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Quentel, François; Filella, Montserrat

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A simple method for quantifying the humic content of commercial products

François Quentel · Montserrat Filella

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Abstract A method based on an analytical technique, initially developed for quantifying aquatic refractory organic matter (often called humics), has been applied to commercial samples claiming to contain humic-type substances. At present, no method exists for quantifying the humic content on this type of sample. The analytical method is based on measuring the peak current obtained by adsorptive stripping voltammetry of the complex formed by refractory organic matter in the presence of trace amounts of Mo(VI). The quantification procedure requires the response obtained for the unknown sample to be compared with the response obtained with International Humic Substance Society (IHSS) reference humic substances. A very simple procedure that enables the humic content of any sample to be expressed as IHSS standard equivalents is described in detail. The method is highly selective, reproducible and suitable for routine analysis.

Keywords Humic commercial products · Quantification · Humic substances · Voltammetry

Introduction

The last few years have seen the introduction of an increasing number of products containing humic substances in the market. These products are intended to be used in a wide range of fields, from agriculture to cosmetics, food, health, etc. However, no standardized, or at least widely accepted, analytical method exists that makes it possible to quantify the humic concentrations in such products, nor is the commercialisation of these products generally regulated or controlled. The existence of a reliable determination method is a prerequisite to assess their quality and, eventually, to regulate their use.

It is important to understand that the lack of a convenient method for determining humic content in commercial products is not surprising, as it is simply the reflection of a lack of suitable, generally accepted methods for quantifying those compounds in natural systems, despite the fact that humic substances are relevant to a wide range of environmental issues, from the toxicity of trace elements and micropollutants, to the carbon cycle, and the production of undesirable disinfectious by-products in drinking waters. Moreover, none of the methods occasionally used in research and in environmental monitoring is without drawbacks (see Ref. [1] for a recent review), not least the fact that most of the methods ‘see’ only a certain fraction of humics [1], the proportion of which does not remain constant from system to system. It is important to point out that this situation is exclusively due to the uncommon nature of these substances: fulvics and humics are operationally defined fractions of natural organic matter and not compounds with reproducible structures and composition. Thus, they are not unique substances but a category of natural organic matter

F. Quentel
Laboratoire de Chimie Analytique, UMR-CNRS 6521,
Université de Bretagne Occidentale,
6 avenue V. Le Gorgeu, CS 93837,
29238 Brest Cedex 3, France

M. Filella (✉)
Institute F.-A. Forel, University of Geneva,
10 route de Suisse,
1290 Versoix, Switzerland
e-mail: montserrat.filella@unige.ch

with common characteristics, one of which is their resistance to degradation. To complicate things further, a myriad of protocols exist for fulvic and humic fractionation (i.e., definition), and their relevance and properties are assessed differently in different fields (i.e., aquatic chemists, soil scientists, ecologists, water treatment engineers, etc.) [2].

As discussed in detail in Ref. [1], any analytical method measures a property of an analyte (or its reaction product), but 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. Recently, an electroanalytical method based on cathodic stripping preceded by the adsorptive collection of Mo(VI)-humic or fulvic acid complexes has been proposed for natural samples [3, 4]. This method is fast, sensitive, needs only small amounts of the sample and, more importantly, is extremely selective: only water, soil and peat fulvic and humic acids, defined as fractions isolated by following the different standard procedures of the International Humic Substance Society (IHSS), give a signal while carbohydrates and proteins do not [3]. So far, the method has been applied to environmental waters [5–7]. In this study, the application of the method to commercial samples has been tested and a procedure that allows the quantification of such samples has been developed.

Experimental

Commercial products

The following commercial products were analysed: Humiron (WSG-6), kindly provided by Humintech (Düsseldorf, Germany); Biovin (Trever, Austria; organic substances, 85–92%; Humusbildner, 70%); Bachhumus Eco (TRABE; organic matter, 40.5%; humic acids, 2.5%; total humic extract, 25%); Bachhumus Bioabono (TRABE; guaranteed contents—total humic extract, 25.00%; humic acids, 5.30%; fulvic acids, 19.70%; analysed—organic matter, 35.43%; total humic extract, 39.98%); H2-Hulmax (Agromed; humic and fulvic extract, 17% (w/v); humic acids, 0.1% (w/v); fulvic acids, 16% (w/v); organic matter, 40.22% dry weight); El milagro de la Naturaleza. Minerales húmicos (www.secretosparatusalud.com; no information about humic content). All products were used as purchased, without further purification. The humic and/or fulvic contents given here are those given by the producers. None give any detail about the technique used to determine humic concentrations. The information given is sometimes confusing. This is the case, for instance, of Bachhumus Eco and Bachhumus bioabono that come from the same producer but were purchased from different retailers.

Measuring method

All voltammetric measurements were taken with a potentiostat/galvanostat μ Autolab II controlled by GPES 4.9 software. A hanging mercury drop electrode (HMDE) Metrohm model 663 VA with a mercury drop size of 0.40 mm^2 was used. All potentials were referred to an Ag/AgCl, 3 mol L^{-1} KCl, reference electrode. The counter electrode was a platinum wire. The square-wave mode (SWV) was used for the measurements. The quartz voltammetric cell was thermostated at $25 \pm 0.1 \text{ }^\circ\text{C}$ during the measurements to ensure that conditions were reproducible.

All chemicals used were of analytical reagent grade except the mineral acid (HCl) and base (NaOH), which were of Suprapur grade. A $1,000\text{-mg L}^{-1}$ stock solution of Mo(VI) (atomic absorption standard, Merck) was diluted as required. Humic and fulvic acid standards were from IHSS.

All solutions were prepared with $18 \text{ M}\Omega \text{ cm}$ Milli-Q water. All glassware and polyethylene bottles were cleaned with 10% (v/v) HNO_3 and 0.5 mol L^{-1} NaOH and rinsed with Milli-Q water. Stock solutions of IHSS standards and solid samples were prepared in 0.01 mol L^{-1} NaOH (concentration, $\approx 250 \text{ mg L}^{-1}$).

The procedure used for the measurements is the same as that described in Ref. [4]: (1) Mo(VI) ($10 \text{ }\mu\text{g L}^{-1}$) is added to an initial acidified (0.01 mol L^{-1} HCl) solution containing a given amount of the compound to be analysed; (2) the solution is deaerated with nitrogen for 10 min; (3) deposition at -0.25 V is carried out with stirring; (4) after the deposition time, the stirring is stopped and, following a 10-s period of rest, the scan is initiated in the negative direction. The operational parameter values used were: frequency, 50 Hz; step potential, 1 mV; and amplitude, 40 mV.

Typical SWV voltammograms have been shown in previous publications [3, 4]. The electrochemical process has been previously studied and described in detail [4, 8]. In SWV mode, the method detection limit, calculated according to Ref. [9], is $3.9 \text{ }\mu\text{g L}^{-1}$ ($2.1 \text{ }\mu\text{gC L}^{-1}$) for 3 min deposition time (for Suwannee River standard fulvic acid I, 1S101F) [4], its sensitivity, $120 \text{ nA mg}^{-1} \text{ L}$ (same standard) [4], and its reproducibility equally good, with a variation coefficient of 5% at the $125 \text{ }\mu\text{g L}^{-1}$ concentration level [4]. Other types of organic matter and trace elements do not interfere [3].

Results

The suggested methodological procedure for solid samples includes two steps. In the first, response curves are obtained for the unknown product (i.e. increasing amounts of the

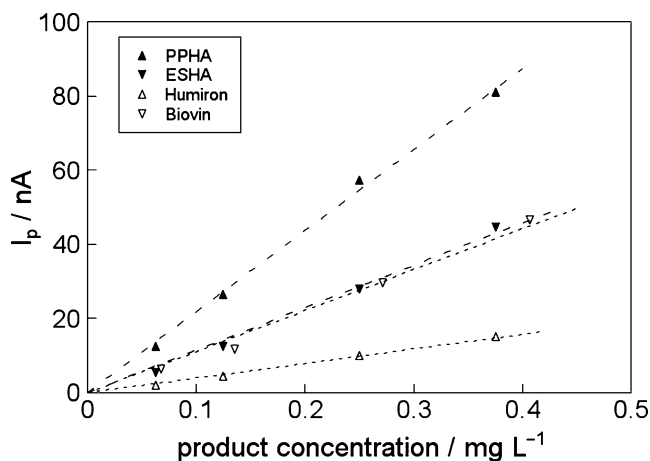


Fig. 1 SWV response curves for solid commercial products containing humic substances (Humiron and Biovin). Substance concentrations are expressed in milligrammes per litre. The response curves for IHSS humic standards closest to the ones observed in the commercial products are also shown (PPHA Pakohee Peat HA (1S103H) and ESHA Elliott Soil HA (1S102H))

given compounds are added to the initial solution and the peak currents obtained measured). In all cases, a linear dependence between the analytical signal and product concentrations was observed in the products under study (Fig. 1). The concentrations of the measured solutions need to be adapted to the linear range of the analytical technique [3] by appropriately diluting the sample if they are outside the linearity range. The same type of response had been obtained for all IHSS standard and reference products ([4]).

The second step of the proposed methodological procedure consists of measuring the response curves of one (or more) IHSS standards. As mentioned in the ‘Introduction’, the intrinsic nature of humic substances means that the results obtained can never be considered absolute measurements but need to be referred to a standard substance. The use of IHSS standard substances is recommended for this purpose. Although any IHSS standard can be used, in principle, we advise using those whose response, and thus whose nature, is closer to the compound being analysed. For this purpose, visual comparison of the response obtained for the unknown

Table 1 Results obtained for the solid commercial substances analysed

Product	Humic content (in mg of ESHA equivalents contained in 100 mg of product)	Humic content (in mg of PPHA equivalents contained in 100 mg of product)
Humiron (WSG-6)	34.5	17.5
Biovin	97.5	47.4

ESHA Elliott Soil HA, PPHA Pakohee Peat HA

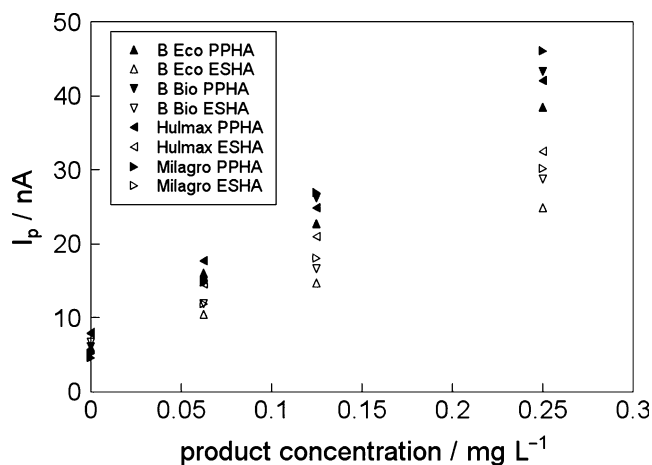


Fig. 2 SWV response curves for liquid commercial products containing humic substances (Bachusmus Eco, Bachhumus Bioabono, H2-Hulmax and Milagro). Substance concentrations are expressed in milligrammes per litre. For each compound, the standard addition curves for two standards (PPHA Pakohee Peat HA (1S103H) and ESHA Elliott Soil HA (1S102H)) are shown

sample with the response of IHSS standards [4, 6] is enough to make a selection. Quantification of the humic contents in the unknown samples requires simultaneous measurement of the standard response curve and the unknown substances *under the same conditions*.

Finally, by combining the responses of the unknown compound and the IHSS standards, the humic contents of the unknown samples can be expressed in ‘IHSS product’ equivalents. Table 1 shows the results obtained here for the two commercial substances tested, expressed as sample weight and standard equivalent. The results can also be expressed in terms of organic carbon content, although this requires the organic carbon content of the unknown samples to be determined. The organic carbon of humic substances can be found at www.ihss.com. In the case of the products tested (Humiron and Biovin), the response was closer to Elliott Soil HA (ESHA, 1S102H) and Pakohee Peat HA (PPHA, 1S103H) standards. Thus, their concentrations were expressed as ESHA or PPHA equivalents.

Table 2 Results obtained for the liquid commercial substances analysed

Product	Humic content (in g L ⁻¹ of ESHA equivalents)	Humic content (in g L ⁻¹ of PPHA equivalents)
Bachusmus Eco	78.5	43.7
Bachhumus Bioabono	74.6	40.5
H2-Hulmax	80.2	58.3
Milagro	2.08	1.10

ESHA Elliott Soil HA, PPHA Pakohee Peat HA

In the case of liquid samples, the procedure recommended is similar to the one used for natural waters [3, 4]: addition of aliquots of a standard solution to the unknown sample. Therefore, in this case, results are also referred to the IHSS standards used. Results obtained for the substances analysed (Bachumus Eco, Bachhumus Bioabono, H2-Hulmax and Milagro) are shown in Fig. 2 and in Table 2.

Conclusions

The application of the quantification method, initially developed for natural water samples, to different solid and liquid commercial humic-containing substances has proved straightforward.

The principle of expressing results as IHSS standard equivalents can be used with other analytical measurement techniques, such as, for instance, UV–vis and fluorescence methods. It is important to point out, however, that the voltammetric-based method proposed here is more sensitive, and, more importantly for the types of samples analysed here, is 100% selective (i.e. other organic matter fractions such as carbohydrates or proteins do not give a signal) [3], which is far from the case for the two techniques mentioned.

On a final note, it should be pointed out that, although the constraint of having to express the results as a function of the response of a standard might appear unsatisfactory to some users, this procedure has been used for years for other

types of natural organic matter, such as carbohydrates (glucose, [10]) and proteins (bovine serum albumin, [11]), the difference being merely that this ‘hidden dependence’ has been often overlooked, or plainly ignored, by the users. Moreover, at least in the case of carbohydrates, the limitations imposed by using glucose considerably exceed those imposed here, in the case of humics. This point has been discussed in detail in Ref. [10].

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