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TOXIKON FINAL GLP REPORT: 09-0302-G2

PHYSICOCHEMICAL TEST FOR PLASTICS – USP

Test Article
WaterSep HF Cartridge

Author
Amtul Qamar, M.S.

Final Report Date
February 19, 2009

COMPLIANCE
21 CFR, Part 58
Good Laboratory Practice for Non-Clinical Laboratory Studies

MANAGEMENT OF THE STUDY

Performing Laboratory
Toxikon Corporation
15 Wiggins Avenue
Bedford, MA 01730

Sponsor
WaterSep Technology Corporation
420 Maple Street, Suite 1
Marlborough, MA 01752

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STUDY SUMMARY

The purified water extract of the test article, WaterSep HF Cartidge, meets the criteria described in the USP Physicochemical Test For Plastics guidelines.

QUALITY ASSURANCE STATEMENT

This study was conducted in compliance with U.S. Food and Drug Administration regulations set forth in 21 CFR, Part 58.

The sections of the regulations not performed by or under the direction of Toxikon Corporation, exempt from this Good Laboratory Practice Statement, included characterization and stability of the test article and its mixture with carriers, 21 CFR, Parts 58.105 and 58.113.

The Quality Assurance Unit conducted inspections on the following dates. The findings were reported to the Study Director and to Toxikon's Management.

INSPECTIONS	DATE OF INSPECTION	DATE REPORTED STUDY DIRECTOR	DATE REPORTED MANAGEMENT
BUFFERING CAPACITY	02/11/09	02/11/09	02/11/09
RAW DATA	02/19/09	02/19/09	02/19/09
FINAL REPORT	02/19/09	02/19/09	02/19/09

Paula Mazzio, B.S., M.P.H.
Quality Assurance

2/19/09
Date

STUDY DIRECTOR SIGNATURE AND VERIFICATION DATES

This study meets the technical requirements of the protocol. The study also meets with the requirements of the Good Laboratory Practice Regulations, 21 CFR, Part 58, with the exemptions as stated in the Quality Assurance Statement.

Protocol Number:	WRP/PCHEM/001–09/000
Study Director:	Amtul Qamar, M.S.
Company:	Toxikon Corporation
Signature:	Amtul Qamar
Date:	2-19-09
Study Supervisor:	Amtul Qamar, M.S.

VERIFICATION DATES:
The Study Initiation Date is the date the protocol is signed by the Study Director.

Test Article Receipt:	01/22/09
Project Log Date:	02/03/09
Study Initiation Date:	02/09/09
Extraction Dates:	02/10/09 – 02/11/09
Technical Initiation:	02/10/09
Technical Completion:	02/12/09

1.0 PURPOSE

The purpose of the study was to determine the physical and chemical properties of a plastic test article using the following tests on the test article extract: Nonvolatile Residue, Residue on Ignition, Heavy Metals, and Buffering Capacity.

2.0 REFERENCES

The study was conducted based upon the following references:

2.1 United States Pharmacopeia 31, National Formulary 26, 2008. <661> Containers, Physicochemical Tests-Plastics.

2.2 ISO/IEC 17025, 2005, General Requirements for the Competence of Testing and Calibration Laboratories.

3.0 COMPLIANCE

The study conformed to the current FDA 21 CFR, Part 58 – Good Laboratory Practice for Non–Clinical Laboratory Studies.

4.0 IDENTIFICATION OF TEST AND CONTROL ARTICLES

The Sponsor supplied the following information on a test requisition form or other correspondence, wherever applicable (excluding confidential or trade secret information). The Sponsor was responsible for all test article characterization data as specified in the GLP regulations.

4.1 Test Article (Sponsor Supplied):

Test Article Name: WaterSep HF Cartridge
CAS/Code #: Not Supplied by Sponsor (N/S)
Lot/Batch #: Product: Investigator 24, Cartridge Lot# 8241
Membrane MWCO/Lot# 100K/102408A, Catalog # WA 100 INV 24 SO
Physical State: N/S
Color: N/S
Expiration Date: N/S
Density: N/S
Stability: N/S
Solubility: N/S
pH: N/S
Storage Conditions: Refrigerate until use
Safety Precautions: Standard Toxikon Safety Precautions

4.2 Control Article (Toxikon Supplied):

Control Article Name: Purified Water (Blank)
Toxikon QC #: 528021009
Physical State: Liquid
Color: Colorless
Stability: Stable at Room Temperature
Storage Conditions: Room Temperature
Safety Precautions: Standard Laboratory Safety Precautions

5.0 IDENTIFICATION OF TEST SYSTEM

The test systems were recommended in the USP guidelines.

6.0 JUSTIFICATION OF TEST SYSTEM AND ROUTE OF ADMINISTRATION

The test article was analyzed through an extracting medium compatible with the test system. The extraction medium was purified water.

7.0 EXPERIMENTAL DESIGN

7.1 Preparation of Test and Control Articles:

7.1.1 The test article was extracted at a ratio of 120 cm^2 per 20.0 mL purified water at 70 ± 2 °C for 24 ± 2 hours. Sponsor supplied "Toxikon letter detailing tox dimensions 012809" for sample preparation dated 2/02/09.

7.1.2 The blank, purified water, was similarly extracted to act as the control.

7.1.3 The extract was cooled to room temperature.

7.1.4 Any other test article preparation was as specified by the Sponsor.

7.2 Pre–Extraction Procedure:
Prior to extraction, the test article was agitated in purified water (150 mL) for 30 seconds. The fluid was decanted and discarded. This washing procedure was repeated.

7.3 Assay Procedure:

7.3.1 Nonvolatile Residue Assay (Table 1):
The test article and blank extracts (50.0 mL) were transferred to separate tared crucibles. The volatile matter was evaporated to dryness on a water bath and dried at 105 ± 2 °C for 1 ± 0.1 hour. The difference between the weights obtained from the test article and the blank did not exceed 15 mg.

7.3.2 Residue on Ignition Assay (Table 1):
This analysis was not performed as the amount of nonvolatile residue did not exceed 5 mg.

7.3.3 Heavy Metals Assay (Table 2):
The test article extract (20 mL) was transferred into one of two 50 mL color–comparison tubes. The pH was adjusted to 3.0 – 4.0 with 1 N acetic acid using a pH meter as an external indicator. The extract was diluted with purified water to 35 mL and mixed.

Into the second color comparison tube containing 20 mL of the blank extract, 2 mL of the Standard Lead Solution was added. The pH was adjusted to 3.0 – 4.0 with 1 N acetic acid using a pH meter as an external indicator. A pH meter was used as an external indicator. The extract was diluted with purified water to 35 mL and mixed.

To each of the tubes containing the *Standard Preparation* and the *Test Preparation*, 1.2 mL of thioacetamide–glycerin base TS and 2 mL of pH 3.5 *Acetate Buffer* were added. The solutions were then diluted with water to 50 mL and mixed. Any brown color produced within 10 minutes in the tube containing the extract of the prepared sample did not exceed that in the tube containing the standard lead solution, both tubes being viewed downward over a white surface (1 ppm in standard lead extract).

7.3.4 Buffering Capacity Assay (Table 2):
The test article extract (20 mL) was titrated with 0.150 mL of 0.01 N Hydrochloric Acid to adjust the pH to 7.0. The blank extract (20 mL) was titrated with 0.20 mL of 0.01 N Hydrochloric Acid to adjust the pH to 7.0. The difference of the two volumes did not exceed 10.0 mL.

8.0 EVALUATION CRITERIA

8.1 The evaluation criteria for each test in the physicochemical test for plastics are as listed below:

Test	Evaluation Criteria
Nonvolatile Residue	≤ 15 mg
Residue on Ignition	≤ 5 mg
Heavy Metals	< 1 ppm
Buffering Capacity	≤ 10 mL

8.2 The study and its design employ methodology to minimize uncertainty of measurement and control of bias for data collection and analysis.

9.0 RESULTS

The results are presented in Tables 1 and 2.

10.0 CONCLUSION

The purified water extract of the test article, WaterSep HF Cartridge, meets the criteria described in the USP Physicochemical Test For Plastics guidelines.

11.0 RECORDS

11.1 Original raw data are archived at Toxikon Corporation.

11.2 A copy of the final report and any report amendments is archived at Toxikon Corporation.

11.3 The original final report, and a copy of any protocol amendments or deviations, is forwarded to the Sponsor.

11.4 All unused test article shall be disposed by Toxikon, per Sponsor's request.

12.0 CONFIDENTIALITY AGREEMENT

Statements of confidentiality were not agreed upon prior to study initiation.

TABLE 1
Analysis: Nonvolatile Residue / Residue on Ignition

Test Article: WaterSep HF Cartridge

Lot/Batch #: Product: Investigator 24, Cartridge Lot# 8241
Membrane MWCO/Lot# 100K/102408A, Catalog # WA 100 INV 24 SO

Nonvolatile Residue (Criteria: ≤ 15 mg)

Sample	(g)		
	Tare	**Wt. 1**	**Weight Difference**
Test Article Extract	50.0059	50.0042	0.0017
Blank Extract	71.2552	71.2552	0.0000

Legend:
Tare = Weight of crucible
Wt. 1 = Weight of crucible + nonvolatile residue / control, post evaporation
Weight Difference = Wt. 1 – tare weight

Acceptable Level: ≤ 15 mg

Test Article Extract (Weight Difference) – Control (Weight Difference) = Nonvolatile Residue

0.0017 g – 0.0000 g = 0.0017 g = 1.7 mg (Meets Criteria)

Residue on Ignition (Criteria: ≤ 5 mg)
Not Applicable

TABLE 2
Analysis: Heavy Metals / Buffering Capacity

Test Article: WaterSep HF Cartridge

Lot/Batch #: Product: Investigator 24, Cartridge Lot# 8241
Membrane MWCO/Lot# 100K/102408A, Catalog # WA 100 INV 24 SO

Heavy Metals (Criteria: ≤ 1 ppm)
Any brown color produced within 10 minutes in the tube containing the test article extract did not exceed that in the Standard Lead Solution. (Meets Criteria)

Buffering Capacity (Criteria: ≤ 10.0 mL)

Sample	Volume	Volume of Titrant	Difference Between the Extract and Control Titrant Volumes
Test Article Extract	20 mL	0.15 mL	0.05 mL
Blank Extract	20 mL	0.20 mL	Meets Criteria

ATTACHMENT A
TFF HF SOP-PRECONDITIONING

WaterSep Technology Corporation

420 Maple Street, Suite 1 Marlborough, Mass. 01752
508- 970 0089, 508-970 0146 (FAX)

WaterSep PES Hollow Fiber

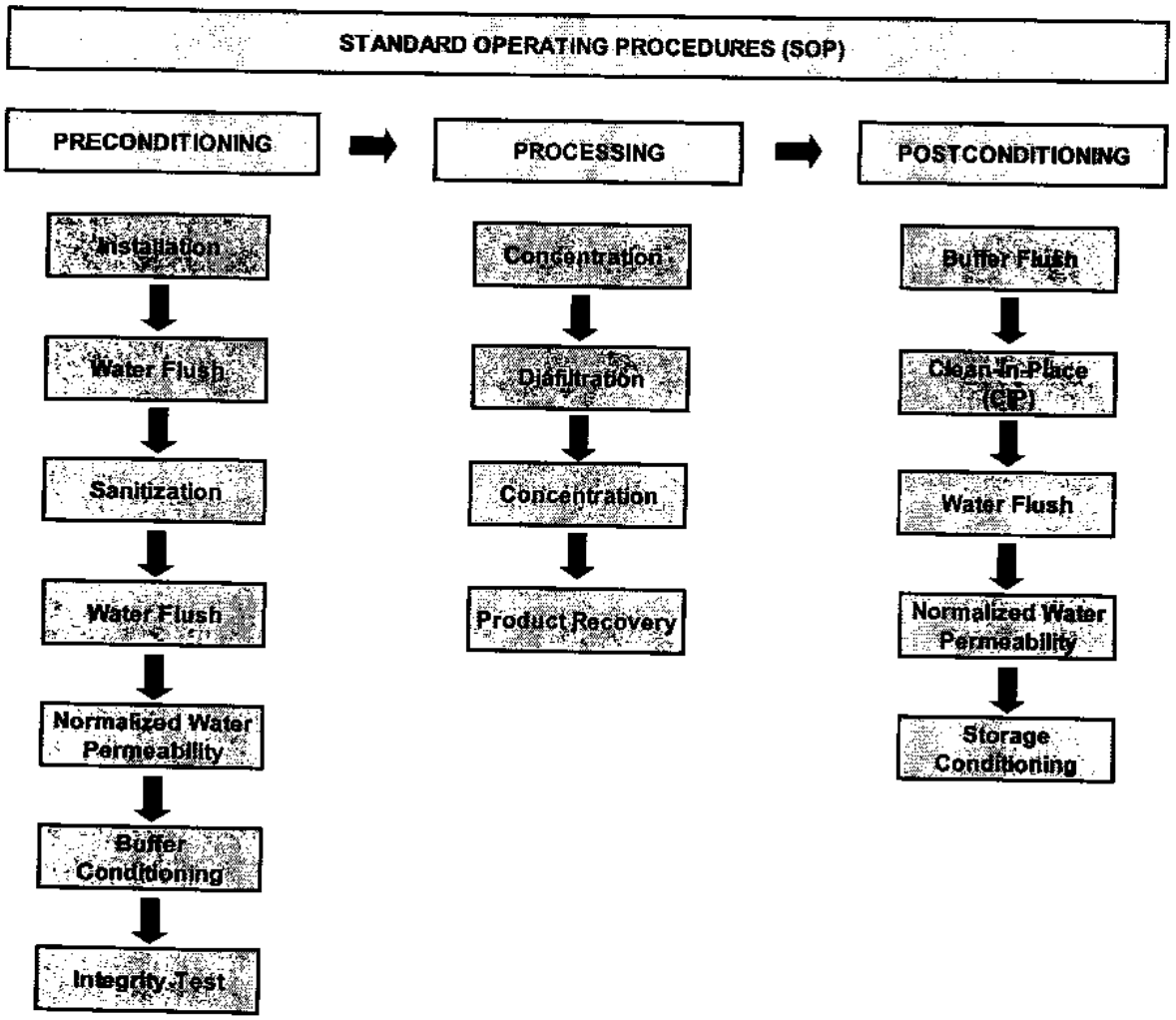
Standard Operating Procedure - SOP

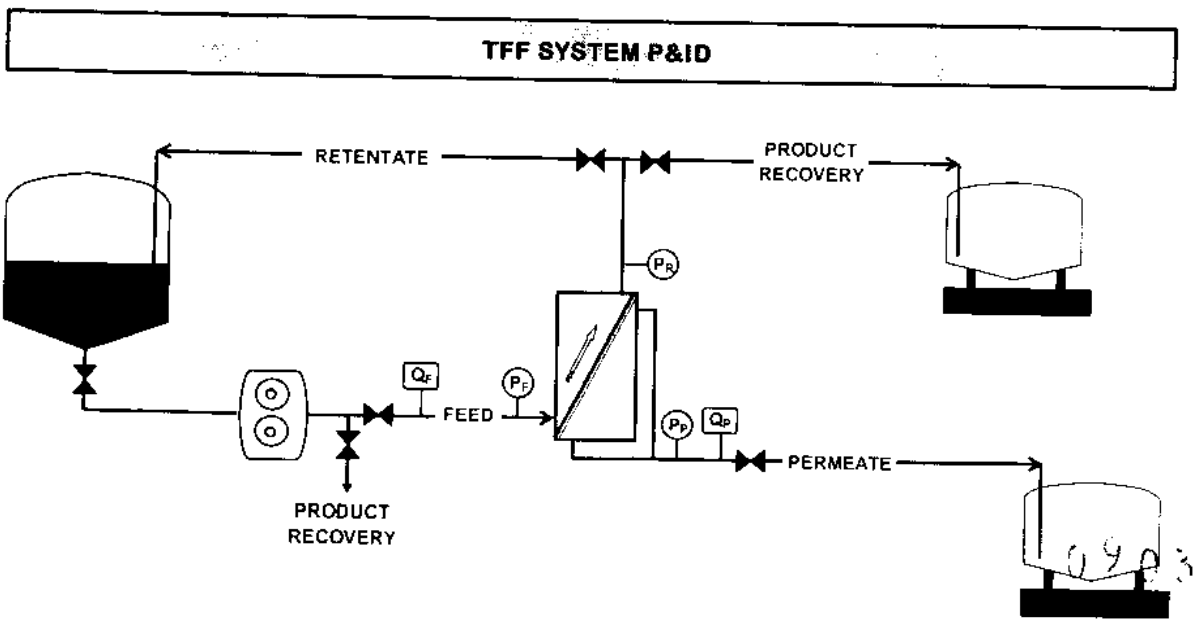
Preconditioning for Use Procedure

Prepared by: John Rozembersky
Revision: January 2009

Tangential Flow Filtration (TFF)

Process Flowsheet for UF / DF





Installation

Hollow Fiber System

Attached the process stream lines to the feed, retentate and filtrate connections of the hollow fiber element.

Water Flush

1. Flush storage agents from membrane element.

1.1. Establish and set process conditions

1.1.1. Add water to feed vessel

Recommended Feed Volume:
50 – 80 liters per m^2 (5 – 8 liters per ft^2)

1.1.2. Open permeate and retentate valves completely.

1.1.3. Direct retentate and permeate streams to waste.

1.2. Flush feed - retentate process stream

1.2.1. Turn pump on and increase feed flow rate speed until a feed-to-retentate pressure differential of 2 - 6 psig is reached (fiber length - FL dependent).

[10" FL ≅ 2 psig, 20" FL ≅ 3 psig, and 40" FL ≅ 6 psig]

1.2.2. Continue to flush feed to retentate stream to waste for 1 - 3 minutes.

1.3. Flush permeate stream process stream

1.3.1. Decrease pump speed and retentate flow rate to ~5 - 10 %.

1.3.2. Close retentate valve completely. Then re-open valve to allow a minor flow rate in the retentate stream to prevent a dead-leg situation.

1.3.3. Increase pump speed and feed flow rate until a feed pressure of 10 – 15 psig reached for UF membranes (10kD – 500kD) or 3 – 5 psig for MF membranes (750kD – 0.45um).

1.3.4. Flush a minimum permeate volumetric throughput of ≥ 40 L/sqm (4 / sqft).

1.3.5. Turn off pump.

1.3.6. Remove water in feed vessel to bottom port. Keep lines flooded to prevent pump cavitation.

Sanitization

2. Sanitization of TFF System

2.1. Establish and set process conditions

2.1.1. Add 0.3 – 0.5N NaOH to feed vessel at 30 – 40°C.

Recommended Fee Volume:
8 – 12 liters per m^2 (0.8 – 1.2 liters per ft^2)

2.1.2. Open permeate and retentate valves completely.

2.1.3. Direct permeate to waste.

2.1.4. Direct retentate back to feed vessel.

2.2. Stabilized Feed-Retentate loop with NaOH

2.2.1. Turn pump on and increase pump speed until feed-to-retentate pressure differential of 2 - 4 psig is reached.

2.2.2. Allow ~ 2 - 3 minutes for retentate recirculation to stabilize.

2.3. Flood permeate with NaOH (Static sanitization of permeate)

2.3.1. Decrease pump speed to ~ 5- 10% of retentate flow rate

2.3.2. Close retentate valve completely. Then re-open valve 5 - 10%

2.3.3. Increase pump speed up until feed pressure is 10 – 15 psig

2.3.4. Continue to flush membrane and flood permeate with NaOH until permeate stream condition reaches equilibrium to feed concentration.

2.3.5. Proceed to next step with pump running.

2.4. Sanitization of Feed – Retentate Stream

2.4.1. Open retentate valve completely.

2.4.2. Close permeate valve completely

2.4.3. Increase pump speed until feed-to-retentate pressure differential of 3-5 psig is reached

2.4.4. Continue to recirculate

Recommended Sanitization Time : 30 – 45 min

2.4.5. Drain NaOH from feed tank and feed/retentate stream

2.4.6. Turn off pump

Water Flush

3. Flush sanitization agent from TFF System

3.1. Establish and set process conditions

3.1.1. Add water at ~ 25°C – 40°C to feed vessel

Recommended Volume:

50 – 80 liters per m^2 (5 – 8 liters per ft^2)

3.1.2. Open permeate and retentate valves completely.

3.1.3. Direct retentate and permeate streams to waste.

3.2. Flush feed - retentate process stream

3.2.1. Turn pump on and increase feed flow rate speed until a feed-to-retentate pressure differential of 5-10 psig is reached.

3.2.2. Continue to flush feed to retentate stream to waste for 1 - 3 minutes.

3.3. Flush permeate stream process stream

3.3.1. Decrease pump speed and retentate flow rate to ~5 - 10 %.

3.3.2. Close retentate valve completely first and then re-open ~10%.

Note: feed pressure should not exceed 30 psig as retentate valve is closed. Decrease pump flow rate more should pressure rise beyond this point.

3.3.3. Increase pump speed and feed flow rate until a feed pressure of 10 – 20 psig reached.

3.3.4. Flush a minimum permeate volumetric throughput of 40 L/sqm (4 / sqft).

3.3.5. Continue to next step

4. Normalized Water Permeability

4.1. Measure water flux rates

4.1.1. Reduce pump speed to feed pressure = 10 - 15 psig

4.1.2. Measure and Record

- Permeate Flow Rate (Q_P) = _____ Lpm
- Feed Pressure (P_f) = _____ psig
- Retentate Pressure (P_r) = _____ psig
- Permeate Pressure (P_p) = _____ psig
- Filtrate Temperature = _____ °C

4.1.3. Calculate

- Calculate TMP = $[(P_f+P_r)/2]-P_p$ = _____ psig
- Calculate filtrate flux rate (J) = Q_P / Area = liters/m^2/hr (LMH)

  J = _____ LMH
- Determine temperature correction factor ($TCF_{20^\circ C}$) = _____

TCF = 0.0005T2 - 0.0449T + 1.7021

- Calculate filtrate flux rate at 20°C $(J)_{20^\circ C}$ = J x $TCF_{20^\circ C}$

  J_{20° = _____ LMH
- Calculate Normalized Water Flux Rate (NWP) = J_{20° / TMP

  $NWP_{20^\circ C}$ = _____ LMH/psig

4.2. Determine Percent Membrane Recovery (%MR)

4.2.1. Initial $NWP_{20^\circ C}$ constant (clean) = _____ LMH/psig

If this is first time use for this membrane lot, the value calculated in 4.1.3. is the initial $NWP_{20^\circ C}$ constant (clean)

4.2.2. If element is in re-use mode, calculate %MR at this point.

%MR = NWP (step 4.1.3) / Initial NWP

%MR = _____ %

5. Buffer Condition TFF System

5.1. Establish and set process conditions

5.1.1. Add Buffer solution to feed vessel with same temperature as feed solution.

Volume required:
10 – 20 liters per m^2 (1 – 2 liters per ft^2)

5.1.2. Open permeate and retentate valves completely

5.1.3. Direct permeate to waste

5.1.4. Direct retentate back to feed vessel

5.2. Stabilized Feed-Retentate loop with buffer

5.2.1. Turn pump on and increase pump speed until feed-to-retentate pressure differential of 3-5 psig is reached.

5.2.2. Allow ~ 2 - 3 minutes for retentate recirculation to stabilize..

5.3. Flood permeate with buffer

5.3.1. Decrease pump speed to 5- 10% of retentate flow rate

5.3.2. Close retentate valve completely first and then re-open ~10%.

5.3.3. Increase pump speed and feed flow rate until a feed pressure of 10 – 20 psig reached.

5.3.4. Flush permeate stream to waste with 10–20 liters per m^2 (1 – 2 liters per ft^2)

5.4. Reduce the buffer in the feed vessel.

5.4.1. Drain buffer down to the bottom of the feed vessel near the exist port.

Feed – retentate lines should remain flooded with buffer. Do not permit air to be drawn in the feed – retentate. Lines should remain flooded and air free.

5.4.2. Turn off pump

6. Integrity Testing of Membrane Element (optional)

Pressure hold test at 10 psig = HOLD for ~1-2 minutes
Air Diffusion at 10 psig < 10 cc/min