# Isoelectric focussing electrophoresis of percent activity of radioisotopes and major constituents incubated in natural colloidal organic matter collected from stations E1, E3, C9, C11

Website: https://www.bco-dmo.org/dataset/764794

**Data Type**: experimental

Version: 1

Version Date: 2019-04-11

#### **Project**

» <u>Biopolymers as carrier phases for selected natural radionuclides (of Th, Pa, Pb, Po, Be) in diatoms and</u> coccolithophores (Biopolymers for radionuclides)

Contributors	Affiliation	Role
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#### **Abstract**

Isoelectric focussing electrophoresis of percent activity of radioisotopes and major constituents incubated in natural colloidal organic matter collected from stations E1, E3, C9, C11. To study the binding mechanisms of radionuclides to organic moieties in colloidal organic matter (COM), marine colloids (1 kDa-0.2 µm) were isolated by cross-flow ultrafiltration from seawater of the west Pacific Ocean and the northern Gulf of Mexico. For the same purpose, exopolymeric substances (EPS) produced by laboratory cultured diatoms were collected as well. In our study areas, colloidal organic carbon (COC) concentrations ranged from 6.5 to 202 μg-C/L in the Pacific Ocean, and were 808 µg-C/L in the Gulf of Mexico. The COM compositions (organic carbon, organic nitrogen, proteins, total hydrolysable amino acids, total polysaccharides, uronic acids, hydroxamate siderophores, hydroquinone) were quantified to examine the relationships between partition coefficients (Kc) of five different radionuclides, 234Th, 233Pa, 210Pb, 210Po and 7Be, and concentration ratios to COC of individual chelating biomolecules that could potentially act as a chelating moiety. The range of partition coefficients (Kc, reported as logKc) of radionuclides between water and the different colloidal materials was 5.12 to 5.85 for 234Th, 5.19 to 6.01 for 233Pa, 4.21 to 4.85 for 210Pb, 4.87 to 5.68 for 210Po, and 4.49 to 4.92 for 7Be, similar to values previously reported for lab and field determinations under different particle concentrations. While any relationship obtained between Kc and abundance of specific moieties could not be taken as proving the existence of colloidal organic binding ligands for the different radionuclides, it could suggest possible organic mojeties involved in the scavenging of these natural radionuclides. Together with results from isoelectric focusing of radiolabeled COM, we conclude that binding to different biomolecules is nuclide-specific, with colloidal hydroxamate siderophoric moieties being important for the binding of Th and Pa radionuclides. Hydroguinones/ guinone (HQ/Q) facilitated redox and chelation reactions seem to be involved in the binding of Pa and Be. However, the actual mechanisms are not clear. Individual amino acids, proteins, total polysaccharides and uronic acids did not yield significant relationships with logKc values of the different radionuclides. Nonetheless, our results provide new insights into the relative importance of different potential ligand moieties in COM in the binding and possible scavenging of specific radionuclides in the ocean.

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## **Dataset Description**

Isoelectric focussing electrophoresis of percent activity of radioisotopes and major constituents incubated in natural colloidal organic matter collected from stations E1, E3, C9, C11. To study the binding mechanisms of radionuclides to organic moieties in colloidal organic matter (COM), marine colloids (1 kDa-0.2 µm) were isolated by cross-flow ultrafiltration from seawater of the west Pacific Ocean and the northern Gulf of Mexico. For the same purpose, exopolymeric substances (EPS) produced by laboratory cultured diatoms were collected as well. In our study areas, colloidal organic carbon (COC) concentrations ranged from 6.5 to 202 μg-C/L in the Pacific Ocean, and were 808 µg-C/L in the Gulf of Mexico. The COM compositions (organic carbon, organic nitrogen, proteins, total hydrolysable amino acids, total polysaccharides, uronic acids, hydroxamate siderophores, hydroguinone) were quantified to examine the relationships between partition coefficients (Kc) of five different radionuclides, 234Th, 233Pa, 210Pb, 210Po and 7Be, and concentration ratios to COC of individual chelating biomolecules that could potentially act as a chelating moiety. The range of partition coefficients (Kc, reported as logKc) of radionuclides between water and the different colloidal materials was 5.12 to 5.85 for 234Th, 5.19 to 6.01 for 233Pa, 4.21 to 4.85 for 210Pb, 4.87 to 5.68 for 210Po, and 4.49 to 4.92 for 7Be, similar to values previously reported for lab and field determinations under different particle concentrations. While any relationship obtained between Kc and abundance of specific moieties could not be taken as proving the existence of colloidal organic binding ligands for the different radionuclides, it could suggest possible organic moieties involved in the scavenging of these natural radionuclides. Together with results from isoelectric focusing of radiolabeled COM, we conclude that binding to different biomolecules is nuclide-specific, with colloidal hydroxamate siderophoric moieties being important for the binding of Th and Pa radionuclides. Hydroquinones/ quinone (HQ/Q) facilitated redox and chelation reactions seem to be involved in the binding of Pa and Be. However, the actual mechanisms are not clear. Individual amino acids, proteins, total polysaccharides and uronic acids did not yield significant relationships with logKc values of the different radionuclides. Nonetheless, our results provide new insights into the relative importance of different potential ligand moieties in COM in the binding and possible scavenging of specific radionuclides in the ocean.

#### Methods & Sampling

Activity concentrations of 234Th, 233Pa, 210Pb, and 7Be were measured by counting the gamma decay energies at 63.5 keV, 312 keV, 46.5 keV, and 477.6 keV, respectively, on a Canberra ultrahigh purity germanium well detector. The 210Po activity was analyzed by liquid scintillation counting (Beckman Model 8100 Liquid Scintillation Counter).

Concentrations of total carbohydrate (TCHO) were determined by the TPTZ (2, 4, 6-tripyridyl-s-triazine) method using glucose as the standard and [Hung and Santschi, 2001]. Protein content was determined using a modified Lowry protein assay, using bovine serum albumin as the standard (Pierce, Thermo Scientific).

Elemental contents of carbon (C) and nitrogen (N), were determined by a Perkin Elmer CHN 2400 analyzer, using cysteine (29.99% C, 11.67% N) as a standard.

In order to examine the specific binding ligands to five different radionuclides in the marine colloids and diatom culture-derived EPS, radiolabeled biopolymers (E1, E3, C9 and C11; suitable due to their high OC content and available sample amount) were subjected to Isoelectric Focusing (IEF) separation with a Gel Electrophoresis apparatus (Amersham Biosciences, Multiphor Electrophoresis System). Briefly, radiolabeled biopolymers and a 140 µL of rehydration solution were loaded onto an IPG strip (GE Healthcare Immobiline™ Drystrip, pH 3–10, 11 cm) and were re-swelled overnight. Afterwards, the strip was loaded into the device for isoelectric focusing for 17.5 h. The strip was then cut into eleven 1 cm-pieces and followed by 1% SDS extraction overnight. Activity concentrations of the five radionuclides in each fraction were subsequently analyzed. Due to the limited amount of each strip fraction, only selected chemical components (TCHO, Proteins and Fe) in individual fractions were characterized by methods described above. To avoid any interference from any background contamination, SDS extractants underwent diafiltration (desalting) using 1 kDa cutoff Microsep™ centrifugal devices (Pall Life Sciences) using ultra pure Milli-Q water for at least three times.

#### BCO-DMO Processing Notes:

- added conventional header with dataset name, PI name, version date
- modified parameter names to conform with BCO-DMO naming conventions
- converted latitude and longitude values from degrees minutes to decimal degrees
- combined major constituents and radioisotope data together.

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#### **Data Files**

#### File

**IEF.csv**(Comma Separated Values (.csv), 2.06 KB) MD5:377babbbd419949394f3b8efdfed00dc

Primary data file for dataset ID 764794

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## **Related Publications**

Chuang, C-Y., Santschi, P. H., Xu, C., Jiang, Y., Ho, Y., Quigg, A., Guo, L., Hatcher, P. G., Ayranov, M., & Schumann, D. (2015). Molecular level characterization of diatom-associated biopolymers that bind 234 Th, 233 Pa, 210 Pb, and 7 Be in seawater: A case study with Phaeodactylum tricornutum. In Journal of Geophysical Research: Biogeosciences (Vol. 120, Issue 9, pp. 1858–1869). American Geophysical Union (AGU). https://doi.org/10.1002/2015jg002970 https://doi.org/10.1002/2015jG002970 Methods

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#### **Parameters**

Parameter	Description	Units
pН	рН	unitless
E1_234Th	percent activity of 234Th at station E1	unitless (percent)
E3_234Th	percent activity of 234Th at station E3	unitless (percent)
C9_234Th	percent activity of 234Th at station C9	unitless (percent)
C11_234Th	percent activity of 234Th at station C11	unitless (percent)
E1_233Pa	percent activity of 233Pa at station E1	unitless (percent)
E3_233Pa	percent activity of 233Pa at station E3	unitless (percent)
C9_233Pa	percent activity of 233Pa at station C9	unitless (percent)
C11_233Pa	percent activity of 233Pa at station C11	unitless (percent)
E1_210Pb	percent activity of 210Pb at station E1	unitless (percent)
E3_210Pb	percent activity of 210Pb at station E3	unitless (percent)
C9_210Pb	percent activity of 210Pb at station C9	unitless (percent)
C11_210Pb	percent activity of 210Pb at station C11	unitless (percent)
E1_210Po	percent activity of 210Po at station E1	unitless (percent)
E3_210Po	percent activity of 210Po at station E3	unitless (percent)
C9_210Po	percent activity of 210Po at station C9	unitless (percent)
C11_210Po	percent activity of 210Po at station C11	unitless (percent)
E1_7Be	percent activity of 7Be at station E1	unitless (percent)
E3_7Be	percent activity of 7Be at station E3	unitless (percent)
C9_7Be	percent activity of 7Be at station C9	unitless (percent)
C11_7Be	percent activity of 7Be at station C11	unitless (percent)
E1_Protein	percent activity of Protein at station E1	unitless (percent)
E3_Protein	percent activity of Protein at station E3	unitless (percent)
C9_Protein	percent activity of Protein at station C9	unitless (percent)
C11_Protein	percent activity of Protein at station C11	unitless (percent)
E1_TCHO	percent activity of total carbohydrates at station E1	unitless (percent)
E3_TCHO	percent activity of total carbohydrates at station E3	unitless (percent)
C9_TCHO	percent activity of total carbohydrates at station C9	unitless (percent)
E1_Fe	percent activity of iron at station E1	unitless (percent)
E3_Fe	percent activity of iron at station E3	unitless (percent)
C9_Fe	percent activity of iron at station C9	unitless (percent)

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# Instruments

Dataset- specific Instrument Name	Perkin Elmer CHN 2400 analyzer
Generic Instrument Name	CHN Elemental Analyzer
Dataset- specific Description	Elemental contents of carbon (C) and nitrogen (N), were determined by a Perkin Elmer CHN 2400 analyzer, using cysteine (29.99% C, 11.67% N) as a standard.
Generic Instrument Description	A CHN Elemental Analyzer is used for the determination of carbon, hydrogen, and nitrogen content in organic and other types of materials, including solids, liquids, volatile, and viscous samples.

Dataset- specific Instrument Name	Amersham Biosciences, Multiphor Electrophoresis System
Generic Instrument Name	Electrophoresis Chamber
Dataset- specific Description	In order to examine the specific binding ligands to five different radionuclides in the marine colloids and diatom culture-derived EPS, radiolabeled biopolymers (E1, E3, C9 and C11; suitable due to their high OC content and available sample amount) were subjected to Isoelectric Focusing (IEF) separation with a Gel Electrophoresis apparatus (Amersham Biosciences, Multiphor Electrophoresis System).
Generic Instrument Description	General term for an apparatus used in clinical and research laboratories to separate charged colloidal particles (or molecules) of varying size through a medium by applying an electric field.

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# **Project Information**

Biopolymers as carrier phases for selected natural radionuclides (of Th, Pa, Pb, Po, Be) in diatoms and coccolithophores (Biopolymers for radionuclides)

#### **NSF Award Abstract:**

Particle-associated natural radioisotopes are transported to the ocean floor mostly via silica and carbonate ballasted particles, allowing their use as tracers for particle transport. Th(IV), Pa (IV,V), Po(IV), Pb(II) and Be(II) radionuclides are important proxies in oceanographic investigations, used for tracing particle and colloid cycling, estimating export fluxes of particulate organic carbon, tracing air-sea exchange, paleoproductivity, and/or ocean circulation in paleoceanographic studies. Even though tracer approaches are considered routine, there are cases where data interpretation or validity has become controversial, largely due to uncertainties about inorganic proxies and organic carrier molecules. Recent studies showed that cleaned diatom frustules and pure silica particles, sorb natural radionuclides to a much lower extent (by 1-2 orders of magnitude) than whole diatom cells (with or without shells). Phytoplankton that build siliceous or calcareous shells, such as the diatoms and coccolithophores, are assembled via bio-mineralization processes using biopolymers as nanoscale templates. These templates could serve as possible carriers for radionuclides and stable metals.

In this project, a research team at the Texas A & M University at Galveston hypothesize that radionuclide sorption is controlled by selective biopolymers that are associated with biogenic opal (diatoms), CaCO3 (coccolithophores) and the attached exopolymeric substances (EPS), rather than to pure mineral phase. To pursue this idea, the major objectives of their research will include separation, identification and molecular-level characterization of the individual biopolymers (e.g., polysaccharides, uronic acids, proteins, hydroquinones,

hydroxamate siderophores, etc.) that are responsible for binding different radionuclides (Th, Pa, Pb, Po and Be) attached to cells or in the matrix of biogenic opal or CaCO3 as well as attached EPS mixture, in laboratory grown diatom and coccolithophore cultures. Laboratory-scale radiolabeling experiments will be conducted, and different separation techniques and characterization techniques will be applied.

Intellectual Merit: It is expected that this study will help elucidate the molecular basis of the templated growth of diatoms and coccoliths, EPS and their role in scavenging natural radionuclides in the ocean, and help resolve debates on the oceanographic tracer applications of different natural radioisotopes (230,234Th, 231Pa, 210Po, 210Pb and 7,10Be). The proposed interdisciplinary research project will require instrumental approaches for molecular-level characterization of these radionuclides associated carrier molecules.

Broader Impacts: The results of this study will be relevant for understanding biologically mediated ocean scavenging of radionuclides by diatoms and coccoliths which is important for carbon cycling in the ocean, and will contribute to improved interpretation of data obtained by field studies especially through the GEOTRACES program. This new program will enhance training programs at TAMUG for postdocs, graduate and undergraduate students. Lastly, results will be integrated in college courses and out-reach activities at Texas A&M University, including NSF-REU, Sea Camp, Elder Hostel and exhibits at the local science fair and interaction with its after-school program engaging Grade 9-12 students from groups traditionally underrepresented.

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## **Funding**

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1356453

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