

Calculation of molecular weights of humic substances from colligative data: Application to aquatic humus and its molecular size fractions

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Abstract—A rigorous mathematical expression for the dependence of colligative properties on acid dissociation of water soluble humic substances is presented. New data for number average molecular weights of a river derived humic material and its gel permeation chromatographic fractions are compared with \bar{M}_n values obtained by a reevaluation of previously published experimental observations on soil and water fulvic acids. The results reveal a remarkable similarity of fulvic acids from widely different sources with respect to number-average molecular weight.

INTRODUCTION

IT HAS been generally recognized that humic substances in soils originate as heteropoly-condensates of such chemically diverse starting materials as phenols, amino acids, carbohydrates, even ammonia (see e.g. FLAIG *et al.*, 1975). The water-mobilizable fraction of soil humic substances contributes significantly to the chemistry of surface waters through interaction with metals, adsorption of hydrophobic substances, etc. and forms an important fraction of the riverine organic carbon flux to the oceans.

One of the major analytical difficulties in the characterization of aqueous humic substances is the lack of any fractionation procedure which will yield pure components which can be subsequently characterized by standard techniques. Consequently, efforts have frequently been devoted to the determination of those bulk physical properties which can be unambiguously defined and determined on complex mixtures. One such parameter is the number-average molecular weight (\bar{M}_n), which can be readily determined from colligative properties such as vapor pressure and freezing point depression. Recently, both vapor pressure osmometry (HANSEN and SCHNITZER, 1969) and cryoscopy (DE BORGER and DE BACKER, 1968) have been applied for the determination of \bar{M}_n values for acid soluble fractions of soil humic substances. In both cases, colligative properties were determined on aqueous solutions of fulvic acids, so that the experimental measurements required corrections for acid dissociation. Lacking a rigorous mathematical expression for the dependence of colligative properties on acid dissociation, different 'correction' procedures were proposed in the two studies. This has led to some confusion about the validity of the approaches chosen (see e.g. WILSON and WEBER, 1977).

In this paper, we wish to present new analytical data for \bar{M}_n values of water-derived humic substances and their molecular size fractions obtained by gel permeation chromatography. We have developed a rigorous mathematical treatment of the dissociation correction applied to the determination of number-average molecular weights for weak polyelectrolytes. This method has been used to re-evaluate analytical data from the above mentioned references. We hope that our discussion will help to remove some misunderstandings and will provide encouragement to generate much needed data on number-average molecular weights of aquatic humic substances.

EXPERIMENTAL

To obtain sufficient organic matter for this study, 200 l. of unfiltered water from a small tributary in the Satilla River flood plain (southeast Georgia) were collected in 20 l. polyethylene carboys and immediately transported to the laboratory. The sample was centrifuged at 10,000 rpm (Sorvall Centrifuge Type SS-3, flow-through mode) and concentrated approximately tenfold under reduced pressure (25 mmHg). The concentrate was freeze-dried, yielding a brown, fluffy powder which contains all but the volatile constituents (e.g. HCl) of the original water. This residue was redissolved in distilled water to a concentration of ~20 g of organic matter per liter. An undissolved residue consisting largely of silica, hydrous oxides of Fe and Al and some high molecular weight organic matter (~3% of total) was removed from the solution. The solute was desalted by shaking with two batches of cation exchange resin (25 ml of Bio-Rad AGX8, 50–100 mesh, H⁺ form, per gram of organic matter) and freeze-dried. The ash content was decreased from an initial 7.7% to a final 2.3%.

Aliquots of the purified river water organic matter were fractionated progressively by exhaustive gel permeation chromatography (GPC) on columns of Sephadex G-50, G-25, G-15, and G-10 using distilled water as eluant. The goal of this fractionation was to obtain chromatographically well defined excluded fractions. Experiments by REUTER (1977) have shown that the ratio of excluded to

retarded fractions is to a high degree also determined by reversible conformational changes of the humic molecules. At low solute concentrations (below 1000 mg/l) conformational changes from extended (or uncoiled) to contracted (or coiled) configurations are affected by the ionic strength and the pH of the solution. Thus, at SHRS concentrations of 100 mg/l, zero ionic strength and pH 7 all of the organic solute was found in the excluded fraction of Sephadex G50. With increasing solute (SRHS) concentrations, the ratio of excluded to retarded fractions decreased until at concentrations above 2000 mg/l a constant ratio was observed. At and above this concentration the distribution between excluded and retarded fractions was found to be stable against changes of pH (± 1 pH unit) and ionic strength. Therefore, no strict control of either parameter was found necessary to obtain reproducible excluded fractions, as long as humic solute concentrations were held above 2000 mg/l.

In order to minimize interactions between the gels and the organic matter, the acidic organic matter solutions were neutralized to pH 7 with NaOH before gel filtration (SCHNITZER and SKINNER, 1968). The effluent from each column was separated into an excluded (E) and a retarded (R) fraction. The excluded fraction was rechromatographed on the same gel type to yield a final excluded fraction for further analyses. The retarded fractions from the first and the second run were combined to be chromatographed on the next lower Sephadex grade. Thus, four excluded fractions (G50E, G25E, G15E, G10E) and one retarded fraction (G10R) were produced. The final Sephadex fractions were desalted to remove added Na^+ using the above named cation exchange resin.

A Mechrolab Model 301A vapor pressure osmometer was used to determine vapor pressures of aqueous solutions of SRHS. These data, together with precise pH measurements on the same solutions were used to calculate number average molecular weights as described in the following section of this paper. The accuracy of the method was tested by determining the \bar{M}_n of a known compound (benzenepentacarboxylic acid).

Infrared spectra were recorded on a Beckman Model 12 Spectrophotometer using KBr pellets. Care was taken to eliminate water from the samples and the potassium bromide (THENG *et al.*, 1966).

MATHEMATICAL TREATMENT OF COLLIGATIVE PROPERTIES OF WEAK POLYELECTROLYTES

The number-average molecular weight (\bar{M}_n) of a mixture of z organic solutes is defined as follows:

$$\bar{M}_n = \frac{\sum_{i=1}^z c_i M_i}{\sum_{i=1}^z c_i} \quad (1)$$

where c_i is the molal concentration of the i th organic solute, whose molecular weight is M_i . The weight concentration of the i th component is $c_i M_i$, so the sum of all $c_i M_i$ values equals the total weight concentration (W) of the mixture of organic solutes. The total molal concentration (C_T) of organic solutes equals the sum of all c_i values. Thus,

$$\bar{M}_n = \frac{W}{C_T} \quad (2)$$

GLOVER (1975) has reviewed the mathematical details of molecular weight determination by absolute colli-

gative property methods (cryoscopy, ebulliometry, vapor pressure osmometry, etc.) for polymeric nonelectrolytes. The instrument response (θ) is related to the total molality of dissolved solute species (m_T) by the equation:

$$\theta = A m_T + B m_T^2 + \dots \quad (3)$$

In the case of nonelectrolytes, m_T and C_T are equal, so θ can be directly related to the total weight concentration (W) of organic solutes by combining eqns (2) and (3).

$$\theta = (A/\bar{M}_n)W + (B/\bar{M}_n^2)W^2 + \dots \quad (4)$$

All the methods described by GLOVER (1975) were based on eqn (4), which was truncated after either the linear or quadratic term. It was pointed out that the statistical significance of derived results is dependent on the order of the equation used to fit experimental data. The constant A , the apparatus constant, is obtained by instrument calibration using a solute of known molecular weight. The \bar{M}_n value of an unknown sample is determined by a least-squares method which yields (A/\bar{M}_n) as one of the regression coefficients.

None of the equations or regression methods described by GLOVER (1975) are directly applicable to solutions which contain weak polyelectrolytes such as humic substances. Suppose the i th organic solute is a weak acid of the general formula $A_i H_{ni}$, which can dissociate in solution to produce a variety of anions $A_i H_{ni-j}$ ($j = 1$ to ni). Despite the complexity of the multiple equilibria, the mass balance constraint requires that:

$$\sum_{j=0}^{ni} (A_i H_{ni-j}) = c_i \quad (5)$$

The concentration of H^+ derived from $A_i H_{ni}$ is given by:

$$(\text{H}^+)_i = \sum_{j=0}^{ni} j(A_i H_{ni-j}) \quad (6)$$

The total molality of ions and molecules (m_i) arising from $A_i H_{ni}$ is:

$$m_i = c_i + (\text{H}^+)_i \quad (7)$$

and in a mixture of z organic acids,

$$m_T = \sum_{i=1}^z m_i = C_T + (\text{H}^+) \quad (8)$$

Combining eqns (2), (3) and (8),

$$\theta = \frac{A}{\bar{M}_n} [W + \bar{M}_n(\text{H}^+)] + \frac{B}{\bar{M}_n^2} [W + \bar{M}_n(\text{H}^+)]^2 + \dots \quad (9)$$

Equation (9) is the fundamental equation which must be used to obtain \bar{M}_n for weak polyelectrolytes. This equation, which stems solely from the constraints of

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