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2008

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### How to cite

QUENTEL, François, FILELLA, Montserrat. Quantification of refractory organic substances in freshwaters: further insight into the response of the voltammetric method. In: Analytical & bioanalytical chemistry, 2008, vol. 392, n° 6, p. 1225–1230. doi: 10.1007/s00216-008-2366-4

This publication URL: <https://archive-ouverte.unige.ch/unige:79047>

Publication DOI: [10.1007/s00216-008-2366-4](https://doi.org/10.1007/s00216-008-2366-4)

# Quantification of refractory organic substances in freshwaters: further insight into the response of the voltammetric method

François Quentel · Montserrat Filella

Received: 8 July 2008 / Revised: 19 August 2008 / Accepted: 20 August 2008 / Published online: 15 September 2008  
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**Abstract** A recently published method for quantifying refractory organic matter (often referred to as humic substances) in freshwaters was applied to a wide range of International Humic Substance Society (IHSS) humic compounds in order to (i) gain a better understanding of the mechanism of the voltammetric response which is the basis of the analytical method and (ii) provide guidance on choosing the optimal standard to be used. At the same time, the sensitivity of the technique has been increased by switching from the pulse mode initially proposed to the square-wave mode. The results obtained show that (i) differences in adsorption onto the electrode rather than differences in complexation strength are responsible for the differences in the intensity of the signal obtained for the different humic compounds, (ii) carboxylate, N- and S-containing groups do not play a role in the voltammetric signal.

**Keywords** Humics · Fulvics · Refractory organic matter · IHSS · SWV · Mo(VI) · Freshwaters

## 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 (pedogenic NOM) or in the body of water (aquagenic NOM). Because of their resistance to degradation, these compounds are sometimes called refractory organic matter (ROM). At present, there is no method for identifying and quantifying all types of NOM present in natural waters. Past studies have focussed mainly on the refractory fraction of NOM, largely because of the key role that it plays in the fate of trace metals and organic micropollutants. It is common practice to isolate two fractions of ROM, the so-called humic (HA) and fulvic acids (FA) on the basis of their different solubility in concentrated acid and base solutions. This is, for instance, the preferred approach of the International Humic Substance Society (IHSS). A wide range of alternative fractionation procedures have also been applied [1]. These result in a wide variety of fractions whose characteristics are not always easy to compare.

An impressive number of studies have focussed on characterizing different ROM-related fractions by using a wide range of experimental methods [2]. However, few methods exist for quantifying ROM, despite the fact that determining ROM concentrations is of the highest importance to issues varying from modelling trace metal speciation to predicting the partitioning of hydrophobic organic contaminants or problems associated with climate change. ROM quantification is not an easy issue because its undefined nature renders the search for a shared and measurable property far from straightforward. So far, the few methods available have proved to be unsatisfactory. Spectroscopic techniques, such as UV–Vis and fluorescence, are the most widely applied. However, their value as

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quantification methods remains doubtful because they ‘see’ only a certain fraction of ROM [3], the amount of which varies among different systems. In addition, UV–Vis is not sufficiently sensitive for samples with a low ROM content. An alternative method, used by a limited number of authors [4–11], is the application of the XAD-based isolation procedure for quantification purposes. However, this method has not proved to be quantitative, and it requires large volumes of water and is tedious and time-consuming, which precludes using it systematically for large numbers of samples. Other recently published methods [10, 11] also cannot be applied to media containing medium to low proportions of ROM. An alternative electroanalytical method based on cathodic stripping preceded by the adsorptive collection of Mo(VI)–humic or fulvic acids complexes has also been proposed recently [12]. This method is quick and reliable and consumes only small amounts of the sample. To our knowledge, it has so far been successfully applied to an extensive study of NOM cycling in an ultraoligotrophic watershed [13] along with some other freshwater systems [14]. The strong interest shown by potential users in this method has prompted us to improve its sensitivity by using the square-wave mode rather than the differential pulse mode initially proposed, and to analyse a large set of IHSS fulvic and humic substances in order to better understand the basis of the method, as well as to guide possible users in choosing the most appropriate standard.

## Experimental

### Apparatus

All voltammetric measurements were performed with a potentiostat/galvanostat Autolab PGSTAT12 controlled using GPES 4.8 software. A static mercury drop electrode (SMDE) Metrohm model 663 VA with a mercury drop size of 0.52 mm<sup>2</sup> was used. All potentials were referred to an Ag/AgCl, 3 mol L<sup>−1</sup> KCl, reference electrode. The counter electrode was a platinum wire. Square-wave voltammetry (SWV) was used for NOM measurements. Alternating current voltammetry (ACV) and differential pulse polarography (DPP) were used to obtain better insight into the response mechanism for the different types of NOM under consideration. The quartz voltammetric cell was thermostated at 25±0.1 °C during the experiments to maintain reproducible conditions.

### Reagents

All chemicals used were of analytical reagent grade except the mineral acid (HCl), which was of Suprapur grade. A 1,000 mg L<sup>−1</sup> stock solution of Mo(VI) (atomic absorption standard, Merck) was diluted as required. Humic and fulvic

acids were from IHSS: Suwannee River standard humic acid II (2S101H), Suwannee River standard fulvic acid I (1S101F), Suwannee River standard fulvic acid II (2S101F), Suwannee River aquatic NOM (1R101N), Elliott soil standard humic acid (1S102H), Elliott soil standard fulvic acid II (2S102F), Pahokee peat standard humic acid (1S103H), Pahokee peat standard fulvic acid II (2S103F), Leonardite standard humic acid (1S104H), Nordic Lake reference humic acid (1R105H), Nordic Lake reference fulvic acid (1R105F), Nordic Reservoir aquatic NOM (1R108N) and Pony Lake reference fulvic acid (1R109F). A 250 mg L<sup>−1</sup> stock solution of each IHSS standard was prepared in 0.01 mol L<sup>−1</sup> NaOH and kept in the dark at 4 °C for subsequent use. The main characteristics of these substances are shown in Table 1.

### Procedure

All standard and sample solutions were prepared with 18 MΩ cm Milli-Q water. All glassware and polyethylene bottles were cleaned with nitric acid (10% v/v) and sodium hydroxide (0.5 mol L<sup>−1</sup>) and rinsed with Milli-Q water. The procedure used for the measurements is the same as that described in ref. [12]. In brief, after adding a small amount of Mo(VI) (10 µg L<sup>−1</sup>) to the acidified (0.01 mol L<sup>−1</sup> HCl) sample in order to ensure the formation of the adsorbed complex, the sample is deaerated with nitrogen for 10 min and deposition at −0.2 V is performed with stirring. After the deposition time, the stirring is stopped and, following a 20-s rest period, 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.

## Results and discussion

### Square-wave versus differential pulse modes

When this method was first described [12], adsorptive differential pulse voltammetry (ADPV) was used. The reported detection limit was 2.4 µgC L<sup>−1</sup> (180-s accumulation time, Suwannee River standard fulvic acid I (1S101F)). In this study, the sensitivity of the method has been further improved by using square-wave voltammetry (SWV) instead of the pulse mode. Figure 1 shows that the reduction current is about five times higher when SWV is used. In the case of Suwannee River standard fulvic acid I (1S101F), the method sensitivity is 120 and 24 nA mgROM<sup>−1</sup> L for SWV and ADPV, respectively. This makes the method extremely valuable for analysing waters containing very low amounts of ROM or using very small samples. The detection limit with SWV, calculated as explained elsewhere [12], is 2.1 µgC L<sup>−1</sup> for the same fulvic acid analysed in ref. [12]. The precision of the method using ADPV was evaluated in

**Table 1** Characteristics of the IHSS humic substances studied: isolation method, elemental composition, acid/base properties, aromaticity

| ROM                  | IHSS cat. no. | Isolation method               | Elemental composition (%) |      |       |      |      | Carboxylate content<br>(meq mgC <sup>-1</sup> ) | Phenolate content<br>(meq mgC <sup>-1</sup> ) | Aromatic<br>composition<br>(%) |
|----------------------|---------------|--------------------------------|---------------------------|------|-------|------|------|---|---|--------------------------------|
|                      |               |                                | C                         | H    | O     | N    | S    |   |   |                                |
| Suwannee River HA II | 2S101H        | XAD-8 + acid-base <sup>a</sup> | 52.63                     | 4.28 | 42.04 | 1.17 | 0.54 | 9.13  | 3.72  | 31                             |
| Suwannee River FA I  | 1S101F        | XAD-8 + acid-base <sup>a</sup> | 52.44                     | 4.31 | 42.20 | 0.72 | 0.44 | 11.44   | 2.91  | 24                             |
| Suwannee River FA II | 2S101F        | XAD-8 + acid-base <sup>a</sup> | 52.34                     | 4.36 | 42.98 | 0.67 | 0.46 | 11.17   | 2.84  | 22                             |
| Suwannee River NOM   | 1R101N        | Reverse osmosis <sup>b</sup>   | 52.47                     | 4.19 | 42.69 | 1.10 | 0.65 | 9.85  | 3.94  | 23                             |
| Elliott soil HA      | 1S102H        | Acid-base <sup>c</sup>         | 58.13                     | 3.68 | 34.08 | 4.14 | 0.44 | 8.28  | 1.87  | 50                             |
| Elliott soil FA II   | 2S102F        | Acid-base <sup>c</sup>         | 50.12                     | 4.28 | 42.61 | 3.75 | 0.89 | 13.24   | 2.27  | ND                             |
| Pahokee peat HA      | 1R103H        | Acid-base <sup>c</sup>         | 56.37                     | 3.82 | 37.34 | 3.69 | 0.71 | 9.01  | 1.91  | 47                             |
| Pahokee peat FA II   | 2S103F        | Acid-base <sup>c</sup>         | 51.31                     | 3.53 | 43.32 | 2.34 | 0.76 | ND  | ND  | ND                             |
| Leonardite HA        | 1S104H        | Acid-base <sup>c</sup>         | 63.81                     | 3.70 | 31.27 | 1.23 | 0.76 | 7.46  | 2.31  | 58                             |
| Nordic Lake HA       | 1R105H        | XAD-8 + acid-base <sup>a</sup> | 53.33                     | 3.97 | 43.09 | 1.16 | 0.58 | 9.06  | 3.23  | 38                             |
| Nordic Lake FA       | 1R105F        | XAD-8 + acid-base <sup>a</sup> | 52.31                     | 3.98 | 45.12 | 0.68 | 0.46 | 11.16   | 3.18  | 31                             |
| Nordic Reservoir NOM | 1R108N        | Reverse osmosis <sup>b</sup>   | 53.17                     | 5.67 | ND    | 1.10 | ND   | ND  | ND  | 19                             |
| Pony Lake FA         | 1R109F        | XAD-8 + acid-base <sup>a</sup> | 52.47                     | 5.39 | 31.38 | 6.51 | 3.03 | ND  | ND  | 12                             |

ND not determined

<sup>a</sup> Method adapted from [15], described at <http://www.ihss.gatech.edu/> and in [16]

<sup>b</sup> Method described at <http://www.ihss.gatech.edu/> and in [17]

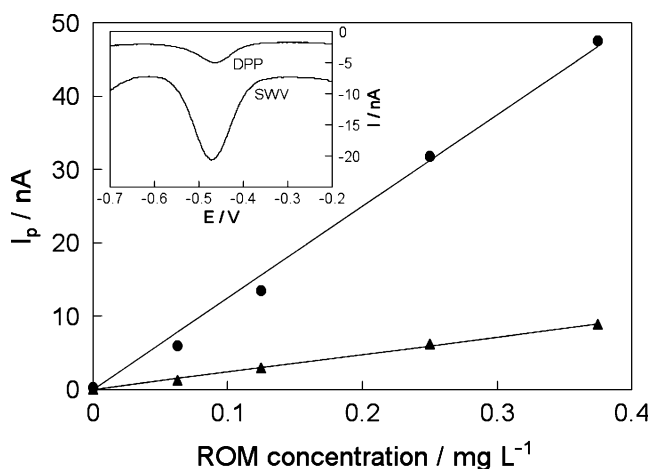
<sup>c</sup> Method described at <http://www.ihss.gatech.edu/> and in [18]

ref. [12]. Reproducibility when SWV is used is equally good: precision was evaluated in replicates ( $n=4$ ) of Suwannee River standard fulvic acid I (1S101F). At the 125  $\mu\text{g L}^{-1}$  level, the average of the signal was 13.7 nA with a relative standard deviation of 0.8 nA.

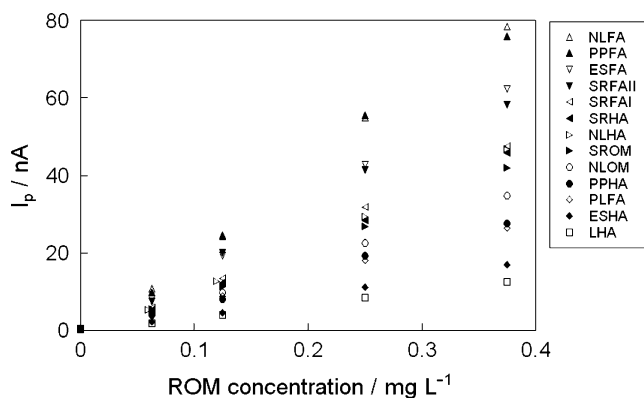
#### Response of different humic materials

When the analytical method was devised, it was found that the intensity of the signal measured depended on the type of humic substance analysed [12]. In order to gain a better understanding of this dependence, 13 different types of IHSS standard humic materials were analysed. The response curves obtained are shown in Fig. 2. Even if, as shown in Table 1, the C content of the various substances differs slightly, similar graphs are obtained when ROM concentrations are expressed as mg of C rather than as mg of ROM as in Fig. 1 (graphs not shown). The strongest signal (Nordic Lake FA) is about eight times higher than the weakest one (Leonardite HA). In general, the signals from fulvic fractions are stronger than those from humic fractions, with the notable exception of Pony Lake FA. However, Pony Lake FA cannot be compared

with other fulvic substances because of its origin. According to the IHSS, this Antarctic FA is formed entirely from biomass that contains no lignin and, as such, it is the sole sample that contains no input from any terrestrially derived



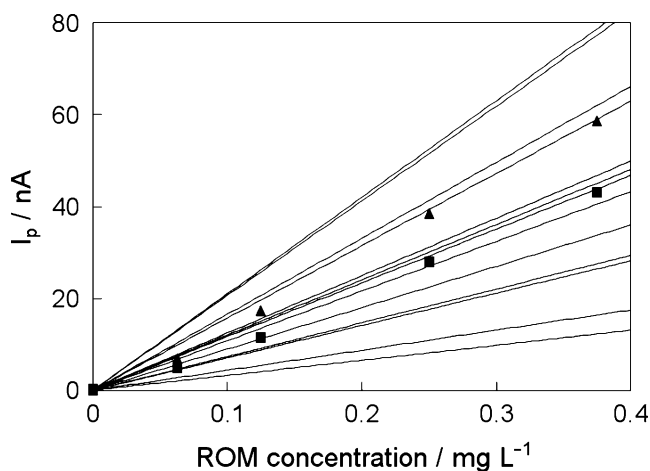
**Fig. 1** Comparison of the intensities obtained by SWV (●) and DPP (▲). DPP and SWV signals are shown in the inset for a solution containing 125  $\mu\text{g L}^{-1}$  Suwannee River FA I after 3-min accumulation at  $-0.200\text{ V}$



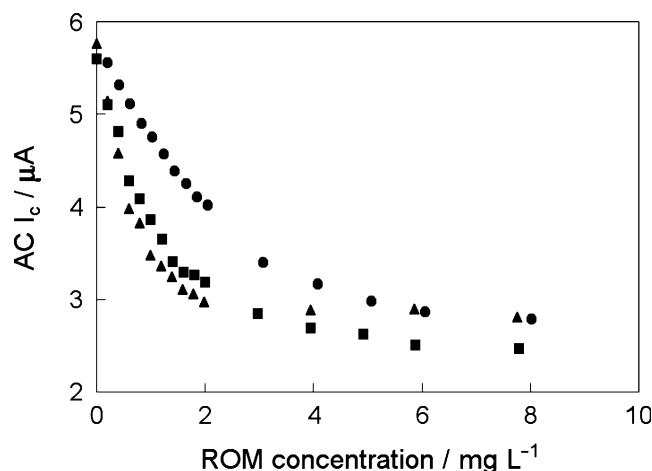
**Fig. 2** SWV response curves for different IHSS standards. Concentrations are expressed in  $\text{mg ROM L}^{-1}$ . *NLFA* Nordic Lake FA (1R105F), *PPFA* Pahokee peat FA (2S103F), *ESFA* Elliott soil FA (2S102F), *SRFAII* Suwannee River FA II (2S101F), *SRFAI* Suwannee River FA I (1S101F), *SRHA* Suwannee River HA (2S101H), *NLHA* Nordic Lake HA (1R105H), *SROM* Suwannee River NOM (1R101N), *NLOM* Nordic Lake NOM (1R108N), *PPHA* Pahokee peat HA (1R103H), *PLFA* Pony Lake FA (1R109F), *ESHA* Elliott soil HA (1S102H), *LHA* Leonardite HA (1S104H)

organic matter. Riverine fulvic and humic acids give very similar responses, confirming previous observations [12]. No statistically significant correlations could be established between the value of the voltammetric signals and parameters such as nitrogen, sulfur, carboxylate or phenolate content or aromaticity (Table 1) for the different substances.

In order to gain further insight into the ROM characteristics generating the signal observed, the SWV response of a synthetic humic substance, HS1500, was tested. HS1500 is an autoxidation product of polyphenols with alkyl bridges. It is characterised by dominant aromatic and quinoid structures with a mean molecular mass of 1.5 kDa. The resulting curve (Fig. 3, squares) is similar to



**Fig. 3** SWV response curves for the synthetic humic substance, HS1500 (■) and ROM isolated from lake water (▲). The response curves for the 13 IHSS substances shown in Fig. 2 are represented as solid lines for comparison

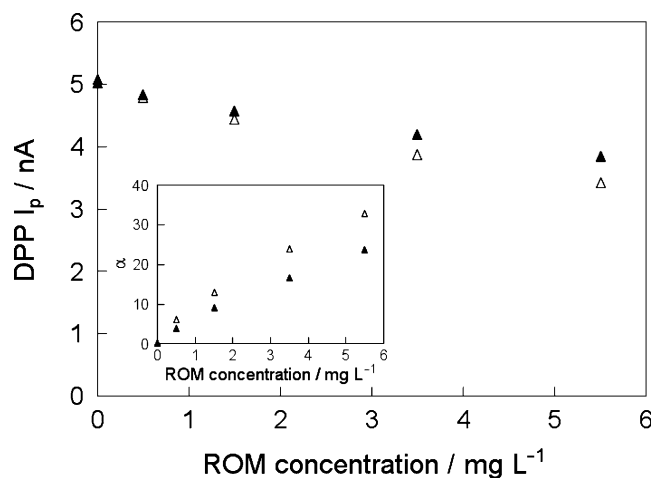


**Fig. 4** Decrease of alternating current as a function of the ROM concentration for Pahokee peat FA II (▲), Suwannee River FA I (■), Leonardite HA (●) after 120-s accumulation at  $-0.200$  V at a phase angle of  $135^\circ$ ,  $f=80$  s $^{-1}$ ,  $\Delta E=20$  mV

the average obtained for natural substances (represented in Fig. 3 by solid lines). Since HS1500 does not have any carboxylate, N- or S-containing groups, this result suggests that these functional groups do not play any role in the voltammetric signal obtained, thereby confirming the abovementioned observation that there was no correlation between the voltammetric signal and these parameters.

#### Electrochemical processes

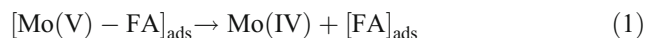
The electrochemical behaviour of Mo(VI) in the presence of FA has already been investigated using SWV [19]. The mechanism proposed involves the adsorption of FA followed by the formation of a surface complex with Mo. This mechanism has been confirmed by obtaining a FA–Mo



**Fig. 5** DPP response as a function of the ROM concentration for Pahokee peat FA II (△) and Leonardite HA (▲). Mo(VI) initial concentration  $5 \times 10^{-6}$  mol  $\text{L}^{-1}$ . The proportion of complexed Mo(VI),  $\alpha$ , as a function of the ROM concentration is shown in the inset



(VI) signal when using different media in the deposition (only ROM present) and the stripping (only Mo(VI) present) steps. At the selected deposition potential, Mo(VI) is reduced to Mo(V) in the vicinity of the electrode and the signal measured in the analytical determination of ROM corresponds to the reduction:



In order to assess the relative weight of the adsorption and complexation processes in the signals obtained from different IHSS substances, additional measurements were taken.

The electrode adsorption of the humic substances was assessed by a series of experiments using ACV. The adsorption of three humic substances that cover the whole range of voltammetric intensities (Pahokee peat FA II, Suwannee River FA I, Leonardite HA) was measured at  $-0.200$  V, which is the potential used for the deposition step in the analytical method. This potential corresponds to a positively charged electrode surface, given that the potential of zero charge is located at  $-0.550$  V in a  $0.5 \text{ mol L}^{-1}$  NaCl medium [20]. Figure 4 shows the decrease in the capacitive current as a function of the concentration of humic substances after 120 s of stirring. The results obtained show that the substances under examination are adsorbed at the potential applied. Adsorption equilibrium parameters were calculated from these data assuming Frumkin and Langmuir isotherms [21]. It was found that, in all cases, adsorption followed Langmuirian behaviour, suggesting that there is no interaction between adsorbed molecules. Langmuir constant values of 2.20, 0.92 and  $0.35 \text{ L mg}^{-1}$  were calculated for Pahokee peat FA II, Suwannee River FA I and Leonardite HA, respectively. Thus, the signal obtained when applying the proposed analytical method is proportional to the degree of adsorption on the electrode.

In order to compare the binding abilities of the different humic substances with Mo(VI), experiments were performed by DPP for the same substances whose adsorption was previously studied. Figure 5 shows that the proportion of Mo(VI) complexed by Pahokee peat FA II, one of the compounds giving the strongest analytical signal, is just slightly higher than that complexed by Leonardite HA, the compound producing the weakest signal. These experiments were performed at a ROM/Mo(VI) ratio ( $5.5 \times 10^{-6}$ ) within the range used in the analytical method (e.g.  $0.4:1 \times 10^{-7}$ ). The complexation results confirm that differences in adsorption onto the electrode rather than differences in complexation strength are most probably responsible for the differences in the intensity of the signal obtained.

#### Choice of the standard

Because of the intrinsic variety of natural ROM, the results obtained using the method are standard-dependent. Al-

though it has been shown that responses for riverine fulvic and humic standards are similar [12], and that these standards are suitable for many freshwaters [13], the choice of standard remains crucial to the application of this analytical method. Clearly, the reference substance used should be as close as possible to the ROM present in the waters being analysed but how to determine which standard is best for any particular case remains an open question. The results of this study bring to light some rules that should be applied when quantifying ROM in freshwaters: (i) in the case of an extensive study of the ROM in a given freshwater system involving many measurements, it is strongly recommended to previously isolate the ROM in the system by following the IHSS procedure and to then use the ROM obtained as the standard; (ii) in cases where this procedure is deemed too cumbersome, or when only occasional measurements in different systems are planned, it is best to use IHSS Suwannee River humic or fulvic standards. For instance, we have isolated ROM from lake water following the procedure applied by Thurman and Malcolm [15] and used it as the standard. The response obtained (Fig. 3, triangles) clearly follows the response obtained by using riverine IHSS standards. As a result, it is clear that using these standards is a sensible choice for most freshwater systems. It is important to point out that the final result varies only slightly whether riverine fulvic or humic acid are used. For instance, analysis of the ROM in a sample of a typical river water gives  $0.19 \text{ mg L}^{-1}$  when using Suwannee River FA I and  $0.20 \text{ mg L}^{-1}$  when Suwannee River HA II is used instead. Needless to say, the reference substance used should always be stated when giving the results.

**Acknowledgements** We would like to thank Thomas Meinelt (Leibniz-Institut of Freshwater Ecology and Inland Fisheries, Berlin, Germany) for the gift of HS1500.

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