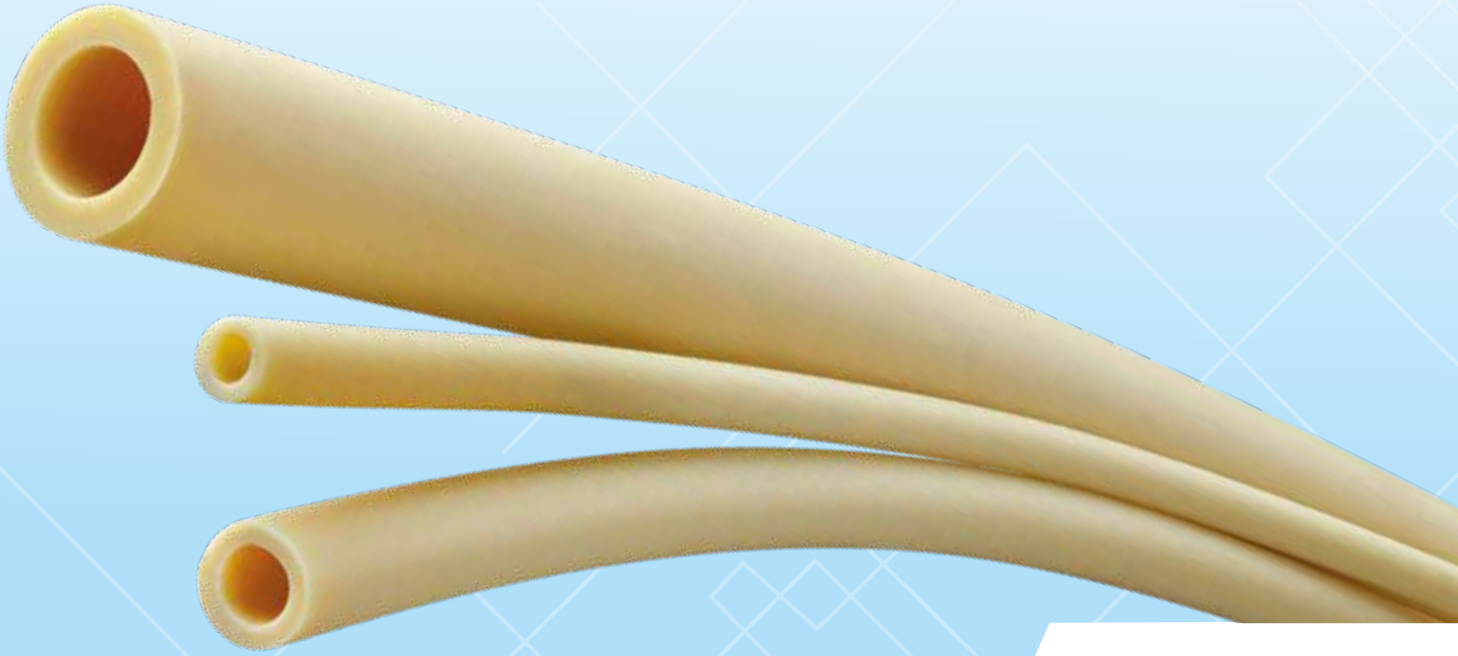
WATSON MARLOW Fluid Technology Solutions



WATSON MARLOW Tubing

Validation guide summary

Bioprene

Contents

1. Introduction

Bioprene is a high performance thermoplastic elastomer tube manufactured from a USP Class VI raw material manufactured within an ISO9001 quality system. Bioprene is manufactured in an ISO 114644-1 Class 7 Cleanroom, is validated to USP Class VI*.

Manufacturing is located at Watson-Marlow Ltd, Bickland Water Rd, Falmouth, UK, TR11 4RU.

Bioprene tubing has the following key features:

- Meets USP Class VI requirements*
- Meets FDA regulations CFR 177.2600 for contact with aqueous food
- Made from NSF51 food compliant raw material
- Long peristaltic life
- Broad chemical compatibility
- Opaque to visible and UV light
- Low extractables; no phthalates added, no latex used

**except for product codes: 088.T400.E0P, 088.T400.EOG. Applies to Bioprene tubing only (not connectors).*

2. Conditions of use

Bioprene can be sterilised by autoclave up to 121°C for 30 minutes and gamma irradiation up to 35 kGy. Functional testing of Bioprene in pump head shows acceptable performance at 40 kGy.

3 Chemical compatibility

A general guide on chemical compatibility of Bioprene tubing can be found on the Watson-Marlow Fluid Technology Solutions website. wmfts.com/chemical

4 Regulation compliance statements and manufacturing conditions

4a Country of Origin

Bioprene is manufactured in Falmouth, Cornwall, United Kingdom.

4b REACH legislation

All raw materials, compounds used in the manufacturing process and the final Bioprene tubing comply with the REACH regulations.

4c RoHS

In compliance with the restriction of hazardous substances (RoHS) directives, no listed substances are used in the manufacture of Bioprene tubing.

4d Working temperature range

Bioprene tubing can be used in a pump from +5C to +80C. In static applications the working temperature range can be extended. The brittle temperature is <-60C.

4e Storage conditions

To maintain the performance of the components throughout their lifecycle, they should be stored in a cool, dry environment away from direct sunlight without exposure to chemicals and not subjected to stress. Normal warehouse conditions of 5C–40C (40F–86F) are acceptable. Wherever possible, original packaging should be maintained. Stock should be rotated on a first in, first out (FIFO) basis.

The performance of any component beyond its use by date, or which has not been stored according to the recommendations outlined above, cannot be assured.

5. Compendial and non-compendial testing*

5a Summary table

Table 1 contains a summary of all the compendial testing and qualifications that Bioprene has been evaluated for. Full test methods and results are available on request. All test work was performed on tubing samples and this was used to represent the Bioprene product family as the components are manufactured using the same material at the same manufacturing site.

Table 1: Summary list of Compendial and non-compendial testing

Test	Result
USP <87> Biological Reactivity tests, In Vitro	Pass
USP <88> Biological Reactivity tests, In Vivo	Pass
USP <85> Bacterial EndoToxins Test	Pass
ISO 10993-4 Hemolysis	Pass
ISO 10993–5 Biological evaluation of medical devices - part 5: tests for In Vitro cytotoxicity	Pass
ISO 10993–6 Short term Intramuscular Implantation Test	Pass
ISO 10993–10 Kligman Maximisation Test	Pass
ISO 10993–10 guidelines for Intracutaneous Injection Test	Pass
ISO 10993–11 guidelines for Systemic Injection Test	Pass
USP <381> Elastomeric closures for injections - physiochemical tests	Pass

5b USP <87> Biological Reactivity tests, *In Vitro*

USP 87 determines the biological reactivity of a cell culture in response to a given test article. Samples of Bioprene tubing were tested in accordance with USP 35, NF 30, <87>, Biological reactivity tests, In Vitro. Extracts, positive control (rubber) and negative control articles were prepared at 37C (98.6F) for 48 hours. Biological reactivity was rated on a scale ranging from Grade 0 (no reactivity) to Grade 4 (severe reactivity).

Results: No reactivity was exhibited by the cell cultures when exposed to Bioprene tubing. Therefore, they passed the requirements of USP 87 biological reactivity tests and have no cytotoxic potential.

5c USP <88> Biological Reactivity tests, *In Vivo*

USP Class VI Plastics Test assesses the potential toxicity of a given test article systemically, intracutaneously and through implantation. Samples of Bioprene tubing were tested in accordance with USP 35, NF30 <88>, Biological reactivity tests, In Vivo. This included the immersion of the test articles in the following solutions: USP 0.9% sodium chloride, cottonseed oil, 1 in 20 ethanol in NaCl and polyethylene glycol 400 at 70C (250F) for 24 hours.

Results: Bioprene tubing extracts and implants showed no toxicity. Therefore, they passed the requirements of USP <88> biological reactivity tests.

5d USP <85> Bacterial EndoToxins Test

USP Limulus amebocyte lysate (LAL) bacterial entoxin assay Endotoxins are lipopolysaccharide complexes in gram negative bacterial cell walls. The limulus ameobocyte lysate (LAL) Kinetic Chromogenic assay is used to determine the quantitative level of endotoxin present within our products. Bioprene tubing was tested in accordance to the requirements of USP 85. Bioprene (27.6.0 cm^2 surface area) was extracted in 50mL of LAL reagent water at room temperature for 60 minutes. LAL-lysate was added to each sample well on an automated microtiter plate and then transferred to a plate reader where the absorbance of each well was read at 405 nm and 37 C at set timepoints. A standard curve was generated using software based on reaction time versus endotoxin concentration which was then compared to the endotoxin concentration and reaction time of the sample extract.

Results: Bioprene extracts had an EU/ml value of <0.009 EU/cm^2 which is comparable to the levels of endotoxin observed in water for injection.

5e ISO 10993-4 Hemolysis

The hemolysis test assesses the potential for indirect contact of a given sample with blood to cause the rupture of erythrocytes (red blood cells).

Bioprene were tested in accordance with ISO 10993-4, Biological Evaluation of Medical Devices – Part 4: Selection of Tests for Interactions with Blood.

Results: Per ISO 10993-4, Bioprene is considered non-hemolytic

5f ISO 10993–5 Biological evaluation of medical devices - part 5: tests for In Vitro cytotoxicity

The biological reactivity of a cell culture, in response to extracts from Bioprene tubing was determined. The maintenance medium on the cell cultures was replaced by extracts of Bioprene tubing or control article.

The cell cultures were incubated for 48 hours at 37±1C (98.6F±33.8F).

Results: Bioprene tubing showed no signs of cytotoxic potential. Therefore, they passed the requirements of ISO 10993–5.

5g ISO 10993–6 Short term Intramuscular Implantation Test

The purpose of this test is to evaluate the solid material in direct contact with living tissue.

Bioprene and the negative control plastics were tested. The test sites were examined for encapsulation, necrosis, haemorrhage and discolouration macroscopically.

Results: Bioprene tubing did not demonstrate any remarkable difference as compared to the control implant sites when in contact with tissue for 2 weeks.

5h ISO 10993–10 Kligman Maximisation Test

The purpose of this test is to detect the allergenic potential of a test article.

Bioprene was tested in accordance with ISO 10993-10. Samples of Bioprene were extracted in USP 0.9% NaCl for injection and cottonseed oil at 70C for 24 hours and then injected intradermally. After two weeks, an additional topical application was introduced to the site of intradermal injections.

Results: The sites that were exposed to the test articles and negative control showed no signs of erythema or edema. Therefore, Bioprene is deemed not to contain any allergy.

5i ISO 10993–10 guidelines for Intracutaneous Injection Test

The intracutaneous test is designed to evaluate local responses to the extracts of Bioprene tubing following intracutaneous injection. Sample was extracted using 0.9% sodium chloride for injection, cottonseed oil, 1 in 20 ethanol in NaCl or polyethylene glycol 400 at 70C for 24 hour.

Results: Bioprene tubing meet the requirements of ISO10993–10 guidelines for the intracutaneous injection test.

5j ISO 10993–11 guidelines for Systemic Injection Test

The purpose of the systemic injection study is designed to screen test articles extracts for potential toxic effects as a result of a single dose systemic injection. Bioprene is extracted using 0.9% sodium chloride for injection, cottonseed oil, 1 in 20 ethanol in NaCl or polyethylene glycol 400 at 70C for 24 hour.

Results: Bioprene tubing meet the requirements of ISO 10993–11 guidelines for the systemic injection test toxicity.

5k USP <381> Elastomeric closures for injections - physiochemical tests

Extracts of Bioprene were prepared according to the requirements of USP 32, NF 27, Chapter 381 as directed under physiochemical tests. The results of the tests are summarised in Table 2.

Results: Based on the evaluation criteria mentioned below, Bioprene tubing meets the requirements of the USP <381> section physiochemical tests.

Table 2: USP <381> Summary

Test	Test Result	Evaluation Criteria (Type One Closures)	Result
Appearance of Solution: Turbidity	0.032 NTU	≤ 6 NTU For Type 1 Closure	Pass
Appearance of Solution: Color	Colour less intense than Colour Standard	Colour is less intense than colour standard (Eu. Ph. GY5 Colour Standard)	Pass
Acidity or Alkalinity	< 0.05 mL NaOH > Blue colour developed	≤ 0.3 mL NaOH 0.01 N to produce a blue colour	Pass
Heavy Metals	Colour is less intense than colour in the Standard	Colour less intense than Standard Solution 2 ppm Lead	Pass
Reducing Substances	0.1 mL	≤ 3.0 mL For Type 1 Closure	Pass
Absorbance	0.024 A.U	≤ 0.20 A.U For Type 1 Closure	Pass
Extractable Zinc	0.067 ppm ZN	≤ 5 ppm Zn	Pass
Ammonium	Colour is less intense than colour in the Standard	Colour is less intense than 2 ppm ammonium Standard Solution	Pass
Volatile Sulfides	No black stain visible	Any Stain is less intense than the Control Solution	Pass

5l USP <788> Particulate testing microscopic particulate count analysis test

This test is used to determine a level of particulates measuring 10 micron (µm) or less and 25 micron (µm) that may be present in any given drug product. A length of Bioprene tubing was filled with low particulate water and shaken 20 times. The extraction fluid was then recovered and the particles were measured using light obscuration.

Results: Extracts from a Bioprene tube contained 571 particles > 10µm and 16 particles > 25µm

6. Extractables

The objective of this study is to generate a comparative extractable profile of Bioprene tubing.

The extractable profile of the samples was analysed using the following methodologies:

- GC-MS headspace analysis on components for volatile extractables.
- Semi-volatile extractables using direct injection GC-MS.
- Non-volatile extractables using LC-DAD-MS (230 nm and ESI +/-ve).

6a Sample Preparation

A summary of the sample preparation conditions is shown in Table 3 and a summary of the extraction conditions is shown in 4.

7. Conclusions

Bioprene has been shown to pass several compendial and ISO testing, as summarised in this guide. For further information with full compliance statements and the test reports, please contact your WMFTS representative.

The compliance summary and the full validation guide for Bioprene are available by filling in a request form on the wmfts.com website:

www.wmfts.com/

Table 3: Sample Preparation Summary

Test Procedure	Hall Sample ID	Extraction Solutions	Extraction Method
Headspace GC-MS Direct Injection GC-MS LC-DAD-MS (ESI)	H80017 and Negative Control	Water	Incubation, 40°C for 21 days
		50% Ethanol(aq)	
		0.1M Phosphoric acid	
		0.5N NaOH	

Table 4: Summary of Extraction Conditions

Component	Number of Components	Extraction Volume (mL)	Reporting Threshold (µg/mL)	Reporting Threshold (µg/cm2)
Bioprene Tubing	1 x 125 cm	60	0.1	0.02

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