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# Protocol for Creating Major Ion Solutions for Freshwater Systems

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## ABSTRACT

Several experimental designs require serial dilutions of site water. To perform dilutions without the use of prefiltered water or without the addition of nutrients and trace metals in freshwater systems, major ion solutions (MIS) are required. MIS provides a nutrient and metal free dilution medium to minimize hypertonic and hypotonic effects on the organisms in the samples during dilutions by balancing major dissolved ions of  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ , and  $\text{Si}^{4+}$  in freshwater systems. MIS are the equivalent of no nutrient artificial sea water for use in freshwater systems. Unlike artificial sea water, MIS formulation for use in freshwater systems changes from site to site. This protocol describes the process of creating a site specific major ion solution from results of elemental analysis data for use in the specified freshwater system.

## DOI

[dx.doi.org/10.17504/protocols.io.bvyxn7xn](https://dx.doi.org/10.17504/protocols.io.bvyxn7xn)

## PROTOCOL CITATION

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## KEYWORDS

Major Ion Solution, MIS, freshwater dilution media

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Jun 21, 2021

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## MATERIALS TEXT

 $\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$  (CAS 13517-24-3) $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (CAS 10035-04-8) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (CAS 10034-99-8) $\text{Na}_2\text{SO}_4$  (CAS 7757-82-6) $\text{K}_2\text{SO}_4$  (CAS 7778-80-5)

Distilled Water

Carboy for the major ion solution

Graduated Cylinders

## Find major ion composition of the freshwater body

- 1 Identify the elemental concentrations of  $\text{Si}^{4+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$ , and  $\text{SO}_4^{2-}$  in the freshwater system for which you wish to create a major ion solution.

Note: USGS reports are helpful for water bodies in the United States, but major ion compositions from elemental analysis are available for many systems from scholarly communication (peer reviewed manuscripts, theses/dissertations, government reports, preprints, etc.).

- 2 Convert the concentrations of the elements to mg/L from  $\mu\text{M}$  if the data is not already in this format.

## Create the formulation of the major ion solution

- 3 Download Freshwater\_Major\_Ion\_Solution\_calculation.csv from Zenodo at <http://www.doi.org/10.5281/zenodo.5019969>.

Calculations and recipes to make major ion solution (MIS) for freshwater systems										
USE FIRST TABLE FOR CORRECT CALCULATIONS (Note: substitute the final concentration of each ion (mg/L) in boxed area below)										
Element	Element FW	as	Compound FW	Final conc. (mg/L of element)	Final conc. (mmol/L of element)	Final conc. (mmol/L of compound)	Final conc. (mg/L compound)	Mass in 20L mg	mass in 10L	mass in 5L
$\text{Si}^{4+}$	28.0855	$\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$	284.2	0.0000	0.0000	0.0000	0.000	0.00	0.00	0.00
$\text{Ca}^{2+}$	40.08	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	147.02	0.0000	0.0000	0.0000	0.000	0.00	0.00	0.00
$\text{Mg}^{2+}$	24.305	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	246.47	0.0000	0.0000	0.0000	0.000	0.00	0.00	0.00
$\text{Na}^+$	22.9898	$\text{Na}_2\text{SO}_4$	142.04	0.0000	0.0000	0.0000	0.000	Total Na (mg/L)	0.0	0.00
$\text{K}^+$	39.0983	$\text{K}_2\text{SO}_4$	174.27	0.0000	0.0000	0.0000	0.000	0.00	0.00	0.00
$\text{Cl}^-$	35.453	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	147.02	0.0000	0.0000	0.0000	0.000	0.00	0.00	0.00
$\text{SO}_4^{2-}$	96.0576	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	246.47	0.0000	0.0000	0.0000	0.000	Total $\text{SO}_4$ (mg/L)	0.0	0.00
MIS Recipe:										
Compound	mg per 20 L	g per 20 L	mg per 10 L	g per 10 L	mg per 5 L	g per 5 L				
$\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$	0.000	0.00000	0.000	0.00000	0.00	0.00000				
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.000	0.00000	0.000	0.00000	0.00	0.00000				
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.000	0.00000	0.000	0.00000	0.00	0.00000				
$\text{Na}_2\text{SO}_4$	0.000	0.00000	0.000	0.00000	0.00	0.00000				
$\text{K}_2\text{SO}_4$	0.000	0.00000	0.000	0.00000	0.00	0.00000				

Screenshot of Freshwater\_Major\_Ion\_Solution\_calculation.csv

- 4 Fill in the mg/L box in the with the values calculated from Steps 1 and 2. The box to add this data is highlighted in purple in the image below.

Calculations and recipes to make major ion solution (MIS) for freshwater systems										
USE FIRST TABLE FOR CORRECT CALCULATIONS (Note: substitute the final concentration of each ion (mg/L) in boxed area below)										
Element	Element FW	as	Compound FW	Final conc. (mg/L of element)	Final conc. (mmol/L of element)	Final conc. (mmol/L of compound)	Final conc. (mg/L compound)	Mass in 20L mg	mass in 10L	mass in 5L
$\text{Si}^{4+}$	28.0855	$\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$	284.2		0.0000	0.0000	0.000	0.00	0.00	0.00
$\text{Ca}^{2+}$	40.08	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	147.02		0.0000	0.0000	0.000	0.00	0.00	0.00
$\text{Mg}^{2+}$	24.305	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	246.47		0.0000	0.0000	0.000	0.00	0.00	0.00
$\text{Na}^+$	22.9898	$\text{Na}_2\text{SO}_4$	142.04		0.0000	0.0000	0.000	Total Na (mg/L)	0.0	0.00
$\text{K}^+$	39.0983	$\text{K}_2\text{SO}_4$	174.27		0.0000	0.0000	0.000	0.00	0.00	0.00
$\text{Cl}^-$	35.453	$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	147.02		0.0000	0.0000	0.000	0.00	0.00	0.00
$\text{SO}_4^{2-}$	96.0576	$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	246.47		0.0000	0.0000	0.000	Total $\text{SO}_4$ (mg/L)	0.0	0.00
MIS Recipe:										
Compound	mg per 20 L	g per 20 L	mg per 10 L	g per 10 L	mg per 5 L	g per 5 L				
$\text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$	0.000	0.00000	0.000	0.00000	0.00	0.00000				
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	0.000	0.00000	0.000	0.00000	0.00	0.00000				
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	0.000	0.00000	0.000	0.00000	0.00	0.00000				
$\text{Na}_2\text{SO}_4$	0.000	0.00000	0.000	0.00000	0.00	0.00000				
$\text{K}_2\text{SO}_4$	0.000	0.00000	0.000	0.00000	0.00	0.00000				

The box to add the concentrations to is highlighted in purple.

- 5 As multiple compounds contribute to the  $\text{Na}^+$  and  $\text{SO}_4^{2-}$  concentrations and  $\text{Cl}^-$  concentrations come from  $\text{Ca}^{2+}$  calculations, check to make sure that the concentrations are not magnitudes off compared to the reference. There is natural variability in the ionic composition of freshwater systems so some deviation is not an issue. The orange and blue highlights under final concentration column show the corresponding compounds between the cations and anions. If the values are several magnitudes off, use the values calculated using the anions. See the example below to see how this works.

The example used in the image below is for the upper Neuse River Estuary as reported in Paerl and Bowles (1987) from USGS data (Harned 1982).

## References:

Harned, D.A., 1982. *Water quality of the Neuse River, North Carolina-Variability, pollution loads, and long-term trends* (USGS Report No. 2185-D). USGPO,.

Paerl, H.W. and Bowles, N.D., 1987. Dilution bioassays: Their application to assessments of nutrient limitation in hypereutrophic waters. *Hydrobiologia*, 146(3), pp.265-273. <https://doi.org/10.1007/BF00016348>

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Note: substitute the final concentration of each ion (mg/L) in boxed area below													
Element	Element FW	as	Compound FW	Final conc. (mg/L of element)	Final conc. (mmol/L of element)	Final conc. (mmol/L of compound)	Final conc. (mg/L compound)	Mass in 20L mg	mass in 10 mass in 5 L				
Si <sup>4+</sup>	28.0855	Na <sub>2</sub> SiO <sub>3</sub> ·9H <sub>2</sub> O	284.2	4.3	0.1531	0.1531	43.512	870.24	435.12 217.56				
Ca <sup>2+</sup>	40.08	CaCl <sub>2</sub> ·2H <sub>2</sub> O	147.02	5.6	0.1387	0.1387	20.542	410.83	205.42 102.71				
Mg <sup>2+</sup>	24.305	MgSO <sub>4</sub> ·7H <sub>2</sub> O	246.47	1.7	0.0698	0.0698	17.239	344.78	172.39 86.20				
Na <sup>+</sup>	22.9898	Na <sub>2</sub> SO <sub>4</sub>	142.04	2	0.0870	0.0435	6.178	123.57	Total Na (mg/L) 9.0 205.42 102.71				
K <sup>+</sup>	39.0983	K <sub>2</sub> SO <sub>4</sub>	174.27	2.6	0.0665	0.0332	5.794	115.89	57.94 28.97				
Cl <sup>-</sup>	35.453	CaCl <sub>2</sub> ·2H <sub>2</sub> O	147.02	9.9	0.2792	0.1396	20.527	410.54	205.27 102.64				
SO <sub>4</sub> <sup>2-</sup>	96.0576	MgSO <sub>4</sub> ·7H <sub>2</sub> O	246.47	6.7	0.0697	0.0697	17.191	343.82	Total SO <sub>4</sub> (mg/L) 14.1 171.91 85.96				
MIS Recipe:													
Compound	mg per 20 L	g per 20 L	mg per 10 L	g per 10 L	mg per 5 L	g per 5 L							
Na <sub>2</sub> SiO <sub>3</sub> ·9H <sub>2</sub> O	870.243	0.87024	435.121	0.43512	217.56	0.21756							
CaCl <sub>2</sub> ·2H <sub>2</sub> O	410.834	0.41083	205.417	0.20542	102.71	0.10271							
MgSO <sub>4</sub> ·7H <sub>2</sub> O	344.784	0.34478	172.392	0.17239	86.20	0.08620							
Na <sub>2</sub> SO <sub>4</sub>	123.568	0.12357	61.784	0.06178	30.89	0.03089							
K <sub>2</sub> SO <sub>4</sub>	115.888	0.11589	57.944	0.05794	28.97	0.02897							

Example from the Neuse River Estuary of checking the relative concentrations in Major Ion Solution creation.

- The concentrations to use when creating a major ion solution are in the MIS Recipe box at the bottom of the document. The recipes are highlighted below. Example is the same as in Step 5.

A				C		E		F		H		I		J		K		L		M	
Calculations and recipes to make major ion solution (MIS) for freshwater systems																					
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K <sub>2</sub> SO <sub>4</sub>	115.888	0.11589	57.944	0.05794	28.97	0.02897															

The MIS Recipe for use in creation of 5 L, 10 L, and 20 L of major ion solution.

## Create the major ion solution

- Add approximately 1/20 of your total desired volume of distilled water to your intended container for the major ion solution.

Example: use a 20 L carboy to create 20 L of major ion solution and use graduated cylinders for volumetric measurements.

- Add in the Na<sub>2</sub>SiO<sub>3</sub>·9H<sub>2</sub>O, CaCl<sub>2</sub>·2H<sub>2</sub>O, MgSO<sub>4</sub>·7H<sub>2</sub>O, Na<sub>2</sub>SO<sub>4</sub>, and K<sub>2</sub>SO<sub>4</sub> in concentrations calculated in Step 6 to the distilled water in the major ion solution container.
- Fill your container to your desired volume using distilled water. Mix the container until all of the salts dissolve.