

THE NEW LOOK AND NANOTUBE CONCEPT OF HUMIC ACIDS¹⁾

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Summary

The micellar and supramolecular self assemblage theories recently introduced in humic acid chemistry have been received with mixed reactions. However, when confirmed with adequate scientific evidence from authentic soil research, they may constitute substantial advances from the traditional concepts. The consequent change in perception on humic acids is called by Tan the *New Look of Humic Acids* in his new 2010 book on Principles of Soil Chemistry. Disagreements on humic acids are in fact not new, but have developed early since the day humic acids were isolated for the first time by Berzelius in 1830. Although many soil scientists were siding with Berzelius, considering humic acids naturally occurring substances that could be extracted from soil organic matter, a great number of scientists were also found at that time voicing strong opposition to Berzelius' proposition above. Since no satisfactory settlements for both parties can be reached over the years, the controversy is unfortunately carried over to the next century. The International Humic Substances Society, founded in 1981 by scientists of the U.S. Geological Survey, and the school of chemistry continue the idea of humic acids being artifacts, by calling them also *operational compounds* or compounds produced during extractions. A few years later, the "operational" concept was drafted into a formal definition by the society above, considered worldwide the proper authority in humic matters. This has only deepened the dispute, which was acknowledged by Hayes and Clapp in their Soil Science 2001 journal article. A more detailed account is provided by the author in several of his publications and books, but which seldom has attracted any attention. The new advances – the micellar and self assemblage theories – may well prove to be, in essence, a serious and final blow to the operational concept. In line of the new grand ideas, the present author is perhaps adding now more confusion by presenting his opinion, true or false, of the *nanotube* concept of humic molecules, suggested from results of his studies and analyses by scanning electron microscopy.

The Conventional Concepts

It is not the intention to address here in detail the traditional concepts of humic acids. The author also uses the term *Humic Acids* for quick reference rather than *Humic Substances* for identification of the materials under question. For a detailed coverage on the traditional issues of humic matter, reference is made to Tan (2003), who has written a detailed account on the principles and controversies, presented systematically, to the point, and easy to read or understand by most people. Another book, favored by others, is written by Stevenson (1994), which in the author's biased opinion is difficult to read and often confusing for the general audience, due perhaps to using interchangeably the concepts of soil organic matter, humus, and humic acids. Perhaps a third reference by Orlov (1985) – edited in English by Tan – is worth mentioning, which may represent the view and vision of scientists in Russia and Eastern Europe, long considered the birth place of humic acid science.

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The traditional concept defines humic compounds as amorphous polymeric colloidal organic substances with yellow to brown-black color of high molecular weights (Felbeck, 1965; Kononova, 1966; Schnitzer and Khan, 1972; Flaig, 1975; Stevenson, 1994; Tan, 2003; Ghabbour and Davies, 2003). The disagreement is in questions on the nature of the compounds being naturally formed in the environment or being synthetically produced during extraction, a dispute that has started the day after Berzelius has isolated humic acid from soil organic matter in 1830. Since no agreement could be reached during the years, the controversy was unfortunately carried over to the next century. The ensuing conditions were noted to become less conducive for conducting humic acid research when reviewers of grant applications and journal articles alike were reluctant in approving research on allegedly *fake* compounds. This has forced Schnitzer, a distinguished humic acid scientist, to take action for a change in attitude from skepticism into optimism at the 1982 International Congress of Soil Science in New Delhi, India (Schnitzer, 1982). The title of his article – *Quo Vadis Organic Matter Research?* – reflected the prevailing mood of despair from many soil scientists, and basically was a plea for better reasoning in dealing with disagreements on humic acids issues. The International Humic Substances Society (IHSS), founded in 1981 by scientists of the U.S. Geological Survey, Denver, Colorado, chose to embrace the artifact concept (Aiken et al., 1985; Hayes and Clapp, 2001). At its first meetings held in 1985 at Estes Park, Colorado, the IHSS issued a definition considering humic substances *operational compounds* (Aiken et al., 1985). It has caught many soil scientists by surprise and has aggravated the dispute even more. Since as an association, the IHSS is considered by the general public the authority in humic matter, it is no wonder that these terms have since then been copied as the magic words all over the world for identifying humic substances. This concept was adopted as the basis for humic acid research by the school of chemistry (Gaffney et al., 1996). Fortunately, the widespread occurrence of humic acids and their abundance in terrestrial and aquatic ecosystems, supported by their overwhelming importance in the environment and industry together with compelling evidence from the few researches still going on, have forced the IHSS to review their operational concept by the turn of the century into the new millennium (Hayes and Clapp, 2001). It was followed several years later by the IHSS issuing an official statement in its web site that “*humic substances are major components of natural organic matter (NOM) in soil and water as well as in geological organic deposits*” (<http://ihss.gatech.edu/ihss2/whatarehs.html>, accessed June 9, 2009). As it reads now, the definition is in essence a reversal from their operational concept, and as such can also be deemed as an advancement in humic acid chemistry.

The Micellar Concept

The micellar model of humic acids was introduced by Wershaw (1986; 1999) and von Wandruska in 1998. A micelle is by definition a submicroscopic aggregate of amphiphilic molecules in a colloidal system with their hydrophilic ends, normally polar, arranging themselves toward the surrounding solution. This head is composed of ions (charged particles), nonionic particles (noncharged) or zwitterions. On the other hand, the hydrophobic part, usually visualized as a tail of the hydrophilic part, is then arranging itself inside, in the center of, the aggregate, away from the outer solution. This tail is nonpolar and contains aliphatic and aromatic compounds. The process of formation is known as *micellization*, and the micelle formed can assume different shapes and sizes from single spheres to cylinders and bilayers. The bilayers are called *membranes*. This concept is

applied in the construction of humic molecule structures by Wershaw (1986) and von Wandruska (1998). Wershaw claims that humic particles form micelles through spontaneous aggregation in solution, but on mineral surfaces they can form membrane-like structures. The hydrophilic parts, arranged on the outside, consist of polar parts of carboxylic acids, whereas the hydrophobic tail, the nonpolar part, in the inner sides, is composed of mostly unaltered parts of plant polymers. Three types of humic molecules, different in composition and behavior, are indicated to be involved and Wershaw also believes that three types of bonding mechanisms are responsible for holding them together in the micelle. They are hydrogen bonding, π -bonding or in Wershaw's words "stacking of planar- π -donor-planar- π -acceptor groups to form a complex," and charge transfer complexation. Because they are all weak bonds, the micelle is expected to disaggregate or disperse easily into several smaller aggregates or eventually into its different humic molecule components. Indications of such a dispersion have been reported by Piccolo et al. (1996), who noted that mono-, di- and tri-carboxylic acids were capable of dispersing the larger humic micelle into smaller segments as a function of concentration of added organic acid and pH. The important implication following this observation is that the broken segments are capable to reform micelles, and micellization and disaggregation may then continue on and on. This accounts for the common complaints for humic acids, yielding upon extraction a confusing array of humic particles, different in sizes and shapes. In nature, such a self assembling process is more common than many people would have believed. It also takes place on a macro scale in the formation of soil structures from sand, silt, clay and organic soil particles. Granular and crumb structures – the best soil structures for agriculture – are also created by aggregation of the four soil constituents stated above through a self assemblage process. The soil structure formed tends to be destroyed by continuous soil cultivation, but when after cultivation the soil is left alone in nature, the soil particles are noted to "regroup" spontaneously reforming some type of soil structure. The common bonds, playing important roles, in the aggregation process are water- and metal-bridging. Liming helps to promote formation of Ca-bridges between the soil particles, favoring formation of the crumb and granular types of soil structure.

The Supramolecular Concept

The supramolecular theory, fundamental in the past in probing the structure of protein, is introduced by Piccolo (2002) in humic acid chemistry. Failure to find a molecular structure for humic substances acceptable by all is perhaps one reason for his attempts to turn humic acid science from a polymer into a supramolecular assembly concept. The very wide range in molecular weights from 500 to 1,360,000 Da, a variation Piccolo believes too large for any of the values to be the characteristic mass of humic substances, is the reason for him to abandon the idea of humic compounds being polymers. In his opinion, the literature data try to convey that humic substances are not high molecular weight polymers, but are groups of small heterogeneous compounds of biological origin, he calls supramolecular associations capable of self assembly. As expected, skepticism is soon voiced, and interesting as the new theory sounds, many are reluctant to summarily embrace Piccolo's new idea awaiting more evidence from authentic soil research (Sutton and Sposito, 2005). As in the micellar theory, this supramolecular association is also capable to dissociate and reassemble spontaneously, because of the similar weak noncovalent hydrophobic and hydrogen bonds holding it together. However, Piccolo's inclusion of weak dispersive forces makes

the issue of self-assembly very confusing, since dispersion is a process of dissolution instead of being a force of attraction. As stated before, water and metal bridging are perhaps the more common forces of attraction, facilitating self-assembly more easily than expected. Water bridges are in fact hydrogen bondings (Tan, 2010). In view of the above, it is to be expected for the supramolecular associations to take also the form of micelles and membranes.

The Nanotube Concept

With the miraculously rapid development of the electron microscope, the science of structural chemistry of humic substances has advanced beyond expectations in the last part of the twentieth century (Tan, 2005; 2010). Flaig and Beutelspacher (1951) are perhaps the first – followed thirteen years later by Visser (1964) – who have discovered by transmission electron microscopy that humic acids are spherical or globular particles of the order of 10 to 15 nm (nanometers) in diameter. Schnitzer and coworkers confirm later in the 1980s the size of 9 to 12 nm, but indicate that only at high concentration and low pH would humic acids behave as rigid spheroids, but otherwise tend to be flexible linear colloids. It was in fact Tan (2003; 2005), who has described humic acids to exhibit a woven network of elongated fibers, coalescing into perforated sheet structures. In view of the supramolecular self-assembly concept introduced by Piccolo, questions arise now whether the past results above have to be reviewed and the interpretation revised according to modern standards of today. As the name implies, a supramolecular self-assembly is an association of molecules, arranging themselves into shapes of structures without guidance or laboratory manipulation. The molecules forming assemblies, normally in the range of nanometers ($1 \text{ nm} = 1 \times 10^{-9} \text{ m}$) to micrometers ($1 \mu\text{m} = 1 \times 10^{-6} \text{ m}$), are called nanoparticles. They can assume different forms of spheres, tubes or sheets. A well-known spherical nanoparticle is *Fullerene*, a spherical molecule composed of carbon atoms. The hexagonal pattern of a soccer ball surface is how a fullerene looks like. The name nanotubes implies that the molecules are tubular, but – for reasons unknown to the present author – are also sheet-like in form. They are also carbon molecules within the fullerene family. The discovery above of humic acid spheres in the nanometer sizes has prompted the present author to restudy and reevaluate the electron micrographs from his and other people's research. To his surprise fulvic acid (Figure 1A), isolated from a Cecil soil (Hapludults) in Georgia, USA, exhibits a network structure closely resembling that of a nanotube (Figure 1B). It also exhibits the features of a self-assembly DNA nanogrid (Figure 1C). The fulvic acid was extracted with established standard methods and prepared for scanning electron microscopy by a rapid liquid nitrogen method developed by the author (Tan, 1985; 2005). It now appears that what was identified in the past as perforated sheet structures are most probably self-assemblages of membranes, which at higher magnification exhibit structures as shown in Figure 1A. The membranes have mistakenly been identified for being formed by coalescence of humic fiber-like structures, but a careful restudy suggests now the membranes to self-assemble side by side with the humic fibers (Figure 2A). They are “hanging” and supported “in space” by the fiber-like materials. The fiber-like materials are in fact most likely to be carbon nanotubes. Carbon nanotubes are defined as allotropes of carbon, usually cylindrical in structure as stated earlier. They are single- or double-walled particles with sizes of a few nanometers (in diam.), but they can be 18 cm long (or more). Figure 2B is a micrograph of fulvic acid from blackwater of the Satilla River in South Georgia, USA. Sampling and isolation were conducted by the present

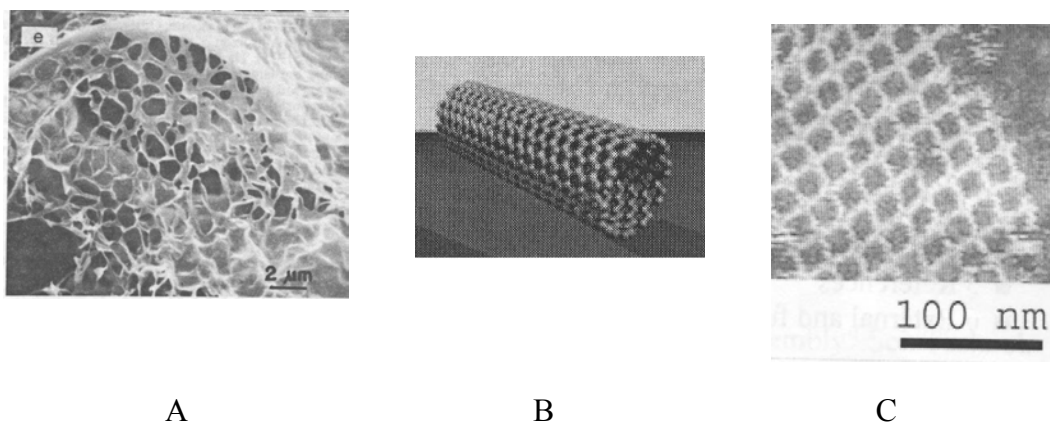


Figure 1: (A) Scanning electron micrograph of Fulvic Acid from a Cecil soil (Hapludults) showing features of carbon nanotube structures (from present author's file); (B) Carbon nanotube, source: http://en.wikipedia.org/wiki/Carbon_nanotube, assessed 10/28/2010; and © self assembly of a DNA nanogrid structure (Strong, 2004).

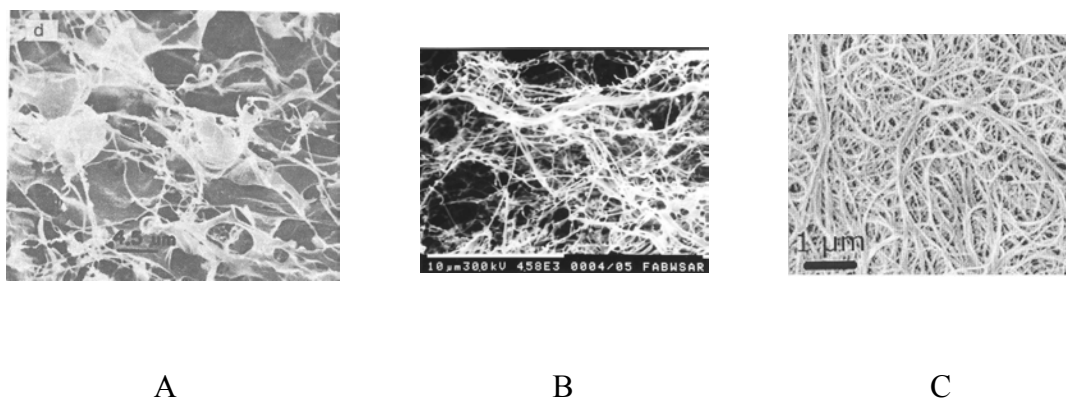


Figure 2: (A) Scanning electron micrograph of Fulvic Acid from a Cecil soil (Hapludults) showing membranes attached to nanotube cylinders, rods or fibers; (B) Scanning electron micrograph of fulvic acid from blackwater of the Satilla River, Georgia, USA showing bundles of carbon nanotubes; (C) Carbon nanotube bundles.

author using established standard procedures for aquatic humic matter (Tan, 2005). At high concentration, the long cylindrical fiber-like fulvic acid molecules are attracted by Van der Waals forces (Figure 2B), assuming bundles with structural features, resembling closely a scanning electron micrograph image of carbon nanotube bundles as shown in Figure 2C. The latter is published in the web site (http://en.wikipedia.org/wiki/Carbon_nanotube, assessed 10/28/2010) from an unknown source. It is expected that critics may claim again that redissolving freeze-dried humic acid and liquid-N preparation for electron microscopy have yielded artifacts. However, would the results above then be repeated again and similar artifacts produced from different batches of humic solutions, extracted from different soils and different sources of black water? Another coincidence worth mentioning is perhaps the findings of scientists of the University of Arkansas. Carbon nanotubes have recently been used in a variety of applications from the production of glue, handle bars, forks, solar cells to batteries, etc. In 2009, scientists from the University of Arkansas have treated tomato seeds with carbon nanotubes and found that they have affected beneficially germination and growth of tomato seedlings (Khodakovskaya et al., 2009), making U.S. commercial companies abuzz in converting carbon nanotubes into superfertilizers. The effects on tomatoes are conspicuously similar to the effects of humic acids on germination of seeds and growth of corn (maize) seedlings reported by Tan and Nopamornbodi (1979) and Tan (2010), suggesting nanotube particles to be perhaps related to the components of the supramolecular association of humic acids.

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