

THE ISSUE OF MOLECULAR WEIGHT IN HUMIC ACID CHEMISTRY

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SUMMARY

Since the day humic substances have been isolated by Berzelius, they are commonly considered polymeric in nature. The *biopolymer theory* became so popular that the concept was embraced by most scientists for more than 1.5 centuries. It has provided a suitable hypothesis that is among other things not running afoul of with the elemental composition of the humic compounds, which shows relatively consistent ranges, especially in their carbon and nitrogen contents. Though the basics of polymer chemistry suggest that the molecular weight of polymers equals the sum of molecular weights of the monomer components minus the moles of water lost in the condensation reaction, many people claim the concept to fail in providing a commonly accepted molecular weight for humic substances. The range, reported in the literature, shows values to vary from a few hundreds to >1,000,000. A range of 500 Da to 1,360,000 Da is also cited by Piccolo and coworkers, a range too large for any of the values to be the characteristic mass of humic substances. This is one reason for Piccolo to introduce an alternative theory, the supramolecular assembly concept. Aside from the interchangeable use of dalton and dimensionless units, addressed presently as creating more confusion, the new hypothesis has created several reactions of *wait-and-see* awaiting future developments. This new concept tries to show humic substances to be composed of groups of small heterogeneous biological compounds, called *supramolecular associations*, capable of self assembly. The idea originates from results of low pressure (LPSEC) and especially high pressure size exclusion chromatography (HPSEC), methods falling in the category of *gel chromatography*. Considered a relatively effective method for the selective separation of humic substances according to molecular sizes, the size exclusion fractionation depends on the type of gel substance used and a large variety of gels are available. Therefore, Piccolo's supramolecular humic assembly — though providing a fascinating model — is derived from the chemical behavior of artificial or operational humic fractions created by HPSEC. The capacity of self assembly of broken humic segments into new humic associations has to be established and confirmed properly by separate analyses. As admitted by Piccolo, no immediate solution is also in sight on the issue of molecular weight.

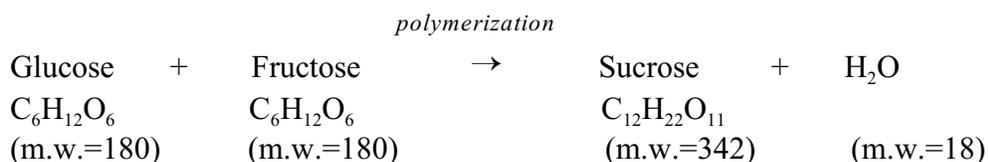
THE BIOPOLYMER CONCEPT AND MOLECULAR WEIGHT

The molecular weight of humic substances is perhaps one of the most controversial issues and the prime reason for many scientists criticizing the well-established polymer concept on humic substances. Since the day humic acids were isolated by Berzelius in 1839, most people believe them to be polymeric in nature. By definition a *polymer* is a large compound composed of a large number of repeating smaller structural units, called *monomers*. The term polymer is derived from the Greek words *poly* (= many) and *meros* (= parts). Therefore, a lignin polymer is built of several or a great

number of monomeric lignin units. Another example is polysaccharide, which is made up of repeating monosaccharides bonded together by *glucosidic bonds* and so are proteins which are formed by amino acids connected together by *peptide bonds*. When two monomers are connected together, a dimer is formed, and when three monomers are linked up, the compound is a trimer and so on. For more details and specific illustrations of the polymerization processes reference is made to Tan (2011; 2003).

Polymers of lignin alone, protein or polysaccharides only do not make up humic compounds. According to the lignoprotein theory, it takes both lignin and protein, or all three components as one chemical unit, serving as the smallest repeating unit that will build a humic compound. The humic molecule formed is called a *biopolymer*, because the monomeric units of lignin, protein and carbohydrate are derived from biological tissues. In some similar fashion polysaccharides in combination with protein units, serving together as the smallest chemical units repeating themselves in a molecule, will create aquatic humic substances. In crystal chemistry, such repeating units are called *unit cells*. Unit cells are applied in building up the frame work of clay minerals in soils (Tan, 2011). Though Kononova (1961) is cited by Piccolo et al. (2003) to have introduced the polymer concept of humic matter, such a polymeric view has in fact been known for some time. It is allegedly introduced as early as 1835 by Jöns Jacob Berzelius, after his historical analyses in extracting humic substances. Berzelius' original definition may, of course, differ from the modern day definition of polymers (Berzelius, 1839). In more recent time, Waksman (1932; 1938) has referred in 1932 humic matter — soil humus was the term used at that time — as large molecular associations of lignin and protein compounds. Perhaps, it is Flaig (1975) in Germany, who has truly defined humic matter to be organic polymers from lignin degradation products in his efforts promoting his well-known lignoprotein theory. Other prominent scientists from the German school, influential in supporting the existence of humic matter in the form of chemical polymers are Scharpenseel (1966) and Beutelspacher (Flaig and Beutelspacher, 1951). Haider and co-workers have also suggested polymerization of phenols into humic substances by autoxidation processes (Haider et al., 1975). In Canada, it is Schnitzer (1976) for suggesting humic matter to be benzene carboxylic acid polymers, whereas in the United States Stevenson (1994) has written in his book about his well-known phenol or quinone dimer theory of humic substances. Today, Swift (1999) is a strong supporter of this polymer concept, whereas Piccolo (2002) has voiced strong reservations in favor of his supramolecular theory.

The biopolymer concept above — advancing the idea of condensing small monomers into large biopolymers of humic substances — became so popular that the concept was embraced by most scientists for more than 1.5 centuries. It seems to provide a workable hypothesis that among other things is not running afoul of with the carbon and nitrogen composition of humic substances. The latter are always reported within relatively consistent ranges. However, a far more important issue is the basics in polymer chemistry related to molecular weights. These principles indicate polymers to acquire molecular weights equaling the sum of molecular weights of the participating monomers, minus the moles of H₂O lost during the condensation reactions (Holleman, 1946). The formation of sucrose, a disaccharide, by condensation polymerization of the monomers, glucose and fructose, serves as an illustration:



However, in the case of polymerization of a huge number of different varieties of monomers — producing *heteropolymers* — the molecular weight can become indeed very confusing, if not unknown. This may perhaps account for the traditional concern in unpredictability and/or the wide variations noted in molecular weights of humic acids. As indicated above, the latter are used by a group of scientists for challenging the applicability of the polymer concept in humic acid chemistry.

THE ISSUE OF MOLECULAR WEIGHT

Molecular weights of humic substances are noted to vary widely from a few thousand to 1,000,000 or more. Molecular weight values of only 1,000 to 2,000 have been reported by Schnitzer and Khan (1972). The very wide range in values has been noted to be associated with the method of determination. Low molecular weight values of 1,000 to 20,000 are usually detected by electron microscopy, whereas viscosimetry and osmometry have yielded high values for humic acids from 26,000 to 36,000. Ultracentrifugation, including sedimentation techniques, gives even higher molecular weight values of 30,000 to 230,000 (Orlov, 1985; Stevenson, 1994). On the other hand, gel chromatography using gels with a series of exclusion limits has been reported to yield a very wide range of molecular weights for soil humic acid from 2,600 to 1,360,000 (Cameron et al, 1972). However, such a gel filtration technique yields lower molecular weight values in the order of 700 to 200,000 for lake and marine sediment's humic acids (Ishiwatari, 1975). The lowest molecular weight values — ranging from 500 to 2,000 for aquatic fulvic acids and from 1,000 to 10,000 for aquatic humic acids — have been detected by analyses using small angle x-ray scattering (Thurman et al., 1982).

In basic polymer chemistry such a wide variation in molecular weights is not uncommon. The range can run from the low hundreds for molecular weights of dimers to the millions for huge polymers. Some believe that the range from low to high molecular weight values may look like a continuum (Stevenson, 1994; Piccolo et al., 2003). Citing values reported in the literature from 500 Da to 1,360,000 Da, Piccolo et al. (2003) indicate that the range is too large for any of those values to be the characteristic mass of humic substances. Though the lack of a commonly agreeable molecular weight is perhaps already a big issue, nonetheless the use of dalton units by Piccolo and coworkers seems to add more noise to the problem. In the discussion above the dimensionless unit was used for molecular weights, and the interchangeable use of *dimensionless* and *dalton units*, as applied by Piccolo and coworkers, has only raised new questions. Though the use of dalton units (Da) seems to be preferred by several “top” scientists for molecular weights, they are very confusing for a great number of “ordinary” scientists. Many chemists also consider erroneously the dalton equivalent with *g/mol*, though the definition says it to be equivalent with *12 g/mol* (or $1/12 \text{ mol} = 1 \text{ gram}$), as can be noticed from the formula below:

$$1 \text{ Da} = 1 \text{ amu} = 1/12 \text{ of the mass of 1 atom of } ^{12}\text{C} = 1.66 \times 10^{-24} \text{ g}$$

in which amu = atomic mass unit (Tan, 2011). Molecular weight values, ranging from 500 Da to 1,360,000 Da, as cited by Piccolo and coworkers, will then assume the following values, respectively:

$$500 \text{ Da} = 5 \times 10^2 \times 1.66 \times 10^{-24} \text{ g} = 8.3 \times 10^{-22} \text{ g}$$

$$1.36 \text{ million Da} = 1.36 \times 10^6 \times 1.66 \times 10^{-24} \text{ g} = 2.3 \times 10^{-18} \text{ g}$$

These are practically inconceivably small values for molecular weights of fulvic and humic acids, raising the question, whether the Da units are perhaps unsuitable for practical use in molecular weights. As indicated earlier, though scientifically correct using *daltons* for molecular masses, it is more common to use the dimensionless weight units in issues of molecular weights. In soil physics, weight is considered a force and many scientists, therefore, object using weight units for expressing the amount of mass. However, according to the *second law of Newton*, the molecular mass is by definition:

$$F = ma \quad \text{or} \quad m = F/a$$

where F = force acting upon a body of matter, m = mass contained in the body, and a = acceleration. On the surface of the earth, it is commonly accepted that a = 1, hence m = F. Therefore, force (= weight) = mass, making it perfectly sound on earth to use weights for molecular masses. The choice depends on the preference of the reader and on the purpose of study, but nonetheless it is more practical to express molecular weights in weight units, and preferably in the form of dimensionless units as stated at the start of this section. The dimensionless units for molecular weights as used above, are then more *user-friendly*, since they are more easily understandable for a large number of people than Da, amu or $\times 1.66 \times 10^{-24}$. Nonetheless, the dimensionless figures are indeed also showing wide variations of molecular weight values for the humic substances.

SUPRAMOLECULAR CHEMISTRY AND MOLECULAR WEIGHT

Failure to find a commonly acceptable molecular weight for humic substances is perhaps one reason for attempts to turn humic acid science from the biopolymer into a micellar concept by Wershaw (1986; 1999), and the supramolecular self assembly concept by Piccolo (2001; 2002; 2003). Several years later the nanotube and nanotube membrane concepts are introduced by Tan (2011a and b). Wershaw's model shows aggregation of broken pieces of biopolymers into well-ordered structures of micelles, whereas Piccolo's model suggests aggregation, called *self-assembly*, into irregular or random assemblies. Therefore, the two theories are closely related with each other, but to keep the story short, the supramolecular idea will be used in this paper.

Supramolecular chemistry is a new field in chemistry which became of importance in the second part of the twentieth century, because of its contribution in the studies of the structures of protein and DNA. The principles based on weak intermolecular forces were discovered in 1873 by *Johannes Diderik van der Waals*. However, this new concept was propelled to its current status after

D. J. Cram, Jean-Marie Lehn and C. J. Pedersen have been awarded the Nobel Prize in 1987 for their contribution in this area (Lehn, 1995; 1993). Supramolecular chemistry considers chemical systems, called *supramolecular assemblies*, as multicomponent systems, assembled from atoms, ions and/or discrete numbers of assembled molecular units. Such assemblies are supposed to form naturally without guidance or laboratory manipulations, hence they are usually also known as molecular *self assemblies* held together by weak forces, such as van der Waals, hydrogen and hydrophobic bonds, pi-pi interaction and electrostatic forces. These forces are considerably weaker than covalent bonding, hence will result in formation of less stable substances that are to break easily apart. The product formed initially is the most stable thermodynamic compound that can be attained by self assembly. A “wrong” product is then easily disassembled and the components rapidly reassembled into the “proper” product with the most stable thermodynamic arrangement, all by themselves.

The supramolecular chemistry concept above is applied by Piccolo and coworkers in humic acid chemistry and has been received with mixed reactions. Several people remain skeptical and prefer to wait for more data of support from continuing research (Sutton and Sposito, 2005; Steinberg, 2003; Hayes and Clapp, 2001). Piccolo’s group arrives at the idea from results of their analyses using low pressure (LPSEC) and especially high pressure size exclusion chromatography (HPSEC). The data are claimed to provide indications of humic substances being molecular associations — called by an impressive name of supramolecular assemblies — rather than being polymeric substances. The methods above, falling in the category of *gel chromatography*, are considered relatively effective procedures for the selective separation of humic substances according to molecular sizes. However, the molecular size fractionation depends on the type of gel substance used and a large variety of gels are available for use in such analyses. For the types of gels and principles of chromatography of humic substances, reference is made to Tan (2011). The most widely used gel materials are the cross-linked polymers of polysaccharides, polystyrene, and polyamides, distributed under the names of *Sephadex*, *Biogel*, and *Cellogel* or the like. Sephadex has been mentioned by Piccolo et al. (2003) as the gel material in their LPSEC and HPSEC analyses. This kind of gel comes in many different types of size exclusion limits, just to name a few only: in the form of Sephadex G-50, G-30, G-20, G-10, and G-2, with a size exclusion limit — in terms of molecular weight — of 50,000, 30,000, 20,000, 10,000 and 2,000, respectively. In gel chromatography, large molecules are eluted first and smaller molecules are eluted last. Piccolo and coworkers claim that in their experiments the amount of large molecules eluted first has decreased, whereas that of the smaller molecules eluted last has increased substantially, due to addition of carboxylic acids. This is taken as an indication that the large molecules were broken down into smaller molecules by the lowering of pH. However, in humic acid chemistry, acidification will affect precipitation of humic acid, unless hydrolysis occurs breaking down the humic substance. This may happen by adding very strong acids, such as HF (hydrofluoric acid) often used for cleaning humic preparations. Questions also arise whether the small broken fragments are reaggregated into new humic assemblies, conforming to the principles of self assembly, the “pillar” of supramolecular chemistry. Their ^{13}C -NMR spectra reflect the presence of loose broken components, often called *tailings*, as examined by the present author (see Tan, 2011, page 122). Nonetheless, whatever has happened, the various size excluded fractions are the results of the analytical operations. The humic substance itself is natural, but the several molecular fractions, separated by the size exclusion

process, are artificial or operational fractions created by the procedures in HPSEC. Hence we are back to the old operational concept of humic substances. The difference is only that the new concept considers the humic fractions to be *molecular associations* or *molecular assemblies*, whereas the old concept calls them *humic acid polymers*.

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