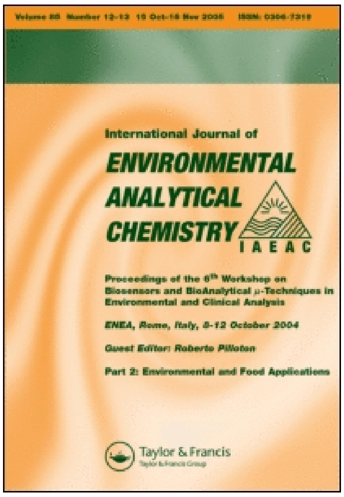

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Application of a refractory organic matter quantification method to wastewater effluents

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An already established analytical method for the quantification of freshwater refractory organic matter (often called humic substances) has been applied to organic matter from a wastewater treatment plant's effluent and a number of downstream locations impacted by the treatment plant. The method is based on measuring the peak currents obtained by adsorptive stripping voltammetry of the complex formed by freshwater refractory organic matter in the presence of trace amounts of Mo(VI). Organic matter is first concentrated by reverse osmosis, then fractionated according to its polarity by the sequential application of DAX-8 and XAD-4 resins. The results obtained show that the voltammetric method measures the refractory organic matter present in the different sewage-derived fractions and that the response obtained follows the trend expected for the fractionation method used (i.e. more hydrophobic fractions largely give the strongest signals). These results have been compared with those obtained from IHSS substances. The results of this study are of particular significance in that it shows that, when applied to surface waters, the voltammetric method measures any refractory organic matter present, irrespective of its origin.

Keywords: refractory organic matter; ROM; HPO; TPI; HPI; SWV; wastewaters; WWTP; ROM quantification

1. Introduction

Many studies have pointed out the importance of organic matter (OM), particularly the fraction which is more refractory to degradation, for many environmental issues ranging from the toxicity of trace elements and micropollutants to the production of undesirable disinfectious by-products to global change. Much research has focused on the fractionation and characterization [1] of the refractory organic matter (ROM), often called humic and fulvic substances (see [2] for a detailed description of the different terms used and their meanings), but very few methods exist for quantifying it. Spectroscopic techniques, such as UV–Vis and fluorescence, are still the most widely used. However, their value as quantification methods remains doubtful because they 'see' only a certain

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fraction of ROM [3], the proportion of which does not remain constant from system to system. In addition, UV–Vis is not sufficiently sensitive for samples with low ROM content. As an alternative, an electroanalytical method based on cathodic stripping preceded by the adsorptive collection of Mo(VI)-humic or fulvic acid complexes has also been proposed recently [4,5]. This method is fast and consumes only small amounts of the sample. It is therefore particularly well suited to environmental studies where a large number of samples needs to be analysed. Moreover, the method has a very low detection limit and can be used reliably in systems which are poor in OM (e.g. [6]).

It is usually assumed that ROM in rivers has two sources: soils and the decomposition of aquogenic biological materials. However, although often not explicitly acknowledged [7], sewage effluents are likely to be major contributors to the refractory pool of OM present in rivers in heavily populated areas. Sewage effluents contain a high concentration of OM, most of which degrades relatively easily in river waters, but they also contain a refractory fraction that will contribute to the riverine pool of ROM. In this study, the voltammetric response of different fractions of OM isolated from the effluent of a wastewater treatment plant (WWTP) and some downstream river locations has been tested in order to ascertain whether this type of ROM is also measured by the analytical method. Prior to analysis, WWTP organic matter was fractionated in operational categories according to its polarity using a well-established method [8]. Since this new analytical method had never before been applied to fractions isolated from river water using this fractionation method, samples from upstream river waters which had not been exposed to WWTP OM were also fractionated and the corresponding fractions analysed.

2. Experimental

2.1 *Apparatus*

All voltammetric measurements were taken with a potentiostat/galvanostat Autolab PGSTAT12 controlled by GPES 4.8 software. A hanging mercury drop electrode (HMDE) Metrohm model 663 VA with a mercury drop size of $0.52\,\mathrm{mm}^2$ was used. All potentials were referred to an Ag/AgCl, $3\,\mathrm{mol\,L^{-1}}$ KCl, reference electrode. The counter electrode was a platinum wire. The square-wave mode (SWV) was used for ROM measurements. The quartz voltammetric cell was thermostated at $25 \pm 0.1^{\circ}\mathrm{C}$ during the measurements to ensure that conditions were reproducible.

2.2 *Reagents*

All chemicals used were of analytical reagent grade except the mineral acid (HCl), which was of Suprapur grade. A $1000\,\mathrm{mg\,L^{-1}}$ stock solution of Mo(VI) (atomic absorption standard, Merck) was diluted as required.

2.3 *Sampling*

Treated water from the Seine-Aval wastewater treatment plant (WWTP), Achères, France, was sampled twice. This WWTP collects over 70% of the dry weather wastewater (combined sewer system) from Paris and the surrounding suburban metropolitan area ($\approx$8 million inhabitants). Wastewaters are treated by primary settling and aerobic activated sludge in this WWTP. Waters from the River Seine were also sampled at two

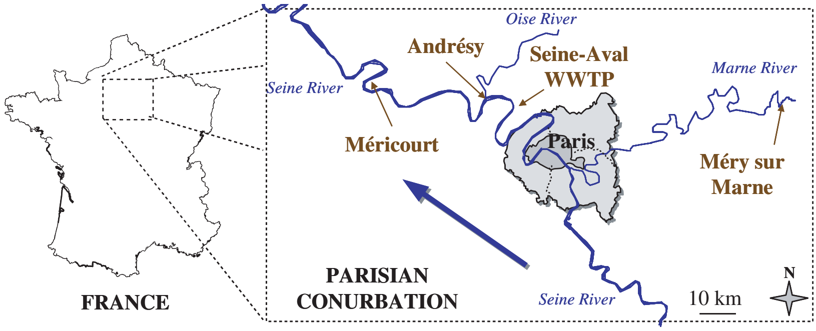

Figure 1. Location of the sampling sites.

Table 1. Location and dates of sampling and main physico-chemical characteristics of the sampled water.

Sampling site	Date	Identifier	Flow/ $m^3 s^{-1}$	Temperature/°C	pH	Conductivity/ $\mu S cm^{-1}$	DOC/ $mg C L^{-1}$
Méry/Marne	30/01/07	M1	80	7	8.5	560	2.0
	27/06/07	M2	35	19	8.3	517	2.0
Seine-Aval WWTP	06/04/06	A1	≈20	17	7.8	1580	38.7
	15/12/06	A2	≈20	14	8.1	1261	17.4
Andrésy	29/06/07	AN	120[a]	18	7.7	606	4.1
Méricourt	03/07/07	MT	120[a]	20	7.6	625	4.0

[a]Measured in Paris (Austerliz).

locations downstream of the WWTP pouring point, Andrésy and Méricourt. Additional samples were taken in the Marne River (Méry-sur-Marne), upstream from Paris. The location of the sampling points is shown in Figure 1. Sampling dates and the main characteristics of the sampled waters can be found in Table 1.

Water samples were collected during a period of dry weather using a double membrane pump (IR ARO®) and sequentially filtered on site through 10 and 0.45 µm polypropylene cartridge filters (Predel®). Subsequent softening and reverse osmosis (RO) concentration were performed in line and on site to reduce operation time and potential OM biodegradation. Prior to RO concentration, samples were softened with a sodium cation-exchange resin in order to eliminate Ca and Mg ions that could co-precipitate with OM and clog RO membranes (Filmtec® TW 30). Conductivity was monitored during RO concentration in order to prevent OM leakage. A final rinsing of the membranes with a 0.05 mol L^{-1} NaOH permitted the recovery of adsorbed OM. Both rinsing waters and RO concentrate were added together and collected in a stainless steel bottle (50 L).

2.4 *Isolation of organic matter fractions*

Once in the laboratory, samples were acidified (0.01 mol L^{-1} HCl) and passed through nonionic macroporous Amberlite® DAX-8 and Supelite® XAD-4 (divynil benzene) resins following the procedure described by Aiken and Leenheer [8]. Hydrophobic (HPO) and transphilic (TPI) fractions were obtained after elution of the resins with a mixture of acetonitrile and water (3:1) and freeze drying. The remaining hydrophilic OM (HPI fraction), neither adsorbed on DAX-8 nor on XAD-4 resins, contains all the salts initially present in the sample and required further purification. The separation of inorganic salts from the HPI fraction was carried out according to a protocol described by Leenheer and co-workers [8,9] which consists of zeotrophic distillation and successive evaporation steps to precipitate the inorganic salts. All Teflon and stainless steel material was washed several times with ultrapure deionised water. Glass material was washed with a detergent (5% TFD4), rinsed thoroughly with ultrapure deionised water and precombusted for 5 h at 500°C. Resins were washed before use by Soxhlet extraction and successive acid and base rinsing in accordance with the protocol described in [10].

2.5 *Measurement procedure*

All solutions were prepared with 18 MΩ.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 each OM fraction were prepared in 0.01 mol L^{-1} NaOH. The procedure used for the measurements is the same as that described in [4]: (i) a small amount of Mo(VI) (10 μg L^{-1}) is added to an initial acidified (0.01 mol L^{-1} HCl) solution containing a given amount of an OM fraction, (ii) the sample is deaerated with nitrogen for 10 minutes, (iii) deposition at –0.2 V is carried out with stirring; (iv) after the deposition time, the stirring is stopped and, following a 20 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; amplitude, 40 mV. Increasing amounts of the given OM fraction were added to the initial solution and the peak currents obtained measured in order to build the corresponding response curve. Typical SW voltammograms are shown in Figure 2. The electrochemical process has been previously described in references [5,11].

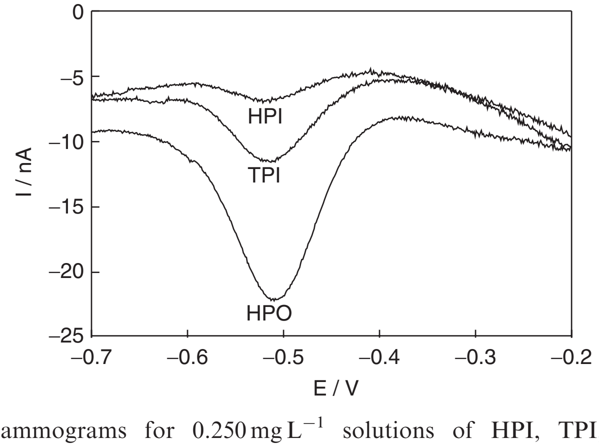

Figure 2. SW voltammograms for 0.250 mg L^{-1} solutions of HPI, TPI, HPO after 3 min accumulation time.

3. Results

Nine different samples of OM related to the WWTP were analysed: four HPO fractions (two from the WWTP and two from downstream samples), four TPI fractions (two from the WWTP and two from downstream samples) and one HPI fraction from the WWTP. The main characteristics of the parent waters as well as the acronyms used to identify samples hereafter and in figures are shown in Table 1. The response curves obtained are shown in Figure 3. Even if, as shown in Table 2, the C-content of the various substances differs slightly, similar graphs are obtained when OM concentrations are expressed as mg of C rather than as mg of OM as in Figure 3 (graph not shown). The results obtained show a linear dependence between the analytical signal and OM concentrations. Signals from HPO fractions are stronger than those from other fractions. The signal from the only HPI

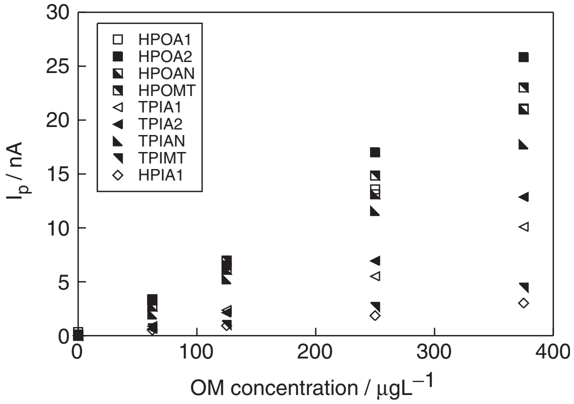

Figure 3. SWV response curves for OM isolated fractions from the WWTP Seine-Aval and the Seine River. OM concentrations are expressed in µg L^{-1}. Meaning of the abbreviations: HPO, hydrophobic; TPI, transphilic; HPI, hydrophilic; A1, WWTP April 2006; A2, WWTP Seine-Aval December 2006; AN: Seine River at Andrésy; MT: Seine River at Méricourt.

Table 2. Elemental composition of isolated fractions [12,13][a].

Sample	C/wt%	H/wt%	O/wt%	N/wt%	S/wt%	Ash
HPOM1	45.0	5.5	35.7	3.0	1.0	9.1
TPIM1	46.0	5.3	36.7	4.8	1.8	2.2
HPOM2	40.4	4.5	29.7	2.8	1.6	12.5
TPIM2	47.3	5.6	37.0	4.9	1.5	4.0
HPOA1	53.6	6.5	27.9	5.7	2.6	2.9
HPOA2	54.1	6.4	29.2	4.4	2.7	4.5
HPOAN	50.2	5.1	32.9	3.4	1.9	5.0
HPOMT	46.5	5.0	31.0	3.1	2.3	4.7
TPIA1	48.4	6.4	28.8	8.4	2.1	4.7
TPIA2	47.3	6.1	34.1	6.9	2.2	2.5
TPIAN	41.4	4.6	35.9	4.9	0.8	14.6
TPIMT	35.4	5.0	29.0	5.5	0.8	7.8
HPIA1	43.8	7.1	29.4	12.3	2.1	5.0

[a] Standard deviation: 0.2.

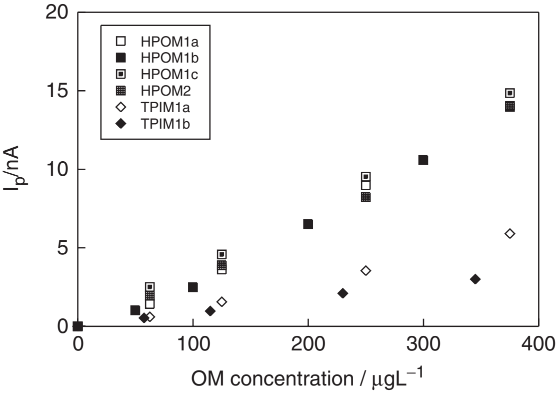

Figure 4. SWV response curves for OM isolated fractions from the Marne River (Méry-sur-Marne). OM concentrations are expressed in $\mu g\,L^{-1}$. Meaning of the abbreviations: HPO, hydrophobic; TPI, transphilic; M1, sampled 30/01/2007; M2, sampled 27/06/2007; a, first analysis; b, purified sample (see text); c, second, independent analysis.

fraction measured is extremely weak. While all HPO signals are fairly similar, there are bigger differences between TPI signals and there is no clear trend among the samples.

River waters which had not been exposed to WWTP effluents were also sampled from the Marne River (Méry-sur-Marne) on two different dates (M1, 30/01/2007, and M2, 27/06/2007). Moreover, (i) one of the HPO fractions was analysed twice on different dates and using independently-prepared solutions (M1a and M1c) and (ii) the HPO and TPI fractions were purified in order to eliminate any remaining inorganic salts (M1b) and analysed again. All response curves are shown in Figure 4. The trends observed for the HPO and TPI fractions were similar to the previous ones. The results obtained for samples M1a and M1c show that the reproducibility of the sampling and fractionation method is good. Differences in HPO and TPI results before and after purification reflect the effect of the presence of excess salt in the unpurified fractions.

4. Discussion

In order to interpret the results obtained, a brief reminder of the basis of the fractionation and measuring methods is required.

The fractionation method – The fractionation scheme applied is based on the definition of operational categories based on polarity: a serial two column array of resins allows the isolation of a 'hydrophobic' (HPO, DAX-8 retained) and 'transphilic' (TPI, XAD-4 retained but not DAX-8 retained) fraction. A relatively complex cleaning procedure makes it possible to isolate a third fraction ('hydrophilic', HPI) not retained by any column. Since the classic operational definition of humic and fulvic acids in water includes the initial isolation of OM by using a DAX-8 resin (and further separation of the fulvic and humic fractions by their pH dependent solubility, thus mimicking the definition initially developed for soils), it is usually assumed that the HPO fraction is equivalent to the fulvic + humic categories and that it, therefore, represents refractory OM, as do fulvics and humics. Nevertheless, it has been known for a long time that isolated humic and fulvic fractions also contain a certain proportion of other hydrophobic, well-defined biochemical categories such as carbohydrates and proteins (see, for instance, the composition of the standard fulvic and humic acids sold by the IHSS at http://www.ihss.gatech.edu/chemistry.html). Similar observations have been published for HPO fractions [14–16]. Moreover, different studies have shown that DAX-8 column elution with water-acetonitrile mixtures elutes about 30% more OM from the column than does NaOH, the traditional eluant in fulvic-humic fractionation procedure [17]. A whole range of organic compounds, ranging from different types of carbohydrates to lipids, proteins and low molecular weight organic compounds are present in the TPI and HPI fractions [13,14]. These compounds will be less hydrophobic and, in principle, more easily degradable than HPO. However, it is important to realise that neither property, hydrophobicity or degradability, shows a clear cut-off in natural OM but rather a continuum that is highly system dependent.

The measuring method – As mentioned in the introduction, the method is based on the cathodic stripping, preceded by the adsorptive collection, of Mo(VI)-ROM complexes. It has been proved that only water, soil and peat fulvic and humic acids, defined as fractions isolated by following the different standard procedures of the IHSS, give a signal, while none of the wide range of carbohydrates and proteins tested do [4].

As expected given the considerations above, the HPO fractions measured in this study for both river and WWTP OM produce a stronger signal than the TPI fractions, highly enriched in carbohydrates and proteins [12], and the HPI fraction gives an even weaker signal. The TPI fractions give a stronger signal than might be expected but this is not really surprising because this fraction 'still' contains a certain number of structures similar to those which are characteristic of the so-called humic substances. Interestingly, these are the fractions that show higher signal variability, which probably reflects the fact that their composition is more highly dependent on the media (i.e. degree of OM decomposition). This point clearly merits further attention. The availability of the voltammetric method may prove very useful for the study of the temporal and spatial dynamics of this OM because it allows for fast analysis of a large number of samples.

Figure 5 shows the comparison of the signals obtained from the different OM fractions measured in this study (hatched zone) and the signal given by 13 IHSS organic compounds studied in [5]. Our fractions give signal strengths between NLOM and peat (PPHA), soil (ESHA) and coal (LHA) humic acids. NLOM is a compound isolated by reverse osmosis and not by following the classic IHSS isolation procedure. It therefore contains all OM fraction considered in this study (i.e. HPO, HPI, TPI). Since WWTP ROM signals fall in the coal-peat zone, it can be deduced that WWTP ROM has a degree of degradation/evolution similar to that of those humic substances.

5. Conclusions

This study confirms that ROM present in wastewater effluents is measured by the voltammetric method. Therefore, when used in real freshwater samples, this analytical method gives a signal that is the composite of the contributions of ROM from any 'natural' origin (soil, aquogenic) and wastewaters. Application of this quantification method presents a clear advantage over the use of empirical corrections of DOC values as is the case nowadays in many fields (e.g. metal speciation modelling, productivity studies). Moreover, a combination of unspecific DOC measurements with specific voltammetric ROM measurements may be an extremely useful tool for following up dynamic processes related to the degradation and fate of wastewater in real systems, as has already happened with the study of the impact of productivity in unpolluted systems [6].

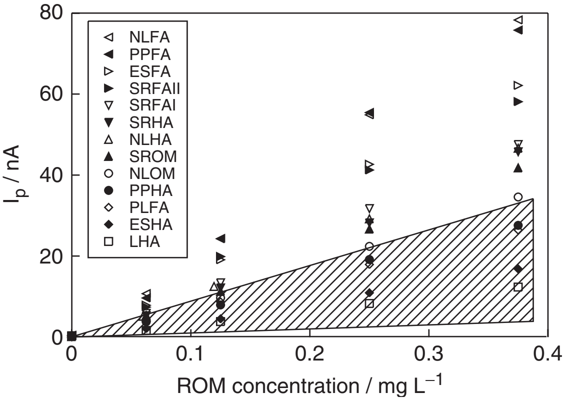

Figure 5. Comparison of SWV response curves obtained for from the WWTP Seine-Aval and the Seine River in this study and 13 IHSS substances (from [5]). Meaning of the abbreviations: NLFA = Nordic Lake FA (1R105F), PPFA = Pakohee Peat FA (2S103F), ESFA = Elliott Soil FA (2S102F), SRFAII = Suwanneee River FA II (2S101F), SRFAI = Suwanneee River FA I (1S101F), SRHA = Suwanneee River HA (2S101H), NLHA = Nordic Lake HA (1R105H), SROM = Suwanneee River NOM (1R101N), NLOM = Nordic Lake NOM (1R108N), PPHA = Pakohee Peat HA (1R103H), PLFA = Pony Lake FA (1R109F), ESHA = Elliott Soil HA (1S102H), LHA = Leonardite HA (1S104H).

Abbreviations

HMDE	hanging mercury drop electrode
HPI	hydrophilic OM fraction
HPO	hydrophobic OM fraction
IHSS	International Humic Substance Society
OM	organic matter
RO	reverse osmosis
ROM	refractory organic matter
SWV	square wave voltammetry
TPI	transphilic OM fraction
WWTP	wastewater treatment plant

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