Supporting Information

Cyanogen Chloride Precursor Analysis in Chlorinated River Water
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Retention Characteristics of Glycine and Suwannee River Fulvic Acid in an IMAC Column

Preliminary IMAC column tests were conducted to examine the relative affinities of glycine and natural humic-like organic matter for the copper-loaded IMAC resin media that used in the main text. The basic approach was to load a solution mixture containing known concentrations of Suwannee River fulvic acid and glycine on a Cu-loaded IMAC resin. After loading the sample analytes on the resin, they were then eluted with deionized water and the elution behavior of glycine and total organic carbon was monitored. Similar elution approaches to isolate proteins from other organic fractions in natural samples have been used by other researchers (see for example reference 26 in the main text). In this study, the objective was to demonstrate the predominantly higher affinity of glycine for the resin relative to fulvic acid constituents. Details and the results of this experiment are described herein.

Experimental Methods

Column Preparation and Metal Loading of IMAC Resin. The polyvalent metal ion binding (PMIB) resin (Affiland, Belgium) was packed in a 10 mm diameter of glass column (Ominfit) to a height of 96 mm (resulting volume was 7.54 cm^3). A 450-mL volume of 5 mM $CuSO_4$ was pumped through the PMIB resin-packed column, followed by a rinse volume of 50 mL Milli-Q water. Based on a copper mass balance, the copper binding capacity of the IMAC column was estimated as 213 $\mu mol\ mL^{-1}$ resin.

Sample Processing with IMAC Column. A model solution mixture of glycine and a reference fulvic acid was prepared that contained nearly equal concentrations of the two compounds on an organic carbon concentration basis (about 10 mg L^{-1} glycine-C, and 10 mg L^{-1} fulvic acid- C). The fulvic acid material was a Suwannee River reference isolate obtained from the International Humic Substance Society, MN.

An 11.3-mL volume of the glycine-fulvic acid solution was processed through the IMAC column at flow rate of 2 mL min^{-1}. After loading the sample, the column was then eluted with deionized water at an elution rate of 0.3 mL min^{-1}. Twenty-three effluent fractions (3 mL each) were collected during the loading and elution period. Each effluent fraction was subsequently analyzed to determine its total organic carbon (high temperature combustion TOC analyzer) and glycine concentrations (HPLC). The analytical methods are the same as those described in the main text.

Results

To conveniently compare the retention of glycine and fulvic acid in the above chromatographic experiment, effluent fraction glycine concentrations were first re-calculated in terms of the equivalent glycine-C concentration. In Figure A-1, the elution profile of both total organic

carbon and glycine-C are compared. Since some the fulvic acid was not retained during the 'loading phase', TOC concentrations of fractions collected during this period are included in the plot. Based on the distinctly longer retention of glycine in the column, fulvic acid constituents appear to have a much weaker affinity for the resin than glycine. Mass balance calculations for the experiment indicate that the recovery of total organic carbon among the collected fractions was 118% (0.208 mg org-C introduced, 0.246 mg recovered). The recovery of glycine was 98%.

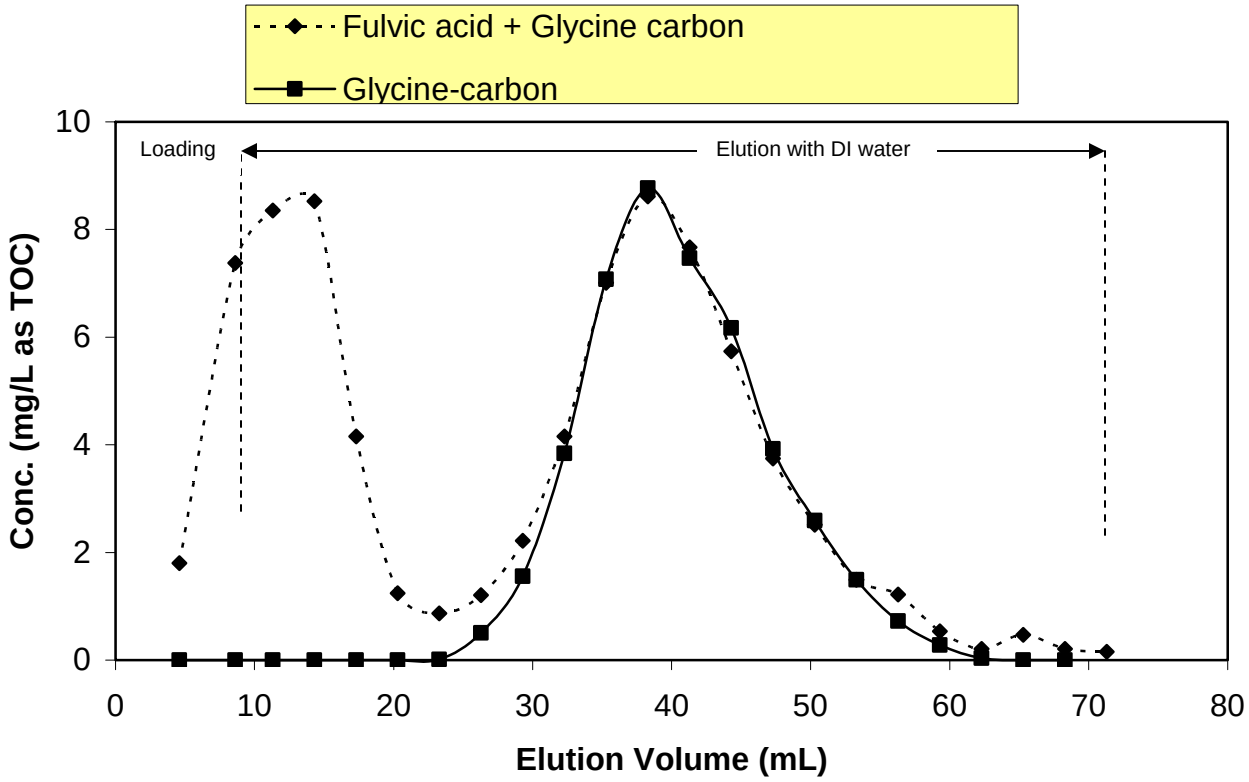

Figure A-1. Elution profiles of total organic carbon (due to fulvic acid and glycine) and glycine-carbon. The loaded sample contained approximately equal concentrations of fulvic acid and glycine on an organic carbon basis.