STANDARD OPERATING PROCEDURE FOR SOLID PHASE ADSORPTION TOXIN TESTING (SPATT) ASSEMBLAGE AND EXTRACTION OF HAB TOXINS

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Standard Operating Procedure for Solid Phase Adsorption Toxin Testing (SPATT) Assemblage and Extraction of HAB Toxins

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Introduction

Due to the severe and ubiquitous nature of harmful algal blooms (HABs) in coastal systems, surveillance and monitoring are critical to mitigate impacts from HAB events. Traditional monitoring programs for HAB toxins typically rely on discrete sampling ("grab" samples). Such methods are inherently biased if the sampling does not capture ephemeral or episodic events and the spatial and temporal variability of the system. In response to this challenge, a passive sampling method, Solid Phase Adsorption Toxin Tracking (SPATT), was developed to monitor toxin concentrations in marine, brackish and freshwater environments. SPATT was first proposed for HAB monitoring in 2004 as a means by which disadvantages associated with shellfish or other indicator organisms might be circumvented (MacKenzie et al., 2004). These passive sampling systems had previously been utilized to detect a wide range of non-HAB environmental pollutants (Górecki and Namieÿnik 2002; Vrana et al. 2005; Kot-Wasik et al. 2007; Seethapathy et al. 2008). The approach has recently been refined as an assessment and monitoring tool to compliment traditional time-series programs and to provide a time-integrated indicator of dissolved toxin presence in a waterbody or coastal location (Fux et al., 2008, 2009, Lane et al., 2010, Kudela, 2011, Wood et al., 2011, Seubert et al., 2013, Gibble and Kudela, 2014, Howard et al., 2017, Kudela, 2017, Peacock et al., 2018). SPATT can be used to elucidate toxin dynamics and environmental drivers, as well as train, validate and improve predictive models (Anderson et al., 2016). Both freshwater and marine toxins have been detected using SPATT when simultaneous discrete water samples have failed to detect them in a given waterway or waterbody, making SPATT a more sensitive indicator of the prevalence of toxins over time than traditional discrete samples (Lane et al., 2010, Kudela, 2011, Gibble and Kudela, 2014, Howard et al., 2017, Peacock et al., 2018).

SPATT has been developed and tested for a variety of resins (see review by Kudela, 2017). These Standard Operating Procedures (SOP) outline the use of the resin DIAON HP20 since it has been demonstrated to quantify microcystins, anatoxin-a, saxitoxin, domoic acid, and okadaic acid in fresh, brackish, and marine waters, all from the same SPATT sampling device (Lane et al. 2010; Miller et al. 2010, Kudela 2011, Gibble and Kudela, 2014, Howard et al., 2017, Kudela, 2017, Peacock et al., 2018). Cylindrospermopsin and nodularin have also been detected using SPATT with DIAON HP20 resin in California, however, the use of DIAON HP20 for these toxins has not

been well characterized in the laboratory. The SPATT methodology is ready for wider adoption by the research, monitoring, and management communities interested in detecting and tracking the dynamics of freshwater and marine toxins. The SOP described herein for the assemblage and construction of SPATT bags can be used for deployment in freshwater, brackish and marine environments and the extraction process described below is for the analysis of both marine toxins and cyanotoxins.

Supplies Required

The following is a list of supplies required for assemblage of SPATT bags and HAB toxin extraction. The current suppliers, item description, catalog numbers and website links are also provided in Table 1.

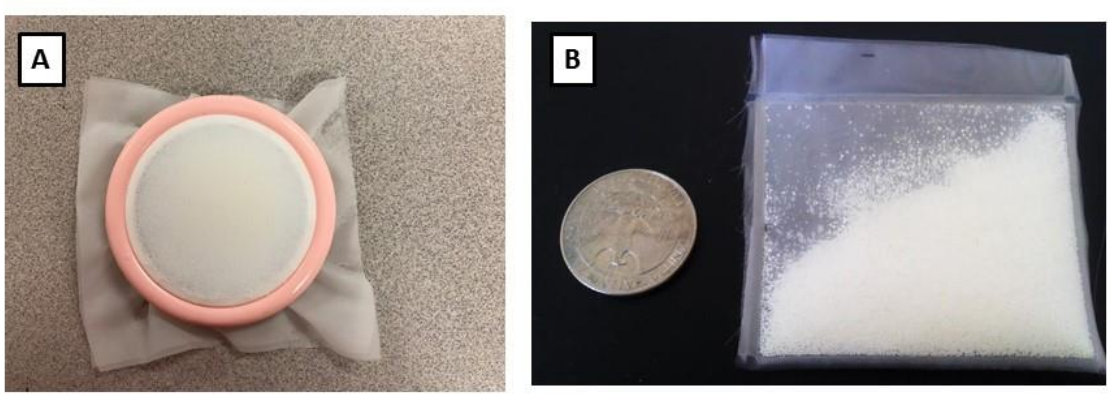
Table 1. List of supplies for SPATT assemblage and HAB toxin extraction.

Material	Supplier	Item Description	Part/Catalog Number	Website link
SPATT Assemblage Supplies				
Mesh	WildCo	Bolt Cloth-Nitex 100 µm 40" W.	3-24-C34	http://shop.sciencefirst.com/wildco/nitex-bolting-cloth/6412-nitex-bolting-cloth-nitex-100m.html
Resin	SorbTech	HP20-1 - Diaion Resin, Styrenic Adsorbent, 1kg	HP20-1	https://www.sorbtech.com/chromatography/adsorbents/polymeric-resins/mitsubishi-resins/synthetic-adsorbents/synthetic-adsorbents/
Methanol	FisherSci	Methanol Optima LC/MS Grade	A456-1	https://www.fishersci.com/shop/products/methanol-optima-lc-ms-fisher-chemical-5/p-3112109
Ultrapure water	Variable	Ultrapure water - 18.2 MΩ·cm	none	
Heat Sealer ('tea-bag')	FisherSci	Cole Parmer HEAT SEALER 8IN L 115VAC	NC9758660	https://www.fishersci.com/shop/products/heat-sealer-8in-l-115vac/nc9758660#?keyword=cole+parmer+heat+seal
Embroidery Hoops	Etsy	Round 2.5" Flexi hoop Embroidery hoop	Etsy	https://www.etsy.com/listing/521574863/flexi-hoop-embroidery-hoop-various-sizes?ref=unav_listing-other-3
	Lakeside NeedleCraft	3" WOODGRAIN Flexi Hoop Frame for Cross Stitch Embroidery Craft Display	none	https://www.lakesideneedlecraft.co.uk/3-woodgrain-flexi-hoop-frame-for-cross-stitch-embroidery-craft-display-1269-p.asp
	Lakeside NeedleCraft	Round 2.5" Flexi hoop Embroidery hoop	none	https://www.lakesideneedlecraft.co.uk/25-round-flexi-hoop-8-colours-red-blue-pink-wood-cross-stitch-embroidery-frame-1274-p.asp
Zipties	Hardware store	Miniature ties 4"X1/8" Large Ties	None	
SPATT Extraction Supplies				
Filter Manifold	J.T. Baker	Baker spe-12G Col Processor	6998-00	https://us.vwr.com/store/product/4635724/spe-glass-column-processors-j-t-baker
Disposable chromatography column	BioRad	Econo-Pac Chromatography columns, 1.5x12cm, polypropylene, 14cm, 20mL bed	732-1010	http://www.bio-rad.com/en-us/sku/7321010-econo-pac-chromatography-columns-pkg-50?parentCategoryGUID=3aaf45bf-6d18-4e09-b553-7484bb8bd5e7
Glass scintillation vial	VWR	Glass scintillation vials, liner-less caps, 20mL	66020-326	https://us.vwr.com/store/product/4618802/scintillation-vials-borosilicate-glass-with-screw-cap-kimble-chase
Disposable Spatula	FisherSci	smartSpatula, 210mm long	Cat No. 50-476-569	https://www.fishersci.com/shop/products/smartspatula-210mm-blue-300pk/50476569#?keyword=Disposable+Polypropylene+Spatulas

SPATT Assembly and Construction

There are 2 ways to assemble SPATT bags, (1) using an embroidery hoop and (2) using a heat sealer that creates a ‘tea-bag’ type of bag (see Figure 1 below).

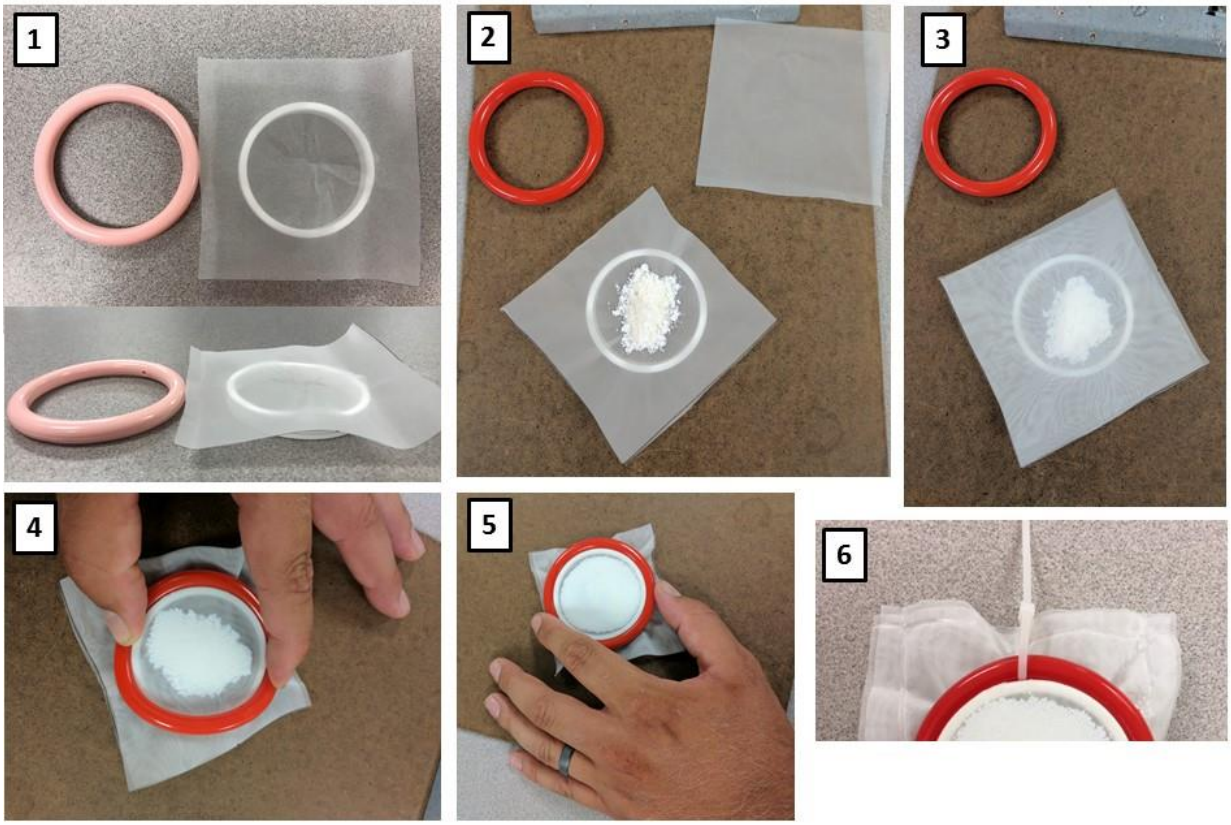
Figure 1: Two SPATT bag designs, (A) embroidery hoop and (B) 'tea-bag'.



SPATT Construction: Embroidery Hoop (Figure 2)

1. Cut two 4” X 4” pieces of mesh. Cut away any portions of the mesh marked with pen or Sharpie, as this will stain the resin once immersed in MeOH (Figure 2 step 1).
2. Separate the hoop into two pieces, inner and outer. Remove the metal hanger and throw away. Drill a 1/8” hole into opposite side of colored hoop from the hanger hole. Hole should be drilled horizontally so that the zip tie will not interfere with the inner white hoop ring (zip tie will be tied here; see step 11).
3. Place a weigh tin on a scale (tare to zero) and weigh out 3g of dry resin using a clean spatula. You can also
4. Take one of the squares of mesh and pinch a cone shape into the center. This allows the resin to stay centered during assembly (Figure 2 step 2).
5. Place the mesh with the cone shape on top of the inner circle of the hoop.
6. Pour resin into the cone indentation.
7. Take care to remove all resin from the tin.
8. Place the second piece of mesh on top and center it (Figure 2 step 3).
9. Snap outer ring of the hoop over both pieces of mesh, trapping the resin between inside (Figure 2 step 4 and 5).
10. Inspect the mesh to make sure there are no creases or folds which could compromise the SPATT resin.
11. Attach miniature zip tie either between the outer and inner hoops OR through hole drilled in step 2 (Figure 2 step 6).

Figure 2. Steps to assemble embroidery hoop SPATT design.

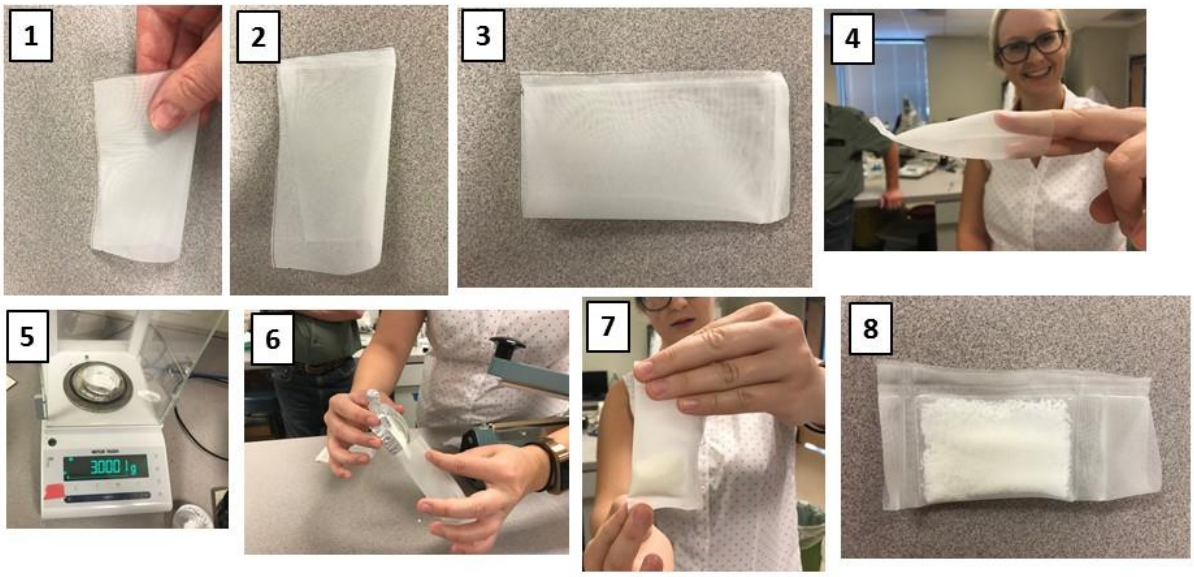


SPATT Construction: 'Tea-Bag'

1. Turn on heat sealer and allow a few minutes to heat up.
2. Cut one 4.5" X 4.5" piece of mesh. Cut away any portions of the mesh marked with pen or Sharpie, as this will stain the resin once immersed in MeOH.
3. Fold mesh piece in half and use heat sealer to seal one of the short edges of the mesh (Figure 3, steps 1 and 2).
    a. Depending on the environment for deployment, a second sealed strip on each side can be applied (see Figure 3, step 8).
4. Heat seal 1 of the long edges of the mesh (Figure 3, step 3).
5. Do a 'finger' test to make sure the 2 heat sealed sides of the mesh do not have any gaps (which would cause the resin to be lost during deployment). (Figure 3, step 4).
6. Place a weigh tin on a scale (tare to zero) and weigh out 3g of resin using a clean spatula (Figure 3 step 5).
7. Pour the resin into the SPATT bag (Figure 3 step 6).

8. Push the resin toward the bottom of the SPATT bag (Figure 3 step 7).
9. Heat seal the open edge of the SPATT bag. Final bag should look like Figure 3 step 8.

Figure 3. Steps to assemble 'tea-bag' SPATT design.



Resin Activation and Deployment

1. Submerge SPATT bag with resin in methanol for ~24 hours.
2. After 24 hours, rinse off methanol with ultrapure water.
    a. Fill a 500ml beaker with ultrapure water and dip the ring in the water, lightly agitate the ring to ensure all the resin contacts the water
    b. The methanol will react with the water and increase the temperature of the water
    c. Pour the water out, refill the beaker, and repeat rinses until the temperature of the water does not increase when the hoop is placed in the beaker
3. Place the SPATT bags in a ziplock bag with ultrapure water covering the resin to ensure the resin does not dry out.
4. Store in a refrigerator until deployment (resin is stable for months in a refrigerator).
5. Use zip tie to attach SPATT to a structure or a weighted line (Figure 4).

Figure 4. SPATT deployment options.

Photo credits: Heath Mash, Carey Nagoda, Meredith Howard, Kendra Hayashi.

Retrieving SPATT Samplers from the Field

1. Upon collection rinse as much silt and debris from the flexi-hoop ring or ‘tea-bag’ as possible using field water.
2. Put SPATT bag into a labeled ziplock bag (does not need to be in water). Writing with sharpie pens directly onto the ziplock bag is recommended.
3. Freeze immediately at < -4°C until extraction of toxins in the lab.

Toxin Extraction from the Resin

The extraction of marine toxins and/or cyanotoxins from SPATT involves different eluents depending on which toxins are of the most interest (see review Kudela 2017). If cyanotoxins, okadaic acid and/or saxitoxin and related paralytic shellfish toxins (PSTs) are the focus, then following the protocol of Kudela, 2011, using 50% methanol for all 3 extractions is recommended. If domoic acid (DA) is the sole analysis, then the 3 extractions should use a combination of eluents, 50% methanol and 1M ammonium acetate in 50% methanol. Table 2 summarizes these extraction protocols.

Table 2. Summary of recommended extraction protocols for SPATT based on the toxins of interest.

Toxins	Extraction	Reference
Cyanotoxins, Okadaic acid, Saxitoxin and related PSTs	10 mL 50% MeOH in MQ	Kudela, 2011
	20 mL 50% MeOH in MQ	
	20 mL 50% MeOH in MQ	
DA and Cyanotoxins	10 mL 50% MeOH in MQ	Lane et al., 2010; Peacock et al., 2018
	10 mL 1M ammonium acetate in 50% MeOH	
	20 mL 1M ammonium acetate in 50% MeOH	
Anatoxin-a[§]	10 mL 100% MeOH with 2% formic acid[¥]	Kudela, unpublished
	20 mL 50% MeOH in MQ	
	20 mL 50% MeOH in MQ	

[§] This protocol is for anatoxin-a specifically, and at the time of this writing, the recovery effects on other toxins from this extraction are being evaluated; Microcystin recovery is not affected (R. Kudela, unpublished data).
[¥]Using 100% MeOH with 2% formic acid for the first extraction improves recovery for anatoxin. This first extract should be diluted back to 50% MeOH before analysis on the LCMS.

1. Retrieve SPATT samples from freezer and only allow samples to warm up for a few minutes because the resin is easier to transfer to the chromatography columns when still frozen.
    a. Label the chromatography columns and scintillation vials (3 extracts per sample).
2. Remove resin from SPATT bags and put into chromatography columns:
    a. Rinse the ring or bag with water – try to get as much dirt/debris off as possible.

b. Embroidery hoop: Break up the frozen resin into smaller clumps while the ring is still in place. Remove the ring and carefully separate the mesh squares. Easiest way is to place mesh with resin on counter top, push all resin to center of square and then scoop resin into column, rinsing spatula with ultrapure water.
c. 'Tea-bag': Push resin to bottom of bag and use scissors to cut the top edge of the mesh bag. Use spatula to scoop resin into the column.

3. Remove top of manifold and place a 20-mL glass scintillation vial underneath the column
4. There will be three extractions with either 10 mL or 20 mL depending on the toxins of interest (see Table 2).
    a. <u>Extract 1</u>
        i. Pipet 10 mL of 50% methanol or 1M ammonium acetate in 50% MeOH into the column.
        ii. Make sure the eluent is pipetted forcefully enough to stir up all of the resin to make sure that extract comes in contact with all resin...
        iii. Apply vacuum and adjust valves to draw the methanol out of the column at a rate of 1 ml min^{-1} (slow drip, not a stream of liquid).
        iv. Once methanol is out of the column, remove scintillation vial and label extract 1.
    b. <u>Extract 2</u>
        i. Insert a new 20 mL glass scintillation vial underneath the column
        ii. Pipet either 10 or 20 mL of eluent into the column. If using the protocol with 20 mL, pipette 10 mL at a time, making sure that it is stirring up the resin. Pipette the second 10 mL without letting the column dry out.
        iii. Apply vacuum and adjust valves to draw the methanol out of the column at a rate of 1 ml min^{-1} (slow drip, not a stream of liquid).
        iv. Once methanol is out of the column, remove scintillation vial and label extract 2.
    c. <u>Extract 3</u>
        i. Repeat steps 4b i-iv and label vial extract 3.
5. Store the three scintillation vials in the dark at -4^{o}C until analysis.
6. Remove column from manifold and let resin dry.
7. Weigh dry resin and use this measurement in the toxin extract calculations (see next section).
8. Once dry, remove resin, and clean column.
    a. Place on manifold and let 10 ml of 100% methanol run through followed by 10 mL of ultrapure water. The column is now ready to be used for another extraction.

Once run, you'll have 3 extract values that should be added together to get a toxin value that is normalized to the weight measured in Step 7, grams of resin and time: ex. ng MCY/g resin/day.

Toxin Extract Calculations

Determine toxin extraction in each of the 3 extracts per SPATT bag via LC-MS or ELISA in unit mass per unit volume, i.e. ng/mL

Example: E1 = 0.00 ng/mL

E2 = 3.92 ng/mL

E3 = 3.24 ng/mL

Divide the dry resin mass (measured in extraction step 7) by each extract concentration to calculate grams toxin per gram of resin (ng toxin/mL * g)

Multiply the resin normalized toxin mass by the extract volume to correct for the extraction solvent extraction volume in each step. For example, multiply E1 by 10, E2 by 20, and E3 by 20, and you will get ng toxin/g resin.

Add the resin normalized toxin concentrations together to get the total toxin extracted from the resin.

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