Organic Acids in Aquatic Ecosystems
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Group Report
How Are Acid-base Properties of "DOC" Measured and Modeled and How Do They Affect Aquatic Ecosystems?

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INTRODUCTION

Dissolved organic carbon (DOC) undergoes an extensive number and variety of interactions in aquatic ecosystems. It exchanges protons, metals, and nutrients, it inhibits or stimulates microorganism growth, it attaches to colloid surfaces, etc. These interactions may appear at equilibrium within observation time scales or may appear reactive. They are studied to understand the basic character and function of DOC and to make predictions about its behavior under a wide range of environmental conditions such as concentration, temperature, pH, alkalinity, and ionic strength.

Fundamental to these studies is the ability to make analytical measurements. Two properties limit the straightforward application and interpretation of most analytical techniques: DOC concentration and composition. High concentrations are often needed for precise analytical measurements. Steps taken to concentrate bring about changes in DOC character because the concentrated sample differs from the original in pH, metal and particulate concentration, and ionic strength. Furthermore, DOC is a mixture of molecules with wide molecular size distribution. Any molecule in this mixture may differ greatly in character and reactivity from any other. Only a very small percentage of this mixture, perhaps about 10%, can be separated and identified as individual molecules; most of it cannot be fractionated to this degree. Thus, almost all analytical measurements of DOC properties are measurements of average properties. These measurements, made on altered DOC and reflecting average properties, are those relied upon for understanding DOC and predicting its behavior. It is in the light of these constraints that all topics in this report must be considered.

Analytical measurements provide data which enable hypotheses about the character of DOC to be tested. These hypotheses may take the form of fairly simple descriptive models, like acid-base equilibria, or more complex models, such as one describing DOC behavior in surface waters which combines photoredox, biological, and mass transport processes. Whenever measurements are made in the laboratory, questions arise about their utility to field studies and to the aquatic ecosystem as a whole. Laboratory measurements are made under controlled and simplified circumstances in which conditions such as temperature, pH, and metal content are carefully controlled. A concentration step which will alter the material may be required as mentioned above. The time frame of laboratory observations is normally no shorter than a few seconds and no more than several days. In an ecosystem, conditions may vary considerably within this time frame and DOC residence times may be months or years.

Laboratory experiments are a necessity; they are designed to describe the system in simplest terms, to understand it at the most fundamental level, and to investigate processes in isolation uncoupled as much as possible from a vast continuum of varying conditions over lengthy time scales. One purpose of this reduction to simplest terms is to understand underlying physical chemical principles. Another equally important purpose is to integrate this knowledge with the environmental ecosystem, i.e., to extrapolate from the simple to the highly complex and variable system. Accomplishment of this latter purpose, extrapolation to the field, is not trivial but is also perhaps not insurmountable. The strategy contains several elements. Whenever possible, properties should be measured on the environmental sample itself, without alteration, and in the field over time. If this is not possible, and it often is not because of some analytical need or purpose that requires concentration, isolation, or fractionation procedures, several procedures should be compared to reveal how the measured properties change and how the sample is altered during sample preparation. Of course, the only assurance that an altered sample has the same properties as the original sample is to compare altered with unaltered samples. If the original sample and its properties can be reconstructed with altered material, the altered material can be used as a surrogate for the original.

This report comments on recent research and on present research needs in the area of proton and metal binding of the organic acid fraction of DOC and discusses application of such research to two problems: the ion balance in colored, low ionic strength lakes and the kinetics of organic acid transformations in aquatic ecosystems. Included is a discussion of concentration methodologies as the first step in a laboratory investigation. Recommendations follow each section. These suggest areas of future research and urge greater cooperation and working arrangements between laboratory-based scientists and scientific investigators in the field.

CONCENTRATION/ISOLATION/FRACTIONATION METHODOLOGIES

The term concentration refers to the necessity to concentrate DOC to a level at which analytical measurements such as IR, NMR, bioassays, etc., are possible. Isolation refers to an additional need, not only to concentrate the DOC but also to separate it from matrix constituents such as metals, silica, or Cl^-. Isolation may be purely an analytical requirement, for example the need to separate DOC from Cl^- to use a Cu ion-selective electrode, or it may be a research design requirement to allow pH or ionic strength to be manipulated. Fractionation refers to a specific research need, as is the need to separate low from high molecular weight acids in order to study their properties separately. The decision to separate DOC from its solvent or its matrix, or to divide it in some way before analysis, depends on the specific purpose in mind. When the purpose is clear, so is the information value of the data. For example, fractionation is required to study low molecular weight organic acids and the data are useful to that fraction only. However, if the purpose is to study the properties of DOC or organic acids as a whole, then the concentration step must not fractionate the DOC unless it can be shown that the fraction has the same properties as the whole.

XAD resins are widely used to concentrate and isolate DOC from aquatic samples. They were quickly adopted to concentrate and desalt gram quantities of dissolved organic matter (DOM) from aquatic samples because they allowed greater convenience and speed than any other method available at the time of their introduction. XAD resin does fractionate DOC and the question remains whether all properties of the XAD extract match the properties of the original DOM. For example, David (unpublished) has found that the carboxyl acidity of the "hydrophilic" fraction (the portion not extracted by the XAD resin) is about 20% higher than the XAD fraction obtained from the same sample. On the other hand, XAD concentrates are found to have the same color and Cl_2 reaction products (Christman, unpublished) as the whole water sample from which they came.

It is unclear exactly how XAD resins fractionate DOC, but the mechanisms appear to be related to the molecular size of the solute and to the exclusion properties of the resin. The reason why "hydrophilic" acids are not extracted may be related to molecular size (sizes greater than about 100,000 daltons may be excluded) or affinities (carboxyl- and hydroxyl-rich molecules with mole ratios of C/COOH less than about 3/1 have low affinities (Thurman et al. 1978; Arhen et al. 1979). Perhaps XAD uniformly extracts only intermediate molecular sizes and does not extract extremely large or extremely small size carboxylic acids. This is speculation, because the molecular size distribution of extracted organic acids is not known precisely. Extraction efficiencies of XAD resins appear to be related to the type of sample which is extracted and this may in turn be related to size distribution (the size distribution of DOC in seawater or groundwater is quite different from the distribution in freshwater).

Recommendations

1. Whenever feasible, analytical measurements on organic acids should be made on unaltered samples.
2. If concentration, isolation, or fractionation is necessary, several techniques should be used and compared for internal consistency of measured properties.
3. Systematic investigations should be undertaken to discover how measured properties change as the sample is modified by pretreatment. Fractions could be added to reconstruct the original material to determine if original properties are obtained.

PROTON AND METAL-DOC BINDING

Data collected from metal-spiked samples indicate that organic complexation of trace metals is prevalent in many aquatic environments, although the specific compounds or binding sites responsible for this complexation are unknown and the actual extent of complexation at natural (unspiked) conditions cannot be directly measured. Empirical thermodynamic models which represent metal chelation in natural waters are available based on titrimetric data, but these cannot be extrapolated confidently to conditions outside the data upon which they are based (Cabaniss and Shuman 1988). A great need exists for data at low natural metal concentrations and within a wide range of pH and ionic strength.

Strong chelation may come either from specific biogenic chelators, such as siderophores or cystein-rich polypeptides, or from humic compounds. While it seems reasonable that the biota should, under some conditions, release specific chelators in their surrounding medium, actual field data on such compounds are lacking. Progress in this field may come from the use of sensitive analytical techniques developed with chelators produced in laboratory cultures of aquatic organisms and applied to field samples.

Strong effective metal chelation by isolated DOM has been demonstrated many times. Once corrected for variations in experimental conditions, the data of many researchers, using various techniques and different samples, are in good agreement. Mathematical representation of these data and hence the chemical interpretation of binding vary widely. Chelation is described equally well as a mixture of discrete ligands, as a continuous distribution of metal affinities, or as a polyelectrolyte (metal affinity of individual ligands enhanced or diminished by long-range coulombic forces from neighboring functional groups). In all cases to date the purpose has been to describe complexation data and their variation with solution conditions (pH, ionic strength, and metal and ligand concentrations) with a minimum set of parameters. Description with the purpose of greatest economy of terms is not very useful in itself, and future progress in thermodynamic models of DOM complexation must be based on a sound understanding of the physical/chemical reality of metal binding. For example, donor atoms involved in reacting ligands should be identified and binding mechanisms understood to distinguish between intrinsic metal affinities (e.g., strong binding attributed to thiol groups) and polyelectrolyte effects. The variation of molecular size distribution with pH, ionic strength, and metal concentrations, as well as the role of polydispersity on metal binding, should also be understood. The need for such fundamental knowledge will probably necessitate new experimental approaches to probe metal binding at the molecular level.

Development of any thermodynamic model of metal-DOM binding is by itself insufficient. The kinetics of coordination reactions in natural waters need not always be rapid. Competing reactions with other ligands and metal exchange reactions may lead to overall kinetics that are slow compared to hydrodynamic residence times (years). For example, metal exchange rates may be very slow if Al and Fe occupy binding sites. In addition, in systems with high alkaline-earth concentrations, such as estuarine and marine water, Ca or Mg in occupied sites may also be slow to exchange (Hering and Morel 1988). Reaction rates will be a function of many parameters including pH (H^+ or OH^- catalyzed reactions), molecular size and charge, etc.

Kinetic methods may be particularly convenient for obtaining thermodynamic data and gaining insight into the nature of the binding process. For example, one may probe the binding properties of humates by following, over a range of concentrations, the kinetics of metal exchange with a strong chelator such as calcein blue or a fluorescent metal such as Europium whose fluorescence is enhanced or quenched over a range of concentrations.

Recommendations

1. New or novel techniques should be applied to probe the molecular size, charge density, and nature of the binding mechanisms of organic acids.

2. New thermodynamic metal-binding models which include metal competition, proton competition, and ionic strength effects are needed that can predict behavior over a wide range of environmental conditions. These models need to be based on the identity of binding groups, molecular configuration, and charge.
3. Actual data sets, not just derived binding parameters, should be reported for future model evaluation and comparison.
4. Laboratory predictions should be tested with field data.

ORGANIC ACID CONTRIBUTION TO CHARGE BALANCE

The ion balance in colored freshwaters that are sensitive to acidification by anthropogenic strong acid inputs can be significantly influenced by the presence of organic acids. Empirical models are used to understand and predict changes from these anthropogenic acidic inputs. The models are based on the ionic composition of the water and require estimating the free organic acid anion concentration (A_f). Organic anions in acidic, organic-rich waters can be a significant or major fraction of the total anions (Hemond 1980; Eshleman and Hemond 1985). Often the models either ignore organic anions or consider them constant during changes in acidic inputs. Major unresolved questions are how organic acid concentrations and their acid-base characters change during anthropogenic acidification, how large these changes are, and whether they are reversible.

A_f is commonly estimated from the difference between the sum of all inorganic cation concentrations (e.g., Ca, Mg, K, Na, NH_4, Al, Mn, and H^+) and the sum of all inorganic anion concentrations (e.g., SO_4, Cl, HCO_3, NO_3, and F). Large errors can be propagated simply from this summing up of 10–15 analytically derived values, each with its own considerable error (typically 2–10% relative error for each [Cronan et al. 1978]). Another way of estimating A_f comes from an empirical expression that attempts to relate A_f to total carboxyl acidity (A_t) and pH (Oliver et al. 1983). However, this approach has problems too. The total concentration of carboxyl acidity (A_t) is the sum of A_f and the concentrations of metal-complexed carboxylic acids (A_c) and protonated carboxylic acids (A_H). Thus $A_t = A_c + A_H + A_f$. The Oliver model does not consider A_c, which leaves this term undetermined unless additional information is available such as independent metal complexation data or a thermodynamic binding model. An alternative method of estimating $A_c + A_f$ based on increase in pH (production of alkalinity) upon photooxidation has also been used (Hemond 1980; Eshleman and Hemond 1985; Gorham et al. 1985). However, interferences need to be considered; for example, sulfuric acid from nonionic sulfur in some bog samples is found to interfere (Gorham et al. 1985).

Recommendations

1. Look for direct, specific analytical methods to determine organic acid anions.
2. Report complete acid-base titrations to better define the protolytic chemistry of whole samples. Report DIC-pH to obtain carbonate species.
3. Investigate the role of metals (e.g., Fe, Al) and their complexes on the proton balance in these aquatic ecosystems.
4. Develop and test empirical relationships to predict organic anion concentrations as a function of pH (i.e., a refined Oliver model).

KINETICS

It is necessary to consider kinetics in all aspects of organic acid chemistry. For example, the effectiveness of ion exchange resins in lowering the ash content of DOC isolates and the protonation of acidic anions by strong acid ion exchange chromatography may be limited by the rate of exchange of proton for metal on metal-occupied carboxylate binding sites. Titration curves of organic acids exhibit irreversible behavior—the so-called "hysteresis effect," in which the base titration is not reproduced when a reverse titration with acid is carried out. The slow exchange or hydrolysis of Al has been hypothesized to explain hysteresis as has bacterial alteration of DOC. Metal exchange reactions may be fast or slow depending on the metals involved, their relative concentrations and binding properties, and the nature of the ligand and its acid-base properties. There may also be specific acid or base catalysis reactions. Water exchange rates may limit the rate of reaction of some metals with donor atoms as they do with $Al(H_2O)_6^{3+}$ at low pH (Eigen 1963). The size, charge density, or molecular configuration of organic acids may affect the rate at which an incoming ion or molecule can approach a reactive site. Kinetics can limit the rate of redox, sorption–desorption, and precipitation reactions. Samples collected in the field may be altered via a number of kinetic processes during transport and storage. The transformation rates of nutrients and other bioactive substances can be affected by pH, light, temperature, and molecular size and shape. In this regard, Jones et al. (1988) demonstrated that a DOC-Fe-PO_4 species, in brownwater lakes that were studied, reduced phosphate availability to aquatic biota by sequestering phosphate and then releasing part of the bound phosphate during illumination.

This discussion of the effects of DOC on the biological availability of nutrients leads to the general question of the relevance of data derived in the laboratory to processes in the natural environment. Environmental samples are manipulated for two major purposes: to facilitate analytical measurements and to eliminate interactive variables. The former has been mentioned above with respect to concentrating DOC for water samples and removing substances which interfere with analytical measurements. The latter manipulation is, in part, necessitated by conceptual considerations. Performing a "controlled" experiment is usually interpreted as the process of holding all known potential variables constant, varying one in a known way, and recording the response of the system. This experimental design has several advantages. The results are easy to interpret and they can be used as the first argument in a logic used to show cause and effect relationships. Results from these types of experiments are valuable. Experiments of this design should and will continue to be performed.

However, if we are to understand the function of natural ecosystems then we must conduct experiments designed to determine which factors dominate in systems governed by interacting variables. One approach, the bottom up approach, is to synthesize models of simple systems. With proper forethought, models derived from "controlled" experiments on environmental processes can be integrated to predict processes in systems of ever-increasing complexity, ultimately ecosystems. The accuracy of ecosystem models can be judged only by comparing model predictions with data derived from long-term ecosystems studies; this is the top down approach.

Recommendations

1. The effects of kinetics on laboratory analytical procedures such as the hysteresis effect on acidity titrations, particularly the possible involvement of Al and Fe, should be studied.
2. The kinetics of reactions involving organic acids themselves (acid-base, complexation, redox, sorption–desorption) should be investigated.
3. Laboratory studies need to be designed so that results can be integrated into ecosystem models.

REFERENCES

Aiken, G.R., E.M. Thurman, R.L. Malcolm, and H.F. Walton. 1979. Comparison of XAD macroporous resins for the concentration of fulvic acid from aqueous solution. *Analyt. Chem.* 51:1799–1803.

Cabaniss, S.E., and M.S. Shuman. 1988. Copper binding by dissolved organic matter. I. Suwannee River fulvic acid equilibria. II. Variation in type and source of organic matter. *Geochim. Cosmo. Acta* 52:185–200.

Cronan, C.S., W.A. Reiners, R.C. Reynolds, and G.E. Lang. 1978. Forest floor leading: contributions from mineral, organic, and carbonic acids in New Hampshire subalpine forests. *Science* 200:309–311.

Eigen, M. 1963. Fast elementary steps in chemical reaction mechanisms. *Pure Appl. Chem.* 6:105–117.

Eshleman, K.N., and H.F. Hemond. 1985. The role of organic acids in the acid-base status of surface waters at Bickford Watershed, MA. *Wat. Res. Res.* 21:1503–1510.

Gorham, E., S.J. Eisenreich, J. Ford, and M.V. Santelmann. 1985. The chemistry of bog waters. In: Chemical Processes in Lakes, ed. W. Stumm, pp. 339–363. New York: Wiley.

Hemond, H.F. 1980. Biogeochemistry of Thoreau's Bog, Concord, MA. *Ecol. Monogr.* 50:507–526.

Hering, J.G., and F.M.M. Morel. 1988. Kinetics of trace metal complexation: role of alkaline-earth metals. *Env. Sci. Tech.* 22:1469–1478.

Jones, R.I., K. Salonen, and H. De Haan. 1988. Phosphorus transformations in the epilimnion of humic lakes: abiotic interactions between dissolved humic materials and phosphate. *Freshwat. Biol.* 19:357–369.

Oliver, B.G., E.M. Thurman, and R.L. Malcolm. 1983. The contribution of humic substances to the acidity of colored natural waters. *Geochim. Cosmo. Acta* 47:2031–2035.

Thurman, E.M., R.L. Malcolm, and G.R. Aiken. 1978. Prediction of capacity factors for aqueous organic solutes absorbed on a porous acrylic resin. *Analyt. Chem.* 50:775–779.