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REVIEW ARTICLE

Quantifying ‘humics’ in freshwaters: purpose and methods

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Natural organic matter (NOM) plays an important role in many environmentally relevant processes. NOM includes many different types of compounds, not all of which behave similarly. Much effort has gone into characterising some fractions of NOM (e.g. humic substances) in the different environmental compartments, in finding tracers to ascertain their origin, etc. However, few methods exist for quantifying the different types of NOM and, as a result, field studies have limited themselves to measuring only total or dissolved organic carbon. In this article, the few existing methods for humic substance quantification are reviewed, and the implications of the current lack of simple measurement methods are discussed in relation to two fields of high environmental relevance: trace element speciation modelling and natural colloid and engineered nanoparticle fate.

Keywords: fulvic acids; humic acids; freshwaters; speciation modelling; colloids; nanoparticles; quantification

1. Introduction

A significant proportion of the natural organic matter (NOM) present in surface freshwaters is composed of fairly stable compounds produced either in the soil or in the water body. Because of their resistance to degradation, these compounds are sometimes called refractory organic matter (ROM) but they are most often referred to as humic acids or humic substances. Historically, this name stems from the extended practice in soil science of isolating two fractions of NOM, the so-called humic and fulvic acids, on the basis of their different solubility in concentrated acid and base solutions. These names have persisted in aquatic sciences even if, in waters, the corresponding fractions are usually obtained by using an isolation procedure based initially on hydrophobicity considerations, followed by an acid–base treatment [1]. To further complicate matters, a variety of alternative isolation schemes exist, all giving fractions that are similar to, but not strictly comparable with, International Humic Substance Society (IHSS) humic compounds, yet some of these fractions are also referred to as ‘humics’. The confusion surrounding the use of this term and of others employed in relation to NOM has recently been discussed in detail [2]. Despite its somewhat fuzzy meaning, and because this nomenclature is so widely used, this article

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uses ‘humics’ as a generic term synonymous with ROM and including all humic and fulvic acids (unless specified otherwise).

However, it is important to keep in mind that ‘humics’ are not the only type of NOM present in surface waters (i.e. see [3] for seawater dissolved organic matter (DOM) and [4] for a review on freshwater colloidal NOM), and are not even the most abundant in many aquatic systems (e.g. oceans – not directly dealt with here – and many surface waters, particularly during productivity periods). There is a wide gap between laboratory studies and field measurements: whereas laboratory studies have traditionally focused mainly on ‘humics’, largely because of the key role that they play in the fate of trace metals and organic micropollutants, field studies have largely relied on the measurement of total organic carbon (TOC) and dissolved organic carbon (DOC), or of some surrogate parameters, for quantification, simply because there are no well-established methods for quantifying the different types of NOM in natural waters. It should be mentioned that, although an impressive number of studies have been devoted to the characterisation of ‘humics’ extracted mainly from soils but also from waters, sometimes by using extremely sophisticated techniques, no comparable effort has been put into developing quantification techniques. Quantification of ‘humics’ in waters is not straightforward because their undefined nature renders the search for a shared and measurable property – one that can be used to obtain an analytical signal – far from simple. In this article, the few existing methods are reviewed and the need for ‘humic’ quantification methods is discussed in two fields; the objective being to promote the use of existing methods and the development of new ones.

2. The purpose or ‘not all DOC is humics’

NOM is known to play a key role in many environmentally relevant processes. These include fundamental issues, such as metal bioavailability and transport in soils and water, or changes in organic carbon production and release linked to climate change, alongside more direct applications, such as water treatment procedures. Most of these areas would benefit from the existence of simple and accurate measurement methods for the different types of NOM. Two of them, trace element speciation modelling in freshwaters and colloid studies, are discussed here in some detail.

2.1. Speciation modelling

In natural waters, trace element binding by NOM has been shown to play a decisive role. Traditionally, only the so-called humic and fulvic acids have been considered in trace metal complexation studies on the grounds that they are ‘the most chemically significant fraction of NOM’ [5] or are ‘widely believed to be representatives of NOM behaviour’ [6]. Although the binding properties of other NOM fractions have not been studied as much, it is currently accepted – and repeated in the introduction of many articles – that humics are largely responsible for the complexation of trace elements by NOM in waters. As a result, only ‘humics’ are considered in models developed to calculate the speciation of trace elements in such systems.

According to the principles on which thermodynamic modelling is based [7], it is necessary to know the total concentration of all components, metals and ligands, in order to perform speciation calculations. However, the apparent capability of some widely used speciation models to reasonably predict free metal ion concentrations in natural systems relies upon modelling methods in which this is not the case for ‘humics’ (i.e. the concentration of ‘humics’ is not known). In practice, this means that ‘humic’ concentrations are either used as a fitting parameter or are imposed as an arbitrary value. In the first case, users adjust their ‘humic’ concentration to get a good fit. In the second case, they use a value that has been calculated previously in other systems. In both

cases, and as effectively pointed out by Cabaniss [8], although the procedure might give good fits, the results obtained provide little mechanistic insight into complexation chemistry. In fact, the method gives an essentially empirical character to a modelling procedure that was deterministic in origin. The NOM concentration values used in the calculations have, in fact, no physical meaning because they confound any other source of variability (or even of error) in the system. The application of two widely used codes, MINTEQA2 and WHAM, is discussed to illustrate the procedures used.

The MINTEQ family includes a collection of codes that evolved from MINEQL [9]. MINTEQA2 is regularly updated and freely distributed by the US Environmental Protection Agency (www.epa.gov/ceampubl/mmedia/minteq/). The traditionally unfriendly interface of this code has led to the development of at least two commercial versions, MINEQL+ (www.mineql.com) and MINTEQA2 for Windows (www.allisongeoscience.com), and a freely distributed version (www.lwr.kth.se/English/OurSoftware/vminteq/). MINTEQA2 incorporates a relatively unsophisticated way of modelling NOM binding: it includes a Gaussian distribution model based on the assumption that NOM is a complex mixture of various functional groups which comprise a population of binding sites and that the probability of occurrence of a binding site is normally distributed with respect to its log K value for metal or proton binding [10,11]. Both publications include data for humic substances. According to MINTEQA2 Help, this model requires as input parameters the DOC concentration (in $\text{mg} \cdot \text{L}^{-1}$) and the site density (in $\mu\text{mol sites} \cdot \text{mg C}^{-1}$). MINTEQA2 advises the user to do the following: 'DOM usually consists of about 50 to 60 percent DOC, so if you have a DOM value in mgL^{-1} , enter about 55 percent of that value as the DOC concentration (mg L^{-1}). DOC is actually the measured quantity'. Concerning the site density, MINTEQA2 Help states: 'The authors of the database of Gaussian reactions have recommended a site density of 1.0×10^{-6} to 2.4×10^{-6} moles of sites per mg DOC'. This means that DOM concentration is taken as synonymous of 'humic' concentration and that the presence of any other type of NOM is precluded. The 'binding activity' of DOM can be modulated by changing the site density which, in any case, is common to all simulated elements, irrespective of their binding characteristics.

WHAM, a very popular speciation code nowadays, treats NOM complexation by including the approach developed in a saga of models by Tipping [5,12–14], the latest being Model VI. For 'humics', Model VI integrates two types of site affinities for cations (types A and B), three types of binding capacities (monodentate, bidentate and tridentate) and association constants describing their interactions with metals. The approach is described in detail in Tipping [5] and more concisely in Filella [15], and is not repeated here. In order to adjust the results, WHAM plays with the possibility of entering different amounts of the two types of 'humics', FA and HA, and of defining the percentage of DOM that is active in binding. In order to illustrate how this approach is applied in practice, all articles published in a well-known environmental journal, *Environmental Science and Technology*, in the period 2005–2009, where WHAM had been applied to surface waters, have been checked. The conditions concerning NOM used in 14 studies are listed in Table S1 (available online only). All authors used measured DOC as the starting parameter and assumed that DOM contained 50% C, which is a reasonable approximation for aquatic 'humics' (i.e. the mean C contents of the 11 aquatic 'humics' offered by IHSS is of 52.7 ± 0.3). Although the existence of other types of NOM was never explicitly recognised, it was implicitly accepted when assuming that not all NOM was 'active'. The percentage of 'active' humic substances ranged from 50 to 100%, depending on the study, with a variety of values having been used (e.g. 50, 60, 65, 80). The ratio FA:HA in the 'active' humic substances was also variable, with 9:1 and 4:1 and 100% FA as choices. In a very few studies, other FA:HA ratios were tested. There was no detectable relationship between the choices of all these parameters and the type of system (lake or river, productive or oligotrophic, etc.) or with the element studied. In some, but not all, cases references were given to justify the choice.

The use of this approach for choosing NOM concentrations is not exclusive to surface waters. It has also been used when WHAM is applied to soils [16–19]. Nor is the approach exclusive to WHAM; other widely used models for soils, ECOSAT [20] and ORCHESTRA [21], which include the NICA-Donnan model for humic ion binding, are based on a similar principle concerning the use of NOM as an adjusting parameter [17,21,22–25].

Quantification of ‘humic’ concentrations in natural water samples and using these measured concentrations in speciation models would eliminate an unnecessary degree of uncertainty from the models and, hopefully, allow them to increase their predictive power in the future.

2.2. *Fate of natural colloids and nanoparticles*

Particles are considered to have colloidal behaviour when their dimensions are roughly between 1 nm and 1 μm in at least one direction [26,27]. The fact that they are small in size implies a long settling time in water, typically days to months, and a large specific surface area. Both features make them good candidates for a significant role in the transport of trace metals, radionuclides and organic contaminants in all environmental compartments. Although colloid residence times are orders of magnitude larger than those of the ‘suspended’ fractions, their behaviour and fate in water systems is clearly distinguishable from that of truly dissolved substances because, unlike truly dissolved compounds, they can undergo relatively fast coagulation and sedimentation processes. The rate of colloid aggregation depends on both the frequency of particle collisions and the efficiency of particle contacts. In turn, the efficiency of particle contacts is controlled primarily by surface forces which introduce an energetic barrier that determines the probability of successful interparticle collisions. This energetic barrier is influenced by the ionic strength and the composition of the water, but also by the adsorption of surface-active species [4]. In this context, a key question is the exact role of NOM in natural colloid coagulation and fate. As early as 1970, Hahn and Stumm [28] stated that NOM would stabilise inorganic colloids in natural waters. Laboratory studies carried out in the 1980s and early 1990s [29–32] confirmed that humic-type substances stabilise inorganic colloids in natural waters. However, on the basis of a synthesis of literature articles and microscopic observations of natural colloids [33,34], Buffle and co-workers [35] later suggested that to understand the formation of aggregates in aquatic systems not only would the role of soil-derived fulvic compounds, or their equivalent in pelagic waters that stabilise colloidal particles in solution, need to be considered, but also that of rigid carbohydrate biopolymers that destabilise them.

There are not many long-term quantitative studies of colloid behaviour at the scale of a whole water body (e.g. a lake) because of the lack of adequate measuring techniques. Studies in which mineral colloids are measured at the same time as the different types of NOM – thus allowing the applicability of this conceptual model to be tested – are even scarcer. A recent example can be found in a study performed within the framework of an extensive research project devoted to the causes and consequences of the current ultraoligotrophic status of a glacial-flour-rich lake (Lake Brienz, Switzerland) [36–38]. Slightly higher coagulation rates (inorganic colloids measured using a single particle counter technique) were recorded in March and April during the spring algal bloom, which suggested that colloid coagulation could be enhanced by the presence of carbohydrates, even in such a low productive system. This effect was confirmed in laboratory coagulation experiments performed in the presence of different types of NOM using natural lake colloids. The contrasting effect of fulvic acid and alginate, a carbohydrate model substance, on coagulation is clearly shown in Figure S1 (available online only). This confirms previous results [39] where, during periods of high productivity in a eutrophic lake, inorganic colloids (not directly measured but indirectly followed through the measurement of Al, Si and Fe colloidal concentrations) were observed to be depleted at depths of maximum polysaccharide concentrations. The routine quantification of

the different types of NOM, instead of the usual measurement of TOC or DOC, would be of great interest in order to be able to predict the role of inorganic colloids in the fate of pollutants in the different aquatic systems and across the seasons.

It should be mentioned that interest in the effect of 'humics' on colloids is experiencing a renaissance with the current boom of studies on the environmental behaviour of engineered nanoparticles. Classical coagulation laboratory studies are now being redone with nanomaterials in the presence of 'humics' [40–46] and, in a few cases, of other types of NOM [42]. The influence of 'humics' on nanoparticle toxicity is also being addressed [47–49]. However, translation of these results to the environmental scale will rely heavily on our ability to measure the different types of NOM present in natural waters.

3. Methods

It is well-known that quantitative analytical chemistry measures a property of an analyte (or its reaction product) in numerical units [50]. However, as mentioned in Section 1, the operationally defined nature of 'humics', together with the concomitant elusive and non-constant composition and structure of these substances, makes it difficult to find such an intrinsic property for them. As discussed below, often the property measured either only characterises a portion of the 'humics' – a system-dependent portion of the 'humics' – or it is also characteristic of other types of NOM present in the system. In the first case, part of the 'humics' is not measured by the technique, which cannot therefore be considered quantitative. In the second case, the method lacks selectivity. These problems appear to be unavoidable so far. However, a good understanding of the different analytical techniques' responses and of how to use several of them in combination to study a particular problem would represent a clear improvement over the current situation, where only TOC or DOC concentrations are measured in the worst case or where, in the best case, UV-vis measurements are made blindly [2].

Spectroscopic techniques, such as UV-vis and fluorescence have been the most widely applied, although often for characterisation purposes rather than for quantification. All other analytical methods have been applied rarely, if at all. Published methods other than those based on UV-vis absorption and fluorescence are given in Table S2 (available online only). Table S2 sets out the principle on which the methods are based, the humic substances used in their development, their detection limits and any existing information about the dependence of the analytical signal on the variable nature of 'humics'. UV-vis and fluorescence are described below, together with most of methods, but are not included in Table S2 because they are simple, well-known techniques and examples of application can easily be found in the literature.

The most widely applied technique is UV-vis. All humic-type substances exhibit a featureless increase in absorbance with decreasing wavelength from 800 to 200 nm [51] that is linked to the compound aromaticity. Given this, absorbance at different wavelengths (e.g. 254, 280 or 285 nm) has commonly been used to index NOM aromaticity. Normalisation of the absorbance based on the mass concentration of DOC has been suggested to be a highly specific indicator of benzene carboxylic acids and phenols, and thus a tracer of soil-derived ROM in natural waters [52–56]. Different bulk spectroscopic quantities – the E2/E3 (quotient of absorbance at 250 and 365 nm), the E4/E6 (quotient of absorbance at 465 and 665 nm) and the E2/E4 (quotient of absorbance at 250 and 465 nm) ratios – have also been used to characterise NOM in natural waters. They give an estimate of the aromaticity percentage and molecular mass [57,58] and are useful for tracking variations in NOM origin and type in a given system. However, the validity of either the direct measurement of UV absorption or any of the mentioned derived quantities as 'humic' quantification methods remains doubtful because these methods only 'see' a certain fraction of 'humics' [59], the amount of which varies among different systems. As early as 1972, Schnitzer

and Khan [60] mentioned that molar absorptivities of 'humics' from different soil sources differed by up to 273%, although lower variability was found in aquatic 'humics' [61]. Some authors have applied empirical relationships linking 'humics' concentrations and UV-vis absorption values (e.g. 'humics' concentration = $64.52A_{326}$ in Croisetière et al. [62]). These empirical relationships are usually derived from correlations established on a certain number of similar water systems. Their general applicability is thus limited. Finally, it is important to keep in mind that UV-vis might not be sufficiently sensitive for samples with a low 'humic' content. Note that published studies concern systems with relatively high organic carbon (OC) content (i.e. $30\text{--}50\text{ mg C} \cdot \text{L}^{-1}$ [53]; $20\text{--}30\text{ mg C} \cdot \text{L}^{-1}$ [58]; $0.1\text{--}20\text{ mg C} \cdot \text{L}^{-1}$ [63]; $10\text{ mg C} \cdot \text{L}^{-1}$ [64]). A detection limit of $\sim 1\text{ mg C} \cdot \text{L}^{-1}$ for the quantification of humic substances from absorbance measurements was determined in Hautala et al. [63] but this can probably be improved, for instance, by increasing the cell path length.

High-resolution fluorescence spectroscopy has also been used extensively in the characterisation of NOM from a variety of freshwater, coastal and marine environments [51,65,66]. Fluorescence has the advantage of being more sensitive than UV-vis light-absorption-based techniques. However, as a quantitative technique for 'humics', fluorescence shares with UV-vis absorption the problem that it only 'sees' a fraction of 'humic' substances, the fluorescent properties of humic substances also being essentially related to their aromatic character. For example, several studies have shown that the intensity of fluorescence for soil humic acid is weaker than for river humic acid [67–69], clearly reflecting their different nature. Moreover, humics are not the only fluorophores present in natural waters. For example, phytoplankton fluorescence has been used historically as a means of assessing phytoplankton biomass and rates of primary production [70,71].

As mentioned above, strictly speaking, 'humics' and 'fulvics' are the names given to some fractions of NOM obtained by following well-defined procedures [2]. This means that the only quantification method that will theoretically measure 'true' humic and fulvic acid concentrations should be based on exactly the same fractionation procedure used in defining them. For aquatic systems, this implies the application of XAD-based isolation procedures for quantification purposes. This approach has been used by a limited number of authors [56,72–76]. However, it requires large volumes of water and is very tedious and time-consuming. This precludes using it systematically for large numbers of samples and probably explains why it has never been widely applied. Moreover, its application in a quantitative way is not easy and requires dressing OC mass balances all along the procedure.

Solid and dissolved organic matter have been characterised quantitatively in terms of three fractions, i.e. HA, FA and hydrophilic acids, using a batch procedure derived from the method currently recommended by the IHSS [77]. However, this method gives low recoveries for HA when present at concentrations $< 50\text{ mg C} \cdot \text{L}^{-1}$, which is the case for most surface waters. In fact, the method as developed was intended for solid samples and liquid samples containing high amounts of OC, such as landfill leachates, influent and effluent water from landfill water treatment plants, etc. It has been applied to municipal solid waste incineration bottom ash [78,79] and polluted soils [80,81], but not to surface waters.

As mentioned in Section 1, the fractionation method proposed by the IHSS is not the only one in the literature; a myriad of different fractionation procedures exist [2]. Any separation method, accompanied by the measurement of the OC contained in the different fractions – which will require a highly sensitive OC detection method – and a satisfactory mass balance, can be used as a quantitative technique in which the different NOM fractions will be quantified as a percentage of the total DOC. This is the case, for example, of Huber's method [82,83], based on the size-exclusion separation of NOM into a large number of fractions, among them 'humic substances, degradation products of humic substances (building blocks), and low molecular weight humics'. This approach is currently applied by a commercial laboratory (www.doc-labor.de). Recently, a

method based on the use of HPSEC with UV detection has also been published [84], but the fact that it can only be applied to waters containing at least 5 mg of C per litre seriously limits its application to surface waters. It is important to stress that, although some fractions measured using these methods have been named 'humics' by their authors, they do not necessarily coincide with IHSS 'humics' because the fractionation methods are based on different principles [2].

The use as a 'humic' quantification method of the non-specific, surface-active interaction of 'humics' with the Hg electrode causing either suppression of the polarographic maximum of dissolved oxygen or a decrease in the capacitive current in different polarographic techniques, was suggested more than 25 years ago by Buffle [85,86]. More recently, two electroanalytical methods based on cathodic stripping voltammetry (CSV) have been proposed. One is based on the formation of Mo(VI)–'humics' complexes at pH 2, which adsorb on to the Hg electrode and are detected by their reduction using CSV [87,88]. The second method is based on the adsorption of Fe–'humics' on to the Hg electrode and their detection by a reduction peak in adsorptive CSV, which is much enhanced by a catalytic effect in the presence of bromate [89]. Both methods give similar results in freshwaters [89], consume only small amounts of the sample and can be applied to samples containing very low amounts of 'humics'. However, it is important to realise that both of these methods, along with others based on different principles and described in this article, give results that are dependent on the type of 'humic' substance present in the sample. The caveats involved specifically in the case of the Mo-based method when choosing the best standard have been studied in detail in Quentel and Filella [88]. Unfortunately, such detailed studies are lacking for most of the methods listed in Table S2 (available online only), even in the studies where the dependence of the analytical signal on the type of 'humic' used is acknowledged (see fourth column in Table S2).

Apart from the methods discussed above, Table S2 contains a certain number of analytical methods based on the measurement of other properties, with a significant number based on the chemiluminescence produced when 'humics' are oxidised by a variety of oxidants. Despite the fact that the chemiluminescence of soil humic acids was described as early as 1967 [90], the usefulness of chemiluminescence-based methods is very doubtful because they completely ignore the fact that different types of 'humics' might give different responses: most of them have been tested with only a single commercial humic acid that, moreover, is very different from 'humics' present in natural waters. Moreover, these techniques are sensitive to a large number of quenchers present in natural waters, such as some trace elements. Despite these problems, these methods continue to be published in peer-reviewed journals. A further point that merits some thought is the fact that none of the methods based on chemiluminescence has ever been applied in field environmental studies after their publication, which can be explained by their limited usefulness. However, the same applies to the vast majority of the methods listed in Table S2 (available online only). It is not possible to ascertain whether this is due to a gap between method developers and potential users or to the intrinsic limitations of the existing methods, but the fact that when a method is applied in real systems the authors of the corresponding studies often belong to the same research group who developed the method pleads in favour of the former.

4. Conclusions

The nature of humic substances will probably preclude the development of any universal, definitive method for quantifying them in any surface water. However, the use of the existing methods and the development of new ones, adapted to the many different aspects where 'humics' play a role in the environment, should be strongly encouraged. Two items that would merit immediate action have been identified in this review article: (1) the need for detailed studies on the dependence of the measured analytical signals on the type of 'humic', as these are currently lacking for most existing

methods and would make it possible to make a well-informed choice of the most suitable standard to be used for any particular case; and (2) the need to improve the communication between method developers and potential users. This article hopes to be a first step in this direction.

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