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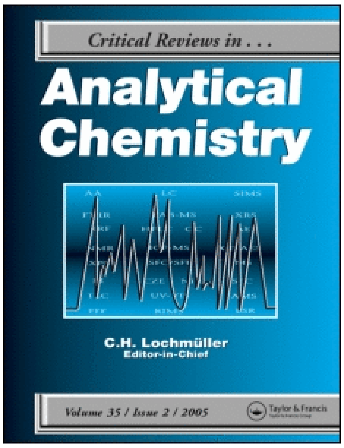

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Methods for Analysis of Aquatic Humic Substances

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ABSTRACT: Methods designed for analysis of aquatic humic substances are summarized and compared. A need to evaluate just the concentration of true humic substances, rather than summary parameters, characterizing dissolved organic substances such as COD, DOC, TOC, and others is stressed. Possible future trends in the studies of dissolved humic substances are touched on.

KEY WORDS: humic substances, analysis, natural waters.

I. INTRODUCTION

Among the various naturally occurring organic substances, humic substances (HS) are the most widespread. Humic substances form most of the organic component of soil, peat, lignite, natural waters, and their sediments. Up to 70% of the soil organic carbon and up to 90% of dissolved organic carbon may occur in the form of humic substances. Humic substances influence groundwater properties and the process of formation of fossil fuels; however, in concert they play a major role in the global carbon geochemical cycle. The global pool of humic matter is an important component in the formation of atmospheric carbon dioxide. The estimated amount of carbon in the atmosphere is 638 to 702 × 10^{15} g C, which is in the same range as carbon accumulated as soil humus (700 to 3000 × 10^{15} g C), peat humus (250 × 10^{15} g C), and aquatic humic substances (850 to 1700 × 10^{15} g C) (Klavins, 1998). Recently, it has been estimated that the global transport of organic carbon to oceans is about 0.4 to 0.9 × 10^{15} g C $yr.^{-1}$ (Hope et al., 1994).

The roles of humic substances is determined by their formation during the humification (decay) process of living matter. Humic substances form an intermediate phase in the transformation process of living matter (organic carbon reservoir) that continue in the organic carbon cycle or be deposited (as fossil materials). Humic substances are known to complex heavy metals and persistent organic xenobiotics. The interaction of humic substances with xenobiotics may modify the uptake and toxicity of these compounds by living organisms and affect the fate of pollutants in the environment. Considering these aspects the roles of humic substances in the environments may be regarded as important. Humus properties depend on its sources. Depending on the character of humification process, the greatest reservoirs of humic matter are distinguished as soil, peat, and aquatic humic substances (AHS).

The present level of knowledge indicates that humic substances form one of the most important groups of biomolecules. However, their properties are confusing from the viewpoint of monodisciplinary sciences — chemistry, biology, and Earth sciences. Humic substances can be regarded as one of the chemical anomalies. This attitude is evident even from the definition of humic substances (Stevenson, 1982). The most common definition is (Humic Substances, 1985): "Humic substances are a general category of naturally occurring, biogenic, heterogeneous organic substances that can generally be characterized as being yellow to black in color, of high molecular weight, and refractory. Humic substances can be divided in three fractions:

- Humin is the fraction of humic substances that is not soluble in water at any pH

- Humic acid (HA) is the fraction of humic substances that is not soluble in water under acidic conditions (below pH 2), but becomes soluble at greater pH
- Fulvic acid (FA) is the fraction of humic substances that is soluble under all pH conditions

The main forms of organic carbon (and humic substances) in aquatic environments are dissolved and particulate (colloidal) organic matter.

The division between dissolved organic matter (DOM) and particulate organic matter (POM) is generally made on the basis of whether it passes through a 0.45-µm filter (Figure 4). The generation of these forms proceeds during the genesis and transformation of organic matter and is linked with processes such as decomposition of living matter, association of organic molecules with inorganic particulate matter and aggregation of smaller particles.

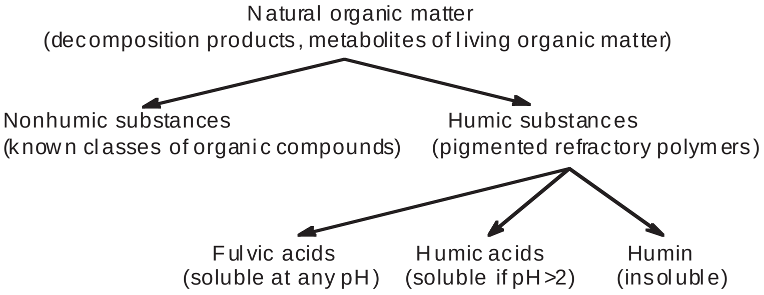


FIGURE 1. Classification of humic substances.

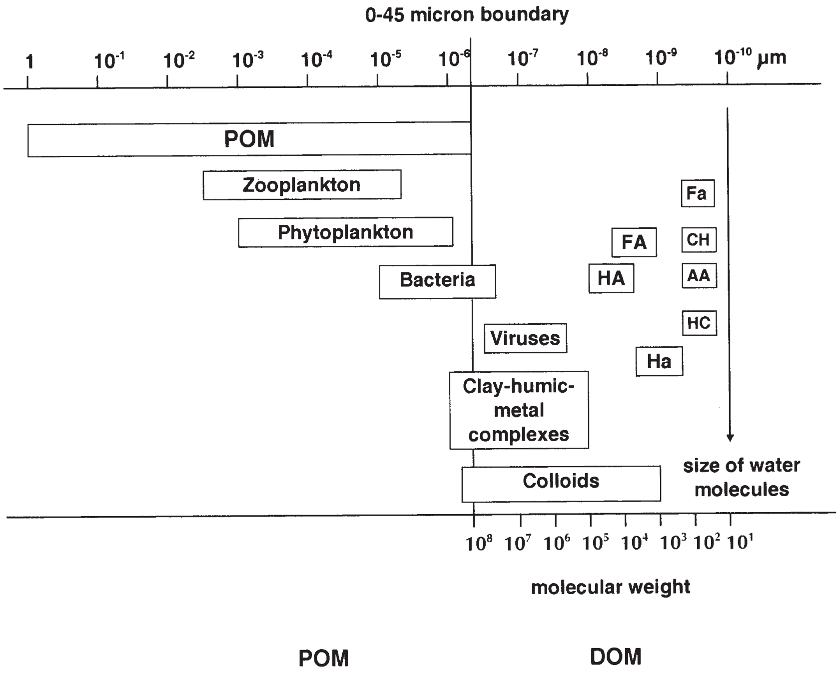


FIGURE 2. Size range of particulate and dissolved organic matter and carbon compounds in natural waters. AA, amino acids; CH, carbohydrates; DOM, dissolved organic matter; POM, particulate organic matter; Fa, fatty acids; Ha, hydrophilic acids; HC, hydrocarbons. (Adapted from Thurman, 1985a.)

Aquatic humic substances originate mostly from three processes:

1. Allochthonous aquatic humic substances derived from terrestrial organic matter
2. Autochthonous aquatic humic substances derived from *in situ* biological production
3. Humic substances derived from agricultural, domestic, and industrial activities (Degens, 1982)

Contemporary structural models considers humic substances as polifunctional macromolecules (Figure 3).

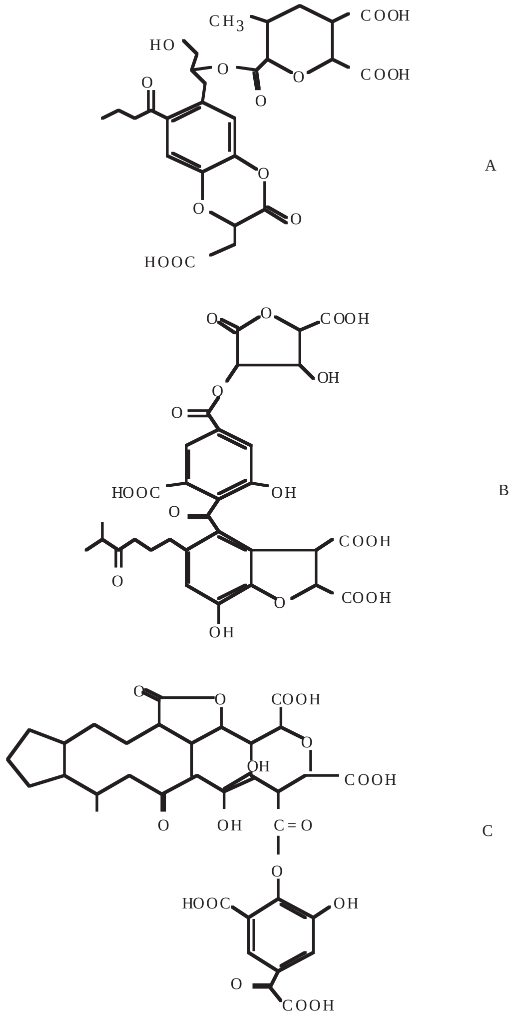


FIGURE 3. Three structural models (A-C) of fulvic acid molecules from the Suwannee River (Leenheer et al., 1995).

The use of a wide variety of analytical methods, including degradative and nondegradative approaches, to characterize Suwanee River fulvic acids, led to a model proposed by Leenheer et al. (1989, 1995b). This model shows the most possible average structural formulas (Figure 3) and also considers the possible precursors in the humification process. The aromatic and aliphatic structures in the proposed formulas indicate hypothetical residues of parent compounds, such as lignins, tannins, carbohydrates, and lipids, which are incorporated into the structure of humic substances. The multitude of possible links of these structural units and their biodegradation degree can explain the large diversity of structural formulas for the possible humus structure. The proposed structures at best can explain the actually found physical and chemical properties of humic substances isolated from the given environments, but the coexistence of HS as complexes with mineral particles is neglected.

Despite the great role of aquatic humic substances in the environment, many aspects of their functions and properties are still unclear, including the analytical determination of AHS.

II. PRINCIPLES FOR ANALYSIS OF HUMIC SUBSTANCES

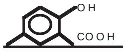
A commonly required water characteristic is the concentration of aquatic humic substances. Despite comparatively high concentrations in natural waters and yet higher concentrations of humic substances in waste waters, their analytical determination is, however, problematic. This is surprising, regarding the importance of humic substances in geochemical cycles of elements and metals, in other xenobiotic speciation, and as indicators of biological processes in waterbodies. In the early period of humus investigations, AHS has been characterized by spectrophotometrical determination of water color, chemical oxygen demand (COD), and biological oxygen demand (BOD). Improved instrumental techniques and organic carbon analysers allows the evaluation of the concentrations of humic substances as organic carbon content (TOC, total organic carbon, DOC, dissolved organic carbon, POC, particulate organic carbon) in waters. Contemporary recommended methods are based on such approaches.

However, all of these analytical methods provide only indirect measures of humus concentration in water.

The problems of analytical determination of concentrations of aquatic humic substances in natural waters are closely related to their properties and the lack of strict boundaries between humic and nonhumic substances. The fundamental properties that may be used for analytical determination of AHS are (Table 1):

TABLE 1

Major Functional Groups and Building Blocks of Aquatic Humic and Associated Substances

Loosely bound substances	
Metal ions: (Ca^{+2}, Fe^{+3}, Na^{+} etc.), adsorbed inorganic particulate materials, low molecular weight organic substances	
Functional groups	
–COOH	Carboxyl groups
–OH	Hydroxylgroups
>C=O	Carbonylgroups
–O–	Ether groups
$-OCH_3$	Methoxylgroups
$-CH_3$	Methylgroups
–C(N	Cyanogroups
–SH, $-SO_3H$	Sulphur containing groups
$-NH_2$, >N–	Amino groups
Major structures of aquatic humic substances	
$-CH_2-CH_2-$, $-CH = CH-$, $-CH_2\ -NH_2$	Aliphatic structures
	Phenoles, phenolecarboxylic acids
	Benzenecarboxylic acids
	Aromatic and polyaromatic structures
	Quinones
	Oxygen containing heterocycles
	Nitrogen containing heterocycles
	Sulphur containing heterocycles
Pentoses and hexoses	Carbohydrates

- Organic carbon as a building element of humic matter (they are organic substances)
- Their acidic character (humic substances are polycationites)
- Ability of humic substances to form insoluble salts with some cations
- The presence of chromophores and fluorophores in their structure
- Presence of hydrophobic structural units in their structure

The most common method for the analysis of humic substances in surface waters is the determination of organic carbon (COD, BOD, TOC, DOC, POC). This is a very powerful and widely used method to study organic matter in natural waters (Datsko, 1959; Degens, 1982; Coel et al., 1984 Agren, 1991), but it neglects the differences between low molecular organic substances and humic substances.

III. CONTEMPORARY ANALYTICAL METHODS OF HUMIC SUBSTANCES

The sorption of humic substances in the UV-Vis spectral region allows this characteristic and water color to be used for their analytical determination. Water color has been determined for many years and hence may be used for trend analysis of humus concentrations in surface waters. Associated analytical problems are sensitivity and interference, particularly from iron and polyphenolic substances (lignin). A more powerful method is the fluorimetric method, which allows the determination of lignin sulfonates and humic substances simultaneously in waters. The fluorimetric method has been used to measure fulvic acids in seawater, after preconcentration on DEAE cellulose and subsequent desorption and final fluorimetric determination (Ivanova et al., 1986). Also, simultaneous absorbance measurements at two wavelengths can be used to determine humic acid and iron concentrations in natural waters (Carpenter and Smith, 1984). This method requires sample volumes of less than 15 ml and is sensitive enough for direct application to most natural waters (limit of detection is 0.01 mg/l for humic acid).

To isolate humic matter from a pool of organic and inorganic substances and to separate humic acids from fulvic acids, chromatographic methods are most successful. Their application is based on the use of polymeric anionites (containing tertiary amino groups or diethylaminoethylgroups) or on the use of hydrophobic sorbents (styrene-divinylbenzene or acrylic acid copolymers). Functional groups in these sorbents may interact with carboxylgroups or hydrophobic structural units in humic substances, allowing their selective isolation from aquatic environments and their separation from accompanying substances. An example of this method is based on the isolation of humic substances from waters by their chromatographic sorption on DEAE cellulose, elution precipitation of humic acids at pH 2, and spectrophotometric determination of both humic and fulvic acids (Krasiukov and Lapin, 1988). Nonionic macroreticular sorbents (Amberlite XAD resins) have been proposed for analysis of humic substances (Sorouradin et al., 1993). Analytical determination of humic substances has been achieved by sorption and desorption with subsequent spectrophotometric analysis. To achieve quantitative desorption, elution of polymers with sodium dodecyl sulfate solution in strongly alkaline media was used.

Methods based on use of DEAE or XAD sorbents for analytical determination of AHS are now standard methods (Standard Methods, 1992). Aquatic humic substances are concentrated by column chromatography on DEAE cellulose or XAD resin, then desorbed and measured as dissolved organic carbon concentrations (Figure 4). The estimated detection limit is 1.1 mg/l for a 50-ml sample. A recommended analytical method based on the use of sorbents is included in the appendix.

Thin layer chromatography on silica gel plates has also been used successfully to analyze humic substances in surface waters after their pre-concentration on Amberlite XAD 4 column (Katalan-Macan et al., 1992).

Later developed method is based on the oxidation of humic acid with hydrogen peroxide in the presence of formaldehyde in alkaline solution (Kitano et al., 1994). This method is sensitive and selective (detection limit 50 ppb and relative standard deviation for five measurements 1.8%) for the determination of HA in natural waters with little interference from substances common in

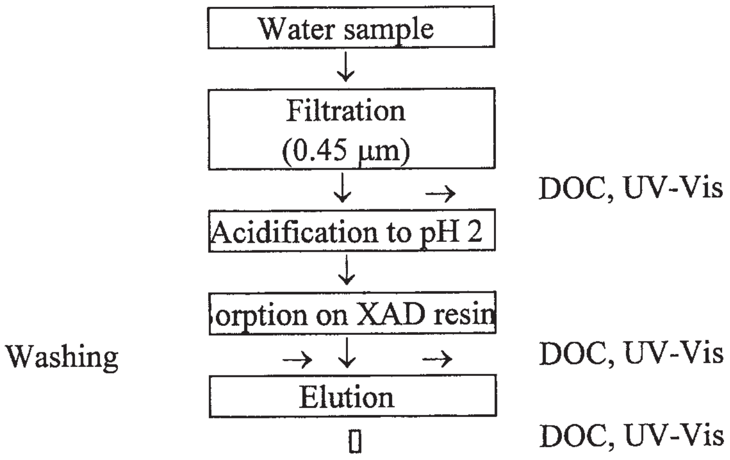


FIGURE 4. Sequence of steps for analysis of aquatic humic substances.

natural waters. The method can also be realized continuously. Humic acids in natural waters have been analysed also by chemiluminiscence produced by the reaction between humic acid and NaBrO in alkaline solution (Hu et al., 1994).

Enzyme-linked immunoassay for the determination of humic acids in natural waters has been proposed (Ulrich et al., 1993) using humic acid coupled to snail *Helix pomatia* hemocyanin and a humic acid horseradish peroxidase conjugate as a tracer. This detection method has achieved high sensitivity (detection limit was estimated to be 0.5 μg/l) and allowed to elucidate structural differences among humic substances of different origin.

Because suspended particles in surface waters are covered by humic substances, it is important determine their amount. This can be achieved by selective desorption of humic substances from particulate matter (Hiraide et al., 1988). It has been found that the major part of metals on or in suspended particles exist as humic complexes (Hiraide, 1992).

IV. CONCLUSIONS

Unless substantial efforts to develop analytical methods for determination of humic substances in surface waters, the problem still is open and versatile, efficient, and unified methods are needed. As the most promising approaches can be regarded use of chromatographic techniques, as well as different approaches based on preconcentraiton of humic substances.

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