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Evaluation of Solid Sorbents for the Recovery of Polyether Toxins (Brevetoxins) in Seawater

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Red tides are blooms of toxic marine algae that occur world wide. The Florida red tide caused by the dinoflagellate, *Gymnodinium breve*, produces up to nine neurotoxins, referred to as brevetoxins, that cause fish kills, contamination of shellfish, and severe respiratory irritation to people along the shore (Baden 1983, Steidinger 1983, Poli et al. 1986, Steidinger 1990, Pierce et al. 1990). High concentrations of red tide (ca. $1X10^6$ cells/liter) cause rapid death to exposed fish, whereas sublethal blooms may cause the toxins to be accumulated in the marine food chain.

This study was undertaken to evaluate particulate sorbent materials for extraction and recovery of the polyether brevetoxins from seawater. Sorbents investigated included an organic copolymer resin, activated charcoal, and silica-bonded octadecane. Of the three types, only the silica-bonded octadecane was suitable for routine use. The resin exhibited inefficient brevetoxin sorption from water, whereas the charcoal adsorbed the toxins so strongly that they were not recoverable by solvent elution. Extraction from seawater with beds of silica-bonded octadecane particles was used to replace conventional liquid/liquid extraction because this approach provided more efficient recovery and avoided many difficulties encountered with liquid/liquid extraction, such as the use of large volumes of water, the formation of intractable emulsions, and accumulation of chlorinated-solvent wastes.

MATERIALS AND METHODS

Aqueous solutions of brevetoxins (Btx) were prepared from seawater cultures of *G. breve* maintained in this laboratory (Pierce et al. 1989). Cultures containing $20\text{-}40X10^6$ cells per liter were filtered through 3um glass-fiber filter paper (Gellman, Ann Arbor, MI) to rupture the cells and release the toxins into aqueous solution, where they became available for adsorption to the solid sorbents. The sorbents evaluated were XAD-2 resin (Rohm and Haas, Philadelphia, PA), a macroreticular non-functional copolymer of styrene and divinyl benzene supplied as 20-50 mesh beads;

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activated coconut charcoal (Altech Chemical, Deerfield, IL) in 50+ mesh size; and three silica-bonded octadecane (Si-C18) sorbents. These Si-C18 sorbents included 100-200um RP18 sorbent (Universal Scientific Co., Atlanta, GA), carbon content unspecified; Polygosil 60-63200 sorbent (Macherey-Nagel, Düren, Germany), carbon content of 12%, and Impaq RG 101150-C_{18} sorbent (PQ Corp., Valley Forge, PA), with a median particle size of 136um and a carbon content of 22.4%.

Solvents evaluated for toxin recovery from the sorbents included methanol, acetone and dichloromethane. All solvents were chromatographic grade (Burdick & Jackson, Muskegon, IL) high purity solvents. Sorbent bed volumes varied depending upon the test performed, and ranged from 0.7 to 7.3 cm^3.

Toxin recovery was tested for each sorbent by passing aqueous toxin solutions through sorbent beds of specified dimensions and flow rates. Then, the loaded beds were washed with water to remove salts and blown superficially dry with air. Toxin retained on the sorbent bed was recovered by solvent elution, and subjected to qualitative and quantitative analysis by high performance liquid chromatography (HPLC).

Liquid/liquid extraction of toxins was used as a comparison with CH_2Cl_2 as the extraction solvent. The extract was evaporated to just dry under vacuum, and the residue taken up in methanol for HPLC analysis.

Chromatographic analysis employed a Varian 5020 HPLC with a UV50 detector (Varian Instruments, Palo Alto, CA) and an OD-5 B&J spherical C_{18} column (Burdick and Jackson, Muskegon, MI). The mobile phase was 85/15, methanol/water, with a flow rate of 1 mL/min. Toxins were detected by ultraviolet absorption at 215 nm. The detector response was integrated by a PE-Nelson 2600 data system (Perkin-Elmer, Norwalk, CT). The chemical structure corresponding to the various brevetoxins has been reported by Poli et al. (1986). Quantitative and qualitative analysis of toxins was provided by comparison with standard toxin solutions prepared by dissolving known amounts of toxins in methanol. Toxin standards were obtained from Chiral Corp. (Miami, FL).

Kinetics were investigated using three identical sorbent beds in series. Toxins were sorbed from aqueous solutions of toxins passed through at a specific rate (residence time). The amount of toxin sorbed by each bed was analyzed separately. The concentration of toxin entering the first bed, Co, was calculated from the total toxins recovered from all three beds. The concentration of toxin leaving the first bed, C, was calculated as the sum of toxins recovered in the second and third beds.

The sorbent column efficiency was determined by calculating the first-order mass-transfer rate constant, k(sec^{-1})

$$k = -1/t \ln(C/Co) = -(u/0.4V) \ln(C/Co)$$

where C/Co is the ratio of the concentration of toxin exiting the column to that entering it, t is the residence time in sec, V is the sorbent bed volume in mL, and u is the flow rate in mL/sec. For these calculations, a void volume of 40% bed volume was used.

The capacity of Polygosil for brevetoxins was measured by developing a breakthrough curve. A toxin solution containing 570 ug/L total toxins (Co) was pumped through a 0.75 mL (0.5 g) bed at 0.5 L/hr for 14 hours. In series downstream from this test bed was a 3-mL analytical bed. This was replaced hourly, and the toxin content was analyzed to determine the amount of toxins exiting the first column (C).

A shorter method was used to estimate the toxin capacity of Impaq Si-C18 sorbent using a concentrated aqueous solution of brevetoxins percolated through a 0.8 g bed of Impaq. The effluent was passed through a second similar bed, and the toxin loading of each bed was determined by methanol elution followed by HPLC analysis.

RESULTS AND DISCUSSION

The ability of each sorbent to remove toxin from seawater was first evaluated using the most abundant toxin, Btx-2, and determining C/Co for specific flow rates. An acceptable sorbent retention was considered to be 95% or greater, corresponding to $C/Co \leq 0.05$.

The results of these tests (Table 1) show that the XAD-2 resin exhibited inefficient recovery of Btx-2 with a C/Co of 0.44, indicating that only 56% was retained by the sorbent. An additional problem with the XAD-2 resin was periodic interference from uv-absorbing compounds apparently extracted from the resin copolymer matrix. These results indicated that XAD-2 resin was not appropriate for routine recovery of the polyether toxins.

Table 1. Btx-2 brevetoxin sorption and subsequent recovery from solid sorbent beds.

	Resin	Carbon	Si-C18
Bed volume, cm^3	7.3	10	6.8
Flow rate, mL/min	13	17	24
Co[1], ug/L	85	750	790
C[1], ug/L	37	0	0
recovery[2], ug/L	40	0	1,200

1. Liquid/liquid extraction of toxins using CH_2Cl_2.
2. Elution of toxins from sorbent using CH_3OH.

The activated carbon granules proved to be highly efficient at removing brevetoxin from water (100% removal); however, the toxin was not recovered from the charcoal by elution with methanol, acetone or dichloromethane. Soxhlet extraction enhanced

desorption, yet complete recovery was not attained. These results showed that carbon sorption was inappropriate for polyether toxin analysis due to incomplete and non-reproducible recovery.

The Si-C18 sorbent, RP-18, used to test toxin recovery exhibited excellent removal of Btx-2 from water and efficient release of toxin from the sorbent for subsequent HPLC analysis (Table 1). Considerably more Btx-2 was recovered from the sorbent than expected from CH_2Cl_2 extraction of the feed to the column. A comparison of toxin recovery using RP-18 sorbent vs. CH_2Cl_2 liquid/liquid extraction of the same toxin solution showed that the RP-18 sorbent method recovered approximately twice as much of three brevetoxins studied as were recovered by CH_2Cl_2 liquid/liquid extraction. In addition, the RP-18 provided a preliminary clean-up by allowing removal of some interfering pigments with hexane elution prior to elution of brevetoxins with methanol.

Results of the sorption kinetics studies are shown in Table 2. Breakthrough occurred at residence times of 1.2 sec./0.75 cm^3 bed volume of Polygosil and 0.7 sec./0.75 cm^3 bed volume for Impaq. Breakthrough from the second column was negligible. The RP18 sorbent was found to be less efficient that either Polygosil or Impaq, so RP18 was not tested further.

Table 2. Sorption kinetics and sorbent capacity for brevetoxins on Si-C18 sorbents.

Sorbent bed #	Flow Rate, mL/sec	Res. Time, sec	Brevetoxin, ug/L Btx-1	Btx-2	Btx-3	Btx-6/9
Polygosil-1	0.25	1.2	79.8	552	35.2	55.5
-2	0.25	1.2	1.4	60.0	5.6	1.4
-3	0.25	1.2	0	3.4	0	0
Total (=Co)			81.2	615	40.8	56.9
k(sec^{-1})			3.4	1.9	1.7	3.1
Impaq-1	0.4	0.7	476	632	5	104
-2	0.4	0.7	53	94	0	8
-3	0.4	0.7	9	19	0	0
Total (=Co)			538	745	5	112
k(sec^{-1})			3.0	2.6	---	3.7
Impaq-1	0.3	0.9	517	662	0	90
-2	0.3	0.9	29	77	0	5
-3	0.3	0.9	6	6	0	1
Total (=Co)			552	745	0	96
k(sec^{-1})			3.1	2.4	---	3.1

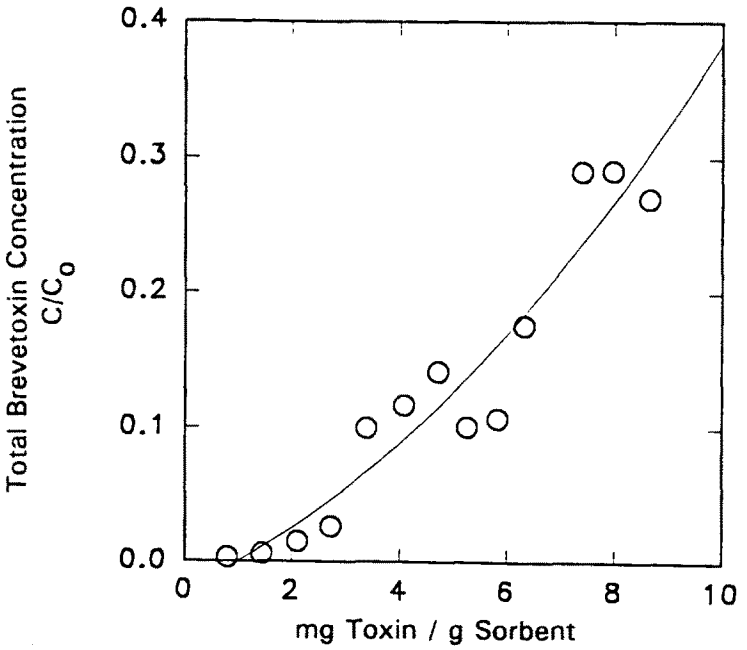

Figure 1. Brevetoxin breakthrough curve for Polygosil Si-C18 sorbent.

The mass transfer rate constants, k, were calculated from these data and are given for each toxin in Table 2. Both sorbents exhibited excellent toxin sorption characteristics. Some variability in toxin recovery was observed for the two sorbents; however, a different red tide culture was used for each sorbent, so a direct comparison of the amount of each toxin recovered cannot be made.

The capacity of Polygosil Si-C18 sorbent was determined by generating a toxin breakthrough curve (Figure 1). Toxin breakthrough was negligible up to 3 mg toxin/g sorbent; 10% breakthrough (C/Co=0.01) occurred at a loading of about 5 mg/g or 0.5 wt % of toxin to sorbent.

Table 3. Capacity of impaq sorbent for brevetoxins.

	Brevetoxins ug/L				
Bed#	Btx-1	-2	-3	-6/9	Total
1	2,524	4,224	591	663	8,000
2	187	188	1	38	410

Si-C18 reversed-phase sorbents provided an efficient alternative to solvent extraction for recovery of brevetoxins from seawater. Efficient residence time for >95% sorption was approximately 2 sec with a capacity of 0.5% for Polygosil and 1% for Impaq.

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REFERENCES

Baden DG (1983) Marine food-borne dinoflagellate toxins Int Rev Cytol 82:99-150.

Pierce RH, deRosset AJ, Hasbrouck PA, Henry MS, King RE, Kotlewski AJ, Nevin CS and Proffitt SL (1989) Mote Marine Laboratory 1988-89 red tide program, final report to Florida DNR, Contract C5547 Oct 2, 1989 55 pp.

Pierce RH, Henry MS, Proffitt LS, Hasbrouck PA (1990) Red tide toxin (Brevetoxin) enrichment in marine aerosol. In: (E. Granéli, B. Sundström, L. Edler and D. Anderson, eds.) Toxic Marine Phytoplankton Elsevier Publishing Co New York pp 397-402.

Poli MA, Mende TJ, Baden DG (1986) Brevetoxins, unique activators of voltage-sensitive Na channels bind to specific site in rat brain synaptosomes. Molec Pharmacol 30:129-135.

Proffitt LS, Pierce RH, Henry MS, deRosset AJ (1989) Solid phase extraction of red tide toxins. Fl Sci 52: Supp 1:28.

Steidinger KA (1983) A re-evaluation of toxic dinoflagellate biology and ecology Prog Phycological Res 2:147-188.

Steidinger KA (1990) Species of the *Tamarensis/Catenella* group of *Gonyaulox* and the Fucoxanthin derivative-containing *Gymnodinioids*. In: (E. Granéli, B. Sundström, L. Edler and D. Anderson, eds.) Toxic Marine Phytoplankton Elsevier Science Publishing Co New York pp 11-16.

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