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IMPLEMENTATION OF USP<665>, USP<1665> & BIOPHORUM (BPOG) PROTOCOLS FOR POLYMERIC SINGLE USE BIOPROCESS SYSTEMS

HYPOTHETICAL CASE STUDY

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ABSTRACT / INTRODUCTION

ABSTRACT

Through a hypothetical case study this Whitepaper provides an overview of USP<665>[1], USP<1665> and the Biophorum (BPOG) Extractable protocol[2] for Single-Use bioprocessing systems used in the production of biopharmaceutical drug products. Additionally, this paper highlights how the risk management process, defined in USP<665> and USP<1665>, can be implemented using a hypothetical scenario.

INTRODUCTION

Pharmaceutical manufacturing processes traditionally used glass and stainless-steel equipment, whilst small molecule manufacturing processes still utilise these materials widely, large molecule (biopharmaceutical) manufacturing processes utilise a much wider range of materials. Polymeric single-use manufacturing equipment (disposable bioprocessing systems) offer several advantages over traditional stainless steel and glass vessels and equipment, such as:

- Cost savings
- Improved flexibility and scalability
- Rapid change over
- Reduced infrastructure requirements
- Reduced potential for cross-contamination

As such there has been an increased use of single-use technologies since the early 2000's and today they are widely adopted in the biopharmaceutical industry. Whilst the end-user (pharma-biopharma) is ultimately responsible for ensuring that the equipment used in their manufacturing processes is appropriate for use i.e., compatible with the product, maintains sterility, and does not release substances which impact patient safety. There has also been a change in the end-user/supplier relationship with a larger emphasis being placed on the supplier to provide high quality data which can be used by the end-user to qualify the Single Use Systems used in manufacturing processes. Stricter quality agreements mean that suppliers need tighter controls on their supply chains and changes to the materials of construction or manufacturing process need to be communicated well in advance of these changes occurring. This means that data from a single component can be used by many different companies, for many different processes and biopharmaceuticals. The BioPhorum extractable protocol was specifically developed to enable suppliers to produce extractable data in a standardised format, which could be used by multiple end users.

Whilst extractable data may have been generated by the supplier, it is still the responsibility of the end user organisation to ensure that the data is generated in a manner which represents their process. Regulatory agencies require end-users to ensure that the materials used to manufacture the pharmaceutical/biopharmaceutical product are suitable for their intended use. In this regard USP<665> has been drafted with the end process in mind, the level of testing and reporting thresholds required for data are therefore, process specific.

[1] Plastic components and systems used to manufacture pharmaceutical drug products and biopharmaceutical drug substances and products

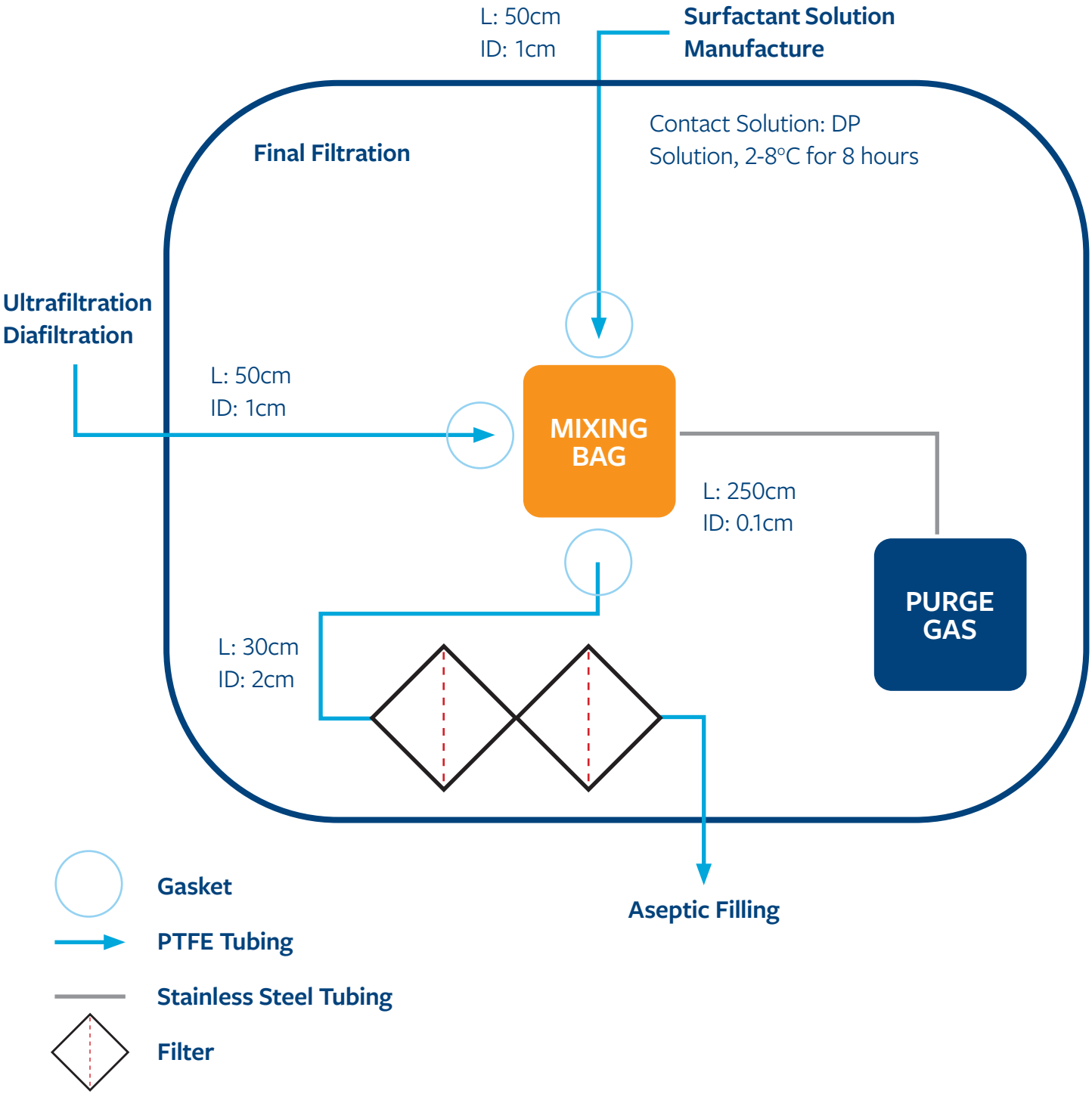
[2] BioPhorum best practices guide for extractables testing of polymeric single-use components used in biopharmaceutical manufacturing

WORKED EXAMPLE

In this whitepaper, we will look at a small variety of single use components, each with a varying range of available extractable data. Through part of a theoretical manufacturing process we will explore the risk evaluation process defined in USP<1665> and potential next steps for addressing any data or knowledge.

To bring context to the examples, a fictional biopharmaceutical manufacturing process has been created, shown in Figure 1. Please note this is not in any way related to any specific drug product manufacturing process.

Figure 1 Example Final Filtration in a Biopharmaceutical Manufacturing Process



PRELIMINARY RISK EVALUATION

As per USP<665> preliminary risk evaluation should be carried out when assessing the requirement for extractable and/or leachable testing of the specific single use component within a process stream. The preliminary risk evaluation determines the level of testing required and can even be used to determine the most appropriate extraction conditions to use for the component within a specific manufacturing process. The risk evaluation as detailed in USP<665> and USP<1665> uses information about the single-use component(s) and how it will be used in the manufacturing process, to determine the theoretical risk of leachable migration. Factors such as if the component contacts liquid or semi-solid streams, is equivalent to a comparator component or system, duration of contact, contact temperature, composition of the contact fluid, and percentage of additives in the component's material of construction are considered.

A risk evaluation was performed using the approach defined in USP<1665> for the manufacturing process shown in Figure 1, for four of the polymeric components in the fluid path. Table 1 captures the process conditions associated with each component and the resulting score.

In this hypothetical example, some of the component suppliers have provided chemical characterisation data. Table 2 details the available extractable data provided by the supplier along with the conditions used to generate the data.

Table 1 Example Risk Evaluation

	COMPONENT			
	Filter	***Mixing Bag***	***Tubing***	***Gasket***
Contact Time	Up to 16 hours	Up to 9 days	Up to 16 Hours	Up to 9 days
Contact Time Risk Rating	1	3	1	3
Contact Temperature	20 ± 2˚C	20 ± 2˚C	20 ± 2 ˚C	20± 2 ˚C
Contact Temperature Risk Rating	2	2	2	2
Contact Fluid	Drug Substance (0.1% PS 80)	Drug Substance (0.1% PS 80)	Drug Substance (0.1% PS 80)	Drug Substance (0.1% PS 80)
Contact Fluid Risk Rating	2	2	2	2
Percentage of additives in MOC	No-data on MOC, Filter is Gamma-Irradiated, no flush performed	No-data on MOC, Bag is Gamma-Irradiated	Low percentage of additives and material	No Data Available (assumed worst case)
Percentage of additives in MOC Risk Rating	3	3	1	3
Overall Score	1,2,2,3	3,2,2,3	1,2,2,1	3,2,2,3
Risk Level	B (moderate)	C (High)	A (Low)	C (High)

Table 2 Available Data with Extraction Conditions

	COMPONENT			
	Filter	***Mixing Bag***	***Tubing***	***Gasket***
Available Data	BioPhorum Protocol	USP<665>	BioPhorum Protocol	No Data
Extraction Time	24 hours and 7 days	24 hours	24 hours and 21 days	N/A
Extraction Temperature	40°C	40°C	40°C	N/A
Extraction Solvent	50% Ethanol, 0.5NaOH, 0.1M phosphoric acid, Water	50% Ethanol, pH 3 Water, pH 10 Water	50% Ethanol, 0.5NaOH, 0.1M phosphoric acid, Water	N/A
Analytical Techniques	GC-MS (VOC & SVOC), LC-MS (NVOC), ICP (Metals)	GC-MS (VOC & SVOC), LC-MS (NVOC), ICP (Metals)	GC-MS (VOC & SVOC), LC-MS (NVOC), ICP (Metals), pH, NVR and TOC	N/A
Reporting Threshold	0.1µg/mL	0.5µg/mL	0.1µg/mL	N/A

KNOWLEDGE GAP IDENTIFICATION

The information provided by each supplier can then be assessed to highlight any data gaps that may be present for each component.

Firstly, we will look at the 'Filter' component, this component was identified as moderate risk and as per USP<665>, requires organic extractable profiling using 50% Ethanol. The supplier provided data in line with the BioPhorum protocol, which in this instance is representative of the components use case. However, the reporting threshold of 0.1µg/mL needs to be verified with a process specific Analytical Evaluation Threshold or 'AET'.

Before completing the extractables protocol, an Analytical Evaluation Threshold or "AET" must be defined. Aligning the Analytical Evaluation Threshold (AET) with a product specific manufacturing process is an important consideration.

To determine the potential impact of a component, a toxicological evaluation needs to be performed. Thus, extractable data should have a reporting threshold which is sufficiently low enough for a toxicologist to confidently determine that analytes below this threshold would present a negligible risk to patient safety. Anything above this threshold can then be evaluated on a case-by-case basis. It is worth noting that if the reporting threshold is greater than the AET, there is a risk that potentially toxic compounds would not be reported.

The Biophorum (BPOG) protocol specifies a general reporting threshold or AET of 0.1µg/mL while USP<665> aligns to a justified AET.

EXAMPLE ANALYTICAL EVALUATION THRESHOLD (AET) CALCULATION

Data from the 'Filter' component was generated using a reporting threshold in line with the BioPhorum (BPOG) protocol of 0.1µg/mL. However, to ensure this component complies with USP<665> the AET was determined using the following calculation:

$$AET = \frac{SCT \; x \; A \; x \; D}{B \; x \; C \; x \; E \; x \; UF}$$

SCT = Safety Concern Threshold (µg/day)

A = number of test items or surface area extracted (items or cm2)

B = number of test items or surface area in the process (items or cm2)

C = extract volume (mL)

D = number of doses manufactured (doses)

E = number of doses a patient receives per day (doses/day)

UF = analytical methods uncertainty factor

Filter AET (µg/mL) Calculation:

$$AET = \frac{1.5 \; x \; 1 \; x \; 60{,}000}{2 \; x \; 1{,}500 \; x \; 10 \; x \; 2}$$

$$AET = \frac{90{,}000}{60{,}000} = 1.5 \; \mu g/mL$$

The same calculation was used for the remaining components in the process where supplier data was provided:

Table 3 Available Data with Extraction Conditions

	COMPONENT			
	Filter	***Mixing Bag***	***Tubing***	***Gasket***
SCT	1.5	1.5	1.5	1.5
A	1	1,150 cm2	200 cm2	1
B	2	21,600 cm2	3,990 cm2	3
C	1,500	1000	660	100
D	60,000	60,000	60,000	60,000
E	10	10	10	10
UF	2	2	2	2
AET	**1.5 µg/mL**	**0.24 µg/mL**	**0.34 µg/mL**	**15 µg/mL**
Reporting Threshold	0.1µg/mL	0.5µg/mL	0.1µg/mL	N/A
Further work required?	No	Yes	No	Yes

Note: It is important to ensure that the reporting threshold used by the supplier is appropriate for how the component is used in the manufacturing process and based on the worst-case daily dose. The reporting threshold should be equal to or below the calculated AET for the component.

KNOWLEDGE GAP EVALUATION

Taking the data from Table 1-3 into account, it is possible to determine if further data is required.

Filter: Was identified as a moderate risk component therefore, as a minimum organic extractable profiling using 50:50 ethanol:water is required as per USP<665>. The extraction study was performed using extraction times, temperatures and solvents which represent a worst-case compared to actual use conditions. Additionally, the reporting threshold was lower than the calculated AET. The supplier data aligned with USP<665> therefore, no further extractable work is required for this component.

Mixing Bag: Was identified as high risk and therefore, required the highest level of chemical testing. Data conforming to USP<665> was available from the supplier for the mixing bag. Based on the manufacturing process conditions, data on a component extracted for at least 9 days would be required. Additionally, the AET is lower than the reporting threshold therefore, data generated with a lower reporting threshold (≤0.34 µg/mL) would be required to determine the risk of using the materials in the process.

Tubing: Was identified as low risk, as per USP<665> only NVR and UV absorbance data would be required. The supplier provided data as per the BioPhorum protocol alongside pH, NVR and TOC data. No UV absorbance data was available. However, the other data available (GC-MS, LC-MS and ICP) meant that additional UV data was not considered to be necessary. The extraction study was performed using extraction times, temperatures and solvents which represent a worst-case compared to actual use conditions. Additionally, the reporting threshold was lower than the calculated AET. The supplier data aligned with USP<665> therefore, no further extractable work is required for this component.

Gaskets: Was identified as high risk and therefore, required the highest level of chemical testing. No data was available for the gasket. Thus, further testing is required with an AET of 15 µg/mL.

USP<665> VERSUS BIOPHORUM EXTRACTABLE PROTOCOL

Table 4 and 5 below can be used to assess the similarities and differences between USP<665> and the BioPhorum (BPOG) extractable protocol.

As summarised below, USP<665> has 3 different levels of testing based on the components risk category whilst the BioPhorum extractable protocol does not use the same risk-based approach (note: this is covered in the BioPhorum's Guide for Evaluating Leachable Risk).

Table 4 Stipulated Test Requirements from USP<665> & BIOPHORUM (BPOG) for each risk designation.

Risk Level	USP <665> Requirements	BPOG Extractables Requirements
 LOW	Non-Volatile Residue (NVR) UV	Extractables data evaluation as per BIOPHORUM (BPOG) protocol.
 MEDIUM	Organic extractables profile	
 HIGH	Three solvent extractables profile. Element analysis (as required)	

Table 5 Overview of Extraction details for USP<665> & BIOPHORUM (BPOG)

COMPONENT	SOLVENTS				TIME (IN DAYS)			
	50% Ethanol	*pH3 0.1M phosphoric acid*	*pH10 0.5 NaOH*	*WFI*	*1*	*7*	*21*	*70*
Containers (storage)	X	X	X	X	X		X	X
Tubing attached to storage containers	X	X	X	X	X		X	X
Bag ports (storage)	X	X	X	X	X		X	X
Closures	X	X	X	X	X		X	X
Containers (Mixing)	X	X	X	X	X		X	
Bag ports (mixing)	X	X	X	X	X		X	
Impellers & molded parts for bioreactors / mixers	X	X	X	X	X		X	
Tangential flow modules for perfusion or continuous processing	X	X	X	X	X		X	
Tubing attached to mixing containers	X	X	X	X	X		X	
Tubing connectors and disconnectors, fittings, over moulded junctions.	X	X	X	X	X		X	
Filtration cassettes	X	X	X	X	X			
Aseptic connectors and disconnectors	X	X	X	X	X	X		
Filters	X	X	X	X	X	X		
Filling needles	X	X	X		X			
Chromatography column housing	X	(X)	(X)		X			
Small components (O-rings, gaskets, etc.)	X	(X)	(X)		X			

KEY		
	X	USP<665> & BPOG
	(X)	USP<665> Only
	X	BPOG Only

DATA EVALUATION

Extractables above the AET should be evaluated by a toxicologist to determine the potential risk to patient safety. Compounds above the reporting threshold defined in the BioPhorum protocol (0.1 µg/mL) may not require toxicological evaluation unless they are detected at levels above the AET.

The table below summarises some example results for studies provided by the supplier or studies that were performed as a result of the gap analysis.

Whilst a number of extractables were reported by the supplier for the components filter & tubing, not all the extractables were found above the AET. Only extractables above the AET were sent for toxicological evaluation.

The reporting threshold for the mixing bag and gasket was aligned to the AET prior to commencing the study. Therefore, all analytes detected were sent for toxicological evaluation.

Table 6 Number of analytes detected above the Reporting Threshold and the AET

Components	*Reporting Threshold*	*Calculated AET*	*Number of Analytes above Reporting Threshold*	*Number of Analytes above AET*
Filter	0.1 µg/mL	1.5 µg/mL	30	2
Mixing Bag	0.24 µg/mL	0.24 µg/mL	10	10
Tubing	0.1 µg/mL	0.34 µg/mL	10	5
Gasket	15 µg/mL	15 µg/mL	1	1

CONCLUSION

To conclude, it is important to ensure the extractable data generated during a study is an accurate representation of worst-case scenarios when qualifying components for use in a particular manufacturing process. Whilst the protocols discussed in this whitepaper can be followed to provide worst-case data for a number of in-use cases, they do not cover all eventualities. Ultimately, it is the end-users responsibility to ensure sufficient data is available to evaluate the potential risk of using a specific component or material in their process stream.

The risk evaluation presented in USP<1665> can be useful for determining what testing is required, although alternative approaches (where justified) can be used (e.g. an individual organisations risk assessment process).

ELEMENT MATERIALS TECHNOLOGY

Element has one of the largest and most experienced extractables & leachables (E&L) practices in the world, in addition to unparalleled experience testing a breadth of products in the pharmaceutical, biopharmaceutical and single-use systems (SUS) markets. As your regulatory and scientific partner, Element will help you to navigate the most efficient path to regulatory approval.

Our team of engaged experts have directly contributed to the industry for years, serving as active participants in several working groups that have developed the PQRI, USP Expert Panels, ELSIE and BIOPHORUM (BPOG) extractable and leachable best practices.

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