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Application Note

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Flexsafe® Bag Family

Mechanical Resistance and Extractable Evaluation of Flexsafe® Storage, Flexsafe® Magnetic Mixer, and Flexsafe® Pro Mixer Bags for High Temperature Applications up to 60°C

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Abstract

The Flexsafe® family including storage, shipping, and mixing systems provide a unique single-use solution for biopharmaceutical manufacturing facilities, fitting all applications from buffer and media preparations, downstream steps to final formulation. The Flexsafe® film offers high standard quality attributes such as biocompatibility, integrity, and robust supply chain.

Heating in single-use bags is required in numerous biopharmaceutical processes. This application study evaluates the suitability of the Flexsafe® bag family for applications at high temperature up to 60°C.

50 L scale Flexsafe® mixing bags are used in this first assessment and robustness after extraction with water at 60°C is evaluated by performing an integrity test with ink. Physical and mechanical properties are also checked.

Finally, an evaluation of the influence of a 60°C process temperature on the extractable profile is shared.

Find out more: www.sartorius.com/flexsafe-pro-mixer

Introduction

Heating in single-use bags is required in numerous biopharmaceutical applications. For example, in buffer and media preparation, WFI can be heated up to accelerate dissolution and thereby optimize the process. Also in downstream, some steps can require higher temperature.

The purpose of this application study is to give first results on the mechanical resistance of Flexsafe® Magnetic Mixer bag and Flexsafe® Pro Mixer bag after extraction at 60°C with water and thus suitability of Flexsafe® Magnetic Mixer bags, Flexsafe® Pro Mixer bags, and Flexsafe® bags for storage to be used in high temperature applications up to 60°C.

A 50 L Flexsafe® Magnetic Mixer bag including TPE C-Flex® 374 and Tuflux® silicone tubing and a 50 L Flexsafe® Pro Mixer bag including drain valve and thermowell are chosen to assess this high temperature process.

An 8 hour contact extraction at 60°C with tap water have been run during mixing phase at 120 rpm on the Flexsafe® Magnetic Mixer bag. Then, film, seals, bag components, and mixing accessories have been visually inspected, measured and tested to check physico-mechanical properties. Infrared spectrometry and an integrity test with ink have also been performed. Tests are adapted from the ASTM D543 "Standard test method for resistance of plastic to chemical reagents" and from internal protocol.

A 60 minutes contact extraction at 60°C with tap water have been run during mixing phase at 200 rpm on the Flexsafe® Pro Mixer bag including critical components. Then, film, seals, bag component, and mixing accessories have been visually inspected and an integrity test with ink have been performed.

Separate technical and application notes on Palletank® cubical jacketed mix and Flexsafe® Pro Mixer heat transfer performance are available.

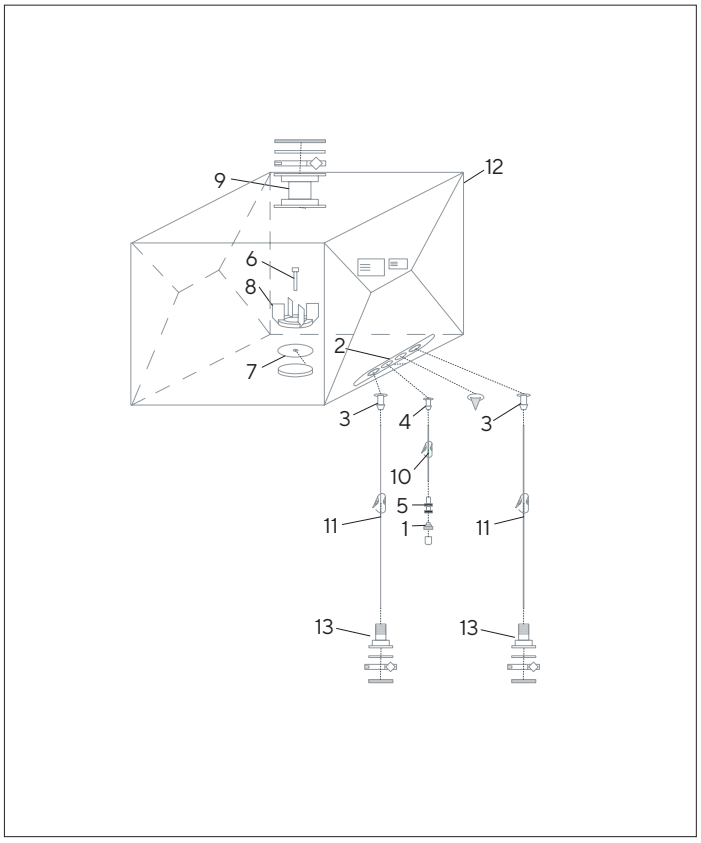
The purpose of this application note is also to evaluate the influence of the process temperature on the extractable profile when the temperature exceeds the one used in regular extractable study conditions (40°C).

Materials

Trial 1

Consumables

- Tap Water
- 50 L Flexsafe® Magnetic Mixer bag including PE port, TPE C-Flex® 374 tubing and Tuflux® silicone tubing (figure 1), sterilized by Gamma irradiation between 25 and 45 kGy.



N°	Description	PN	Material
1	Clave Connector	ST 107530	Acrylic | PBT | PP | SI
2	4 Ports Flange	TC 112124	PE
3	Port ½"	TC112128	PE
4	Port ¼"	TC112131	PE
5	Luer Female ¼"	TC112313	PP
6	Impeller Post	TC112343	PE
7	Impeller Seat	TC112344	PE
8	Impeller Ø6.35" (161 mm) Magnetic Mixer + Shield	TC122855	PE | PVDF
9	Single Port Tri Clamp 8"	TC112877	PE
10	Tube ¼" ID X ⅜ OD L6" (150 mm)	TU 110775	TPE C-Flex® 374
11	Tube ½" ID X ¾" OD L60" (1500 mm)	TU 113416	SI (Pt) Tuflux®
12	Bag Chamber	SF 113810	PE
13	TC 1-½" × ½" HB	TR 114165	HDPE

* C-Flex® is a registered trademark of Saint-Gobain Performance Plastics Corporation

Figure 1: 50 L Flexsafe® Magnetic Mixer bag drawing and extract from the bill of material

Equipment

- Palletank® cubical jacketed mix 50 L
- Magnetic Mixer drive unit
- Balance 3T Combics Pro
- Unichiller Huber
- Temperature probe
- Dynamometer and sensor
- FTIR spectrophotometer
- Calliper
- Micrometer
- Profile projector
- Light table

Trial 2

Consumables

50 L Flexsafe® Pro Mixer bag including pH and conductivity sensors, drain valve and thermowell (figure 2), sterilized by Gamma irradiation at 50 kGy.

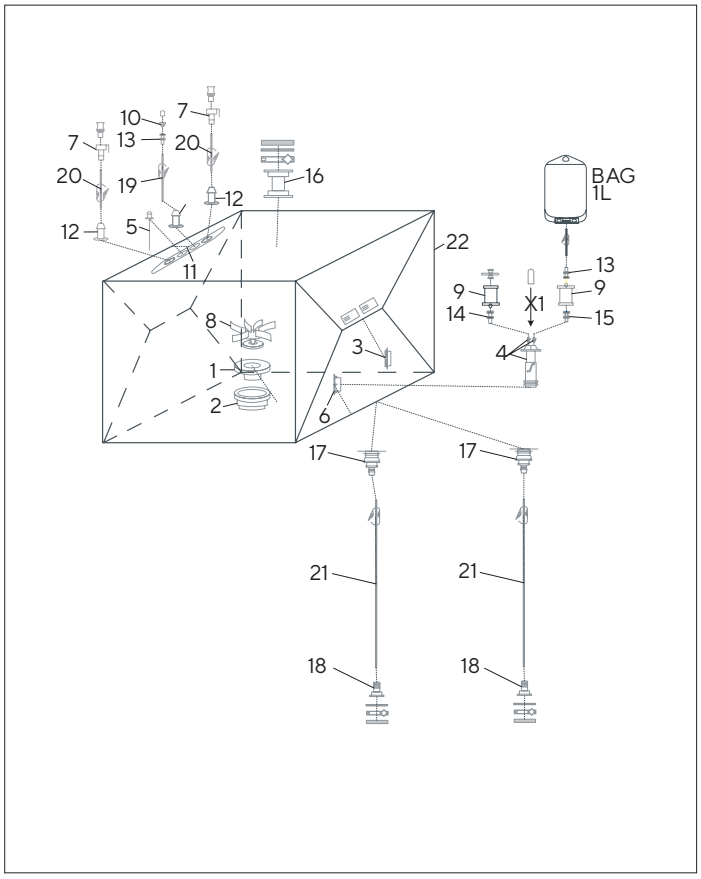


Figure 2: 50 L Flexsafe® Pro Mixer bag drawing and extract from the bill of material

N°	Description	PN	Material
1	Cup for Mixer	1000024443	HDPE | PE
2	Shield of Mixer	1000034379	PET
3	Conductivity Sensor	1000034792	PE
4	Injected Device with PH Sensor Gen II	1000034812	HDPE | SI(Pt)
5	Thermowell ID 3 mm (L 135)	AX112667	PE | SI(Pt)
6	Bag port PH Sensor	AX112957	PE
7	Coupling Female¼	CK211010	PSU
8	Flexsafe® Impeller Assembly	1000034856	PE
9	Check Valve Male (out) and Female (in)	LC113161	PC | SI
10	Clave Connector	ST107530	ABS | PS | SI
11	4 Ports Flange	TC112124	PE
12	Port ¼"	TC112131	PE
13	Luer Female ¼"	TC112313	PP
14	Luer Female 5/32"	TC112315	PP
15	Luer Male 5/32"	TC112320	PP
16	Single Port Tri Clamp 8"	TC112877	PE
17	Bottom Draining Valve ½" HB	TC114240	PE
18	Tri Clamp 1-½" × ½" HB	TR210911	PP
19	Tube ¼"ID X 3/8" OD L4" (100 mm)	TU113234	SI(Pt) Tuflux®
20	Tube ¼"ID X 7/16" OD L4" (100 mm)	TU 113260	SI(Pt) Tuflux®
21	Tube ½"ID X ¾" OD L60" (1500 mm)	TU113416	SI(Pt) Tuflux®
22	Bag Chamber	SF113810	PE

Equipment

- Palletank® for Mixing with Weighing 50 L
- Flexsafe® Pro Mixer drive unit
- Recirculation heater
- Temperature probe
- Datalogging thermometer

Methods

Method

Trial 1

1. Connect the Palletank® for Magnetic Mixer with jacket to the chiller system.
2. Install the 50 L Flexsafe® bag for Magnetic Mixer in the Palletank®.
3. Fill up the bag to nominal volume with tap water.
4. Insert temperature probe via the top port and position it at the regular thermowell location.
5. Couple the Magnetic Mixer drive unit to the Palletank®.
6. Set impeller speed at 120 rpm and start mixing.
7. Set the chiller to reach and maintain 60 ± 5 °C inside the bag.
8. Once, the 60 °C target temperature is reached, continue mixing for 8 hours.
9. After the 8 hour period, perform a cooling step down to 40°C and make sure that the system and the water have cooled down sufficiently, to prevent scalding, before proceeding with bag emptying.
10. Once the bag is fully drained and disconnected, perform an integrity test with ink and inspect the bag for leaks (components welds, film welds).
11. Take some samples from the bag and perform additional tests as listed in table 1.

Trial 2

1. Install the 50 L Flexsafe® Pro Mixer bag in the Palletank®.
2. Fill up the bag to nominal volume with water.
3. Insert temperature probe through bag's thermowell and connect to datalogging thermometer.
4. Couple the Flexsafe® Pro Mixer drive unit to the Palletank®.
5. Set impeller speed at 200 rpm and start mixing.
6. Connect the bag to the recirculation heater.
7. Start heating until temperature probe reads at least 60°C and keep 60 ± 5 °C for at least 60 minutes while continuing mixing.
8. Then, to prevent scalding allow system to cool for at least 48 hours before draining.
9. Once the bag is fully drained and disconnected, perform an integrity test with ink and inspect the bag for leaks (components welds, film welds).

Results

Trial 1

The graph of the temperature measured inside the bag during the test confirm that the bag stayed at 60°C for at least 8 hours (figure 3).

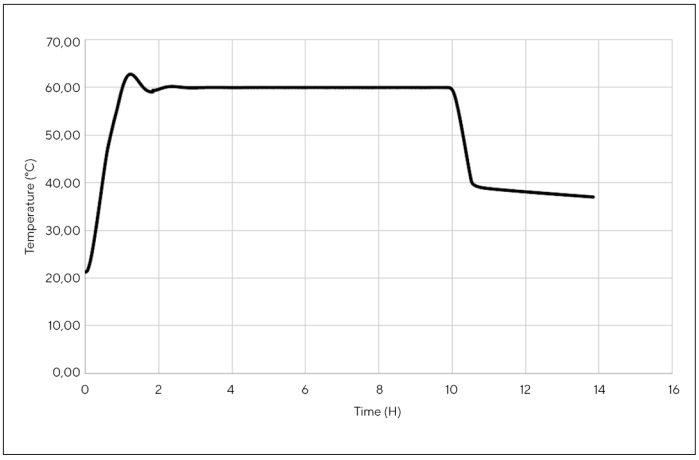


Figure 3: Temperature inside the 50 L Flexsafe® Magnetic Mixer bag during the test.

The result of the bag inspection and integrity test with ink shows no visual defect and no leak of the Flexsafe® Magnetic Mixer bag after an 8 hour-contact time extraction with tap water at 60°C.

The additional physical and mechanical tests performed pass Sartorius specifications and show no differences before and after application (table 1).

Table 1: Results obtained for each test performed on the 50 L Flexsafe® Magnetic Mixer bag, accessories and impeller seat.

Tests	Results Pass|Fail	Observations
Visual inspection	Pass	**Visual inspection:** There is no modification of the visual bag|component properties of Flexsafe® bag and mixing accessories before and after filling and extraction at 60°C with tap water for 8 hours. **Physical | mechanical properties:** There is no modification of the physico-mechanical film|component properties of Flexsafe® bag and mixing accessories after filling and extraction at 60°C with tap water for 8 hours. There is no significant difference between the IR spectrum of the Flexsafe® film and PE seat and the IR Spectrum of the Flexsafe® film and PE seat after filling and extraction at 60°C with tap water for 8 hours.
Integrity by ink	Pass	
Film tensile strength	Pass	
Tensile strength of seals	Pass	
Tensile strength of connections	Pass	
IR on film and PE seat	Pass	
Film thickness	Pass	
Tubing thickness	Pass	
Impeller PE seat dimensions	Pass	

Trial 2

The result of the bag inspection and integrity test with ink shows no visual defect and no leak of the Flexsafe® Pro Mixer bag after a 60 minutes-contact time extraction with tap water at 60°C.

Discussion

The results show that the Flexsafe® film and mixing accessories from the 50 L Flexsafe® Magnetic Mixer bag have an excellent mechanical resistance in a worse case process scenario after an 8 hour-contact time extraction with tap water at 60°C.

The test on the 50 L Flexsafe® Pro Mixer bag, which constitutes a worst case in terms of bag configuration as it includes several critical components in mixing applications like sensors, confirm good performance of the system at 60°C.

The tests performed establish a good evaluation and first assessment concerning the suitability of the Flexsafe® bag family for applications up to 60°C. The tests are not exhaustive, for example, in terms of configurations like larger bag volumes, and therefore do not constitute a comprehensive validation of process conditions across the entire mixing range. Thus, before using a bag from the Flexsafe® family for such applications, it is recommended to proceed to further testing that is representative of specific process conditions and bag configuration. The Sartorius Confidence® Validation Services can offer such possibilities.

Usually, most applications using Flexsafe® mixing or storage bags can be classified as low-risk application according to BPOG best practice guide for leachables risk evaluation. Therefore, compliance toward USP class IV or EP is sufficient and no extractables or leachables data is required. The following chapter will provide additional considerations on the risk assessment applying extractables and leachables information, which are required for applications in higher risk scores, e.g. drug substance preparation and/or storage.

Evaluating Relevance of Process Temperature Exceeding Extractables Study Conditions

Extractables studies are performed to provide characterization and quantification data on chemical compounds - extractables - that are released by materials of construction. The aim is to generate a comprehensive qualitative and fully elucidated extractables profile of the test item including an estimation of the quantitative level of the extractables. This information is used for the toxicological assessment of a single-use system (SUS) during the qualification of a biopharmaceutical process.

Further, knowledge of the extractables is required to identify compounds that are potentially released into a process stream - process equipment-related leachables (PERLs) - or that that are present in the final drug product - leachables.

It is not possible (purpose) to bracket all potential process conditions a SUS can encounter with an Extractables study. The design of the SSB' standardized extractables approach covers most process conditions of the biopharmaceutical industry. For that purpose, several solvents including a strong organic extraction solvent such as ethanol, high extraction temperatures, and long extraction times are applied.

In recent years, a harmonization between different approaches has been achieved. An extraction temperature of 40°C is accepted within industry group,[1] regulators,[2] and suppliers.[3] This temperature was selected to provide reasonable quantities of extractables in an adequate extraction time allowing a reliable material characterization without the risk of influencing materials' integrity.

It is known that for extraction times longer than seven days, equilibrium concentrations of the extractables are approached. Their level depends to some extent on the temperature. Typically increasing with higher temperatures and is reached after a shorter time in that case.[4] This was confirmed analytically during the developing phase of the Sartorius Extractables approach were 60 °C was selected as one extraction temperatures along with 40 and 23 °C. The time and temperature dependency is shown in Figure 1 for a SU bag film material for the total extractables amount. The individual compounds behave similarly. As an example, the extractables data obtained for 40 °C at seven days can be used to assess a process below or equal to one day at 60°C. The level of the extractables are identical. Qualitatively, the extractables profile remains unchanged and new extractables are not "generated" nor do others "disappear".

At longer contact times going into equilibrium a maximum factor of two can be assumed between the level of extractables between 40 and 60°C. In context of equilibrium it needs to be emphasized, that after a media preparation (e.g. 60°C) with a subsequent longer storage at a lower temperature (e.g. 40-23°C), the extractables concentration after storage will approach the equilibrium level at the lower, the storage temperature and not the level of the higher mixing temperature.

At customer application scale - usually below 1 day - the graph shows that the amount of extractables is significantly lower at short contact times compared to equilibrium conditions. Further, the temperature has a lower impact on the extractables level in the first few hours than for long contact time. In consequence, the amount of the extractables at 60°C/1 day is equal to the amount at the extraction conditions 40°C/7 days. This observation is in line with the extrapolation method given in ASTM 1980-16, which is based on the Arrhenius concept.

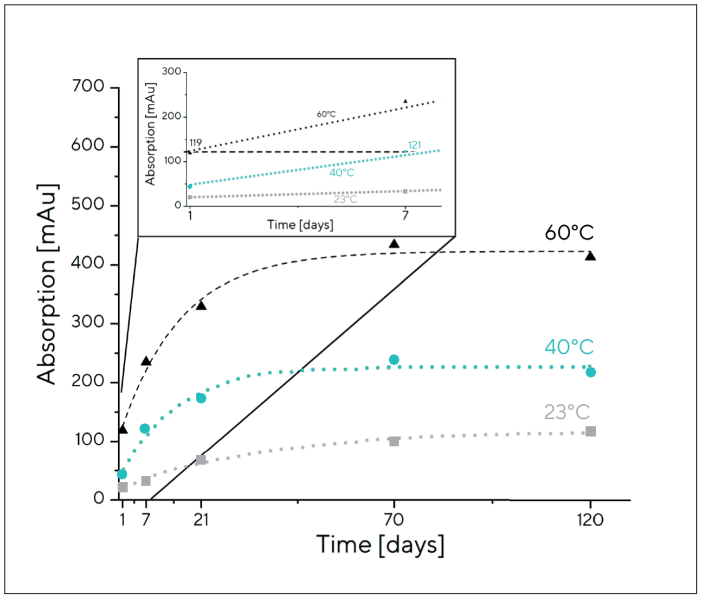


Figure 4: Results from a kinetic experiment of the developing phase for the Sartorius Extractables Approach. Total extractables amount measured by HPLC-UV analysis of a film ethanol extract. Level of extractables - the quantity - between 40 and 60° C in equilibrium is different by approximately a factor of two. As an example, extractables level at seven days/40 °C (blue circle) is identical to level at one day/60 °C (black triangle). Taken from Pahl et al.[3]

In addition, the temperature dependent release of migrants for fluid contact materials for SUS can be modeled as it is already performed for food contact materials.[5]

Note: Extractables quantities are exaggerated and worst-case under any circumstances particularly for the pure ethanol extracts. The outcome of the extractables assessment obtained during qualification of the biopharmaceutical process should be unaffected by higher extractable quantities by a factor of two for temperatures (60°C). Especially, since a extractables assessment of fully elucidated extractable profiles - as they are provided by Sartorius Stedim Biotech - lead to high exposure margin for the individual extractable compounds using permitted daily exposure (PDE) values.

Further, a leachables study is recommended for critical applications to demonstrate toxicological compliance of the SUS during operation.

Conclusion

Flexsafe® bags are suitable for the mixing, storage and shipping of all biopharmaceutical applications from buffer and media preparations, downstream process intermediates to final formulation.

This application note demonstrates the excellent mechanical resistance of the 50 L Flexsafe® Magnetic Mixer bag and the 50 L Flexsafe® Pro Mixer bag configurations tested after extraction at 60°C with water. It also gives insights on extractable evaluation at this high temperature. This is a good baseline in the evaluation of the Flexsafe® storage, Flexsafe® Magnetic Mixer and Flexsafe® Pro Mixer bag's suitability for high temperature applications up to 60°C.

Flexsafe® pave the way towards your single-use manufacturing facility of the future.

Reference

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