGy?

RESULT: All samples met the specification for Type 1 per the USP and Ph.Eur. chemical testing at time zero. All samples did not meet the specification for JP chemical testing at time zero. There was a failure for the pH test and data trended high for UV Spectrum % Absorbance with increasing energy level. These results indicated the gamma sterilization process at the higher energy level had an immediate negative impact to elastomeric pistons.

TABLE 2: GAMMA STERILIZATION IMPACT ON CHEMICAL PROPERTIES OF ELASTOMERIC CLOSURES AT TIME ZERO

Test	Specification	Control (Unprocessed)	20 kGy	40 kGy
		Reducing Substance (mL)	Type I ≤ 3.0 mL	0.0 to 0.1
Absorbance (Abs.)	Type I ≤ 0.2 Abs.	0.0	0.0	0.0
Residue on Evaporation (mg)	Type I ≤ 2.0mg/50mL of Solution S	0.4 to 0.5	0.3 to 0.5	0.5 to 0.6
Appearance of Solution	Color ≤ GY5 Opalescence: ≤ 6 NTU	Color ≤ GY5 0 NTU	Color ≤ GY5 0 NTU	Color ≤ GY5 0 NTU
Extractable Heavy Metals	Brown color of samples solution is ≤ the intensity of the standard	Pass	Pass	Pass
Extractable Zinc (ppm)	≤ 5 ppm Zn ²⁺	None Detected	None Detected	None Detected
Ammonium	The color of the Solution S is ≤ the yellow color of the 2 ppm standard	Pass	Pass	Pass
Acidity or Alkalinity (mL)	≤ 0.3mL of 0.01M NaOH or ≤ 0.8mL of 0.01M HCl	0.1 mL NaOH	0.1 mL NaOH	0.1 mL NaOH
Volatile Sulphides	Black stain on acetate paper is ≤ the reference	Pass	Pass	Pass
Foam Test	Foam almost disappears, 3 min	Pass	Pass	Pass
UV Spectrum (AU)	Type I ≤ 0.2 Abs.	0.02	0.02	0.03
Description (%)	% Transmission may not fall below 99.0% at both wavelengths	99.9% to 100% at both	99.9% to 100% at both	99.8% to 99.9% at both
Reducing Substance (mL)	KMnO4 may not exceed 2.0mL	0.6 to 0.7	0.7 to 0.9	0.7 to 0.9
pH	Range of pH difference between -1.0 to + 1.0	0.3 to 0.5	0.3 to 0.8	1.2 to 1.6
Residue on Evaporation (mg)	May not exceed 2mg/100mL	0	0	0.0 to 0.1
Zinc	Absorbance of the sample not more than the standard (≤ 0.5 ppm Zn)	Pass	Pass	Pass

To examine how the extractable profile is impacted by sterilization processes and to compare the steam process to the gamma process, sample extracts were prepared and subsequently analyzed via a variety of analytical techniques. The sample population consisted of one (1) lot each of two different modern halobutyl rubber formulations in both 1mL standard and 1mL long style pistons, both styles having a fluoropolymer laminate. Samples that had been gamma irradiated at both 20 kGy and 40 kGy energy levels were compared to samples that had been steam sterilized at 121°C for 30 minutes and dried for three (3) hours. Unprocessed samples were used as the control. Table 3 illustrates the data that has also been shown in chart format, Chart 1.

Comparing to the control, the RP-HPLC results shows a decrease in antioxidant (BHT) content. The antioxidant (BHT) content further decreased in the samples irradiated at 20 kGy then disappeared in the samples irradiated at 40 kGy. The antioxidant inhibits polymer degradation by scavenging radicals and is intended to be a sacrificial ingredient. Its consumption in the irradiated samples indicates degradation of the polymer that could result in potential loss of desired properties. Headspace analysis illustrates that extractables increase with increasing gamma energy level. Comparing to the control, the ICP results show a decrease in magnesium content in steam processed sample but an increase in magnesium content with increasing irradiation energy.

Gamma irradiation caused a noticeable increase in the total number of extractables detected. The increase in the number of extractables coincided with an increasing gamma energy level. Samples that had been irradiated at 40 kGy displayed the greatest change in the extractable profile as compared with the control samples. Detailed below in Table 3 are results with quantitative data for HPLC, ICP and IC and qualitative data for GC/MS and Headspace. Within the qualitative data, chemical extractables are listed by their generic family name and the number of species/types within that family.

TAKE AWAY #3

How was the extractable profile impacted by various sterilization processes: steam for 30 minutes, gamma at 20 kGy and gamma at 40 kGy?

RESULT: Extractables increased with increasing gamma energy level coupled with a decrease in antioxidant content which indicates greater oxidizing stress is created by the gamma irradiation process. The steam sterilization process showed the lowest number of extractables compared to the gamma irradiation process.

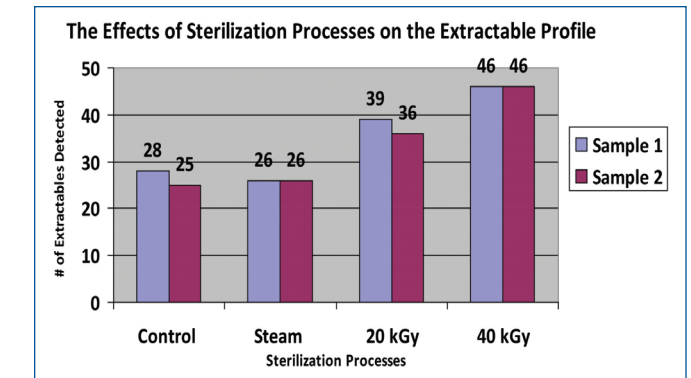
TABLE 3: EXTRACTABLE PROFILES FOLLOWING VARIOUS METHODS OF STERILIZATION

	RP-HPLC (ppm)		GC/MS*		GC Headspace*		ICP (ppm)		IC (ppm)		Total # Extractables	
	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
Control	S 2ppm BHT <1ppm	BHT 3ppm	Butyl Oligomer - 2 Alkane - 1 Unknowns - 14	Butyl Oligomer - 2 Alkane - 1 BHT Unknowns - 12	Alkanes - 3 Cycloalkanes - 2 Butyl Oligomer - 1 Unknowns - 2	Alkanes - 3 Cycloalkanes - 2 Butyl Oligomer - 2 Unknowns - 3	Mg 2.6ppm	S 2.1ppm	ND	ND	28	25
Steam	S 1ppm	BHT 2ppm	Butyl Oligomer - 2 Alkane - 1 Unknowns - 12	Butyl Oligomer - 2 Alkane - 1 BHT Unknowns - 10	Alkanes - 3 Cycloalkanes - 2 Butyl Oligomers - 2 Siloxane - 1 Unknowns - 1	Alkanes - 3 Cycloalkanes - 2 BHT Butyl Oligomer - 2 Unknowns - 1	Mg 2.5ppm	S 3.4ppm	ND	Cl <1ppm	26	26
20 kGy	ND	BHT 1ppm	Butyl Oligomer 2 Alkane - 1 Unknowns - 14	Butyl Oligomer - 2 Alkane - 1 BHT Unknowns - 15	Carbon Disulfide Alkanes - 4 Alkene - 1 Acetone - 1 Cycloalkane - 2 Butyl Oligomers - 2 Unknowns - 8	Alkanes - 4 Cycloalkanes - 2 Acetone - 1 Butyl Oligomers - 2 Unknowns - 4	Mg 3ppm S 1.1ppm	S 1.5ppm	Fl <0.5ppm	Cl <1ppm Fl <0.5ppm	39	36
40 kGy	ND	ND	Butyl Oligomer 2 Alkane 1 Unknowns - 21	Butyl Oligomer - 2 Alkane - 1 BHT Unknowns - 15	Carbon Disulfide Alkanes - 4 Alkene - 1 Acetone - 1 Cycloalkane - 2 Butyl Oligomers - 2 Unknowns - 8	Alkanes - 4 Cycloalkane - 2 Acetone - 1 Alkene - 1 Siloxane - 1 Butyl Oligomer - 2 Unknowns - 13	Mg 4ppm S 1ppm	<MQL	Br <1ppm Fl <0.5ppm	Cl <1ppm Fl <0.5ppm	46	46

(Mg) Magnesium; (S) Sulfur; (BHT) Butylated Hydroxy Toulene; (F) Fluoride; (Br) Bromide; (Cl) Chloride; (ND) None Detected; (ppm) parts per Million; (Sample 1) Bromobutyl 1mL Long; (Sample 2) Chlorobutyl 1mL Standard

*These compounds are tentatively named because standards were not analyzed with the samples for confirmation.

CHART 1: A COMPARISON OF THE NUMBER OF EXTRACTABLES DETECTED FROM VARIOUS STERILIZATION PROCESSES



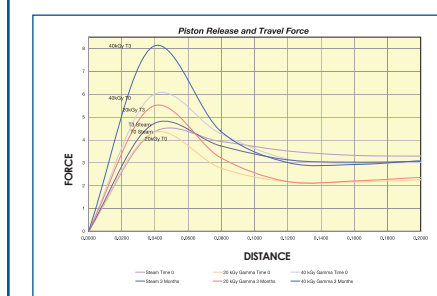
To understand the effect of the sterilization process on the functionality of components used in prefilled syringe systems, a study was performed to compare piston release and travel force among components that have been sterilized by steam at 121° for 30 minutes and gamma irradiation at two energy levels: 20 kGy and 40 kGy. The graphs below display the force profile of 1mL long siliconized pistons made in a modern halobutyl elastomer formulation without and with fluoropolymer film laminate. The forces were evaluated over a three-month period. For non-fluoropolymer laminated samples, the highest forces are observed with the highest irradiation level and the greatest delta between forces is also noted at the highest irradiation level. The steam and lower irradiation level were comparable. For fluoropolymer laminated samples, steam again had the lowest forces over all, however the converse was observed: the higher irradiation level had lower forces than the lower irradiation level and this was observed at all timepoints 0, 1, 3 months. The speed at which the test is usually performed is 100mm per minute. In this study, we used a speed of 300mm per minute since this is closest to field conditions.

TAKE AWAY #4:

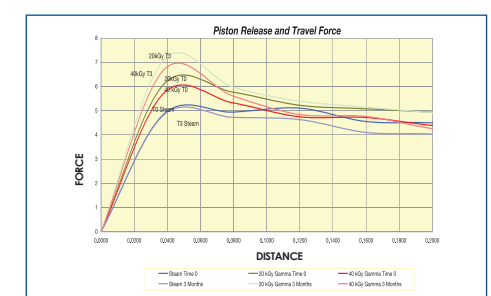
How was the functionality of pistons impacted by various sterilization processes: steam for 30 minutes, gamma at 20 kGy and gamma at 40 kGy?

RESULT: Overall steam samples had the lowest forces. Consistent and predictable extrusion force in all steam samples was observed. However, inconsistent and variable forces were observed in the irradiated samples. This variation is unpredictable and could be attributed to elastomer breakdown by chain scission resulting from the irradiation.

GRAPH 1: FORCE PROFILE OF 1mL LONG SILICONIZED PISTON



GRAPH 2: FORCE PROFILE OF 1mL LONG PISTON WITH FLUOROPOLYMER LAMINATE



CONCLUSION

The results indicate that steam sterilization had little effect on the chemical, physical and extractable profiles of the elastomeric closures, other than a slight decrease in antioxidant content. Gamma irradiation has a greater impact to the elastomer than steam, as noted by the higher number of extractables for gamma irradiated samples, a JP pH failure at 40 kGy, and inconsistent piston release and travel forces at the higher gamma level. As expected, the number of extractables increased with increasing irradiation energy level. This confirms the importance of developing and validating a low dose gamma level when this technique is used as the method of sterilization. The studies carried out also confirm the importance of extractable testing following the sterilization process and the importance of testing closures representative of the full process when performing drug product leachable studies.