Dynamic properties of humic matter by dynamic light scattering and voltammetry

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Abstract

The diffusion coefficients of humic matter samples (Purified Peat (PP) Moss and Fluka) in solution were obtained by both dynamic light scattering (DLS) and voltammetric methods. The diffusion coefficients from voltammetric measurements are greater than those determined by DLS, due to the different way each technique responds to the size distribution of polydisperse samples. Large aggregates are present in both PP humic acid (HA) and Fluka samples (diameters larger than 30 nm), their size depending on the origin and/or preparation procedure of the sample, as well as on the experimental conditions of pH and ionic strength. The influence of ionic strength on the diffusion coefficient of PPHA was analysed. Similar results were obtained by DLS and voltammetry in samples with low polydispersity up to ionic strength of 0.1 M. For higher ionic strengths aggregation may occur, leading to an increase in the apparent molecular size. The influence of pH on the diffusion coefficient of PPHA shows one population formed by large aggregates (185 nm diameter) at pH 5 in the absence of added salt. When pH is lowered below 2.5, coagulation occurs quickly leading to the precipitation of PPHA. Some irreversible disaggregation (120 nm diameter) is promoted by pH increase up to 10. PPHA samples, initially set to pH 10 and then adjusted to pH between 3 and 5, present somehow different characteristics from samples directly set to the required pH, which stresses the influence of the preparation method on final sample properties.

Keywords: Voltammetry; Humic matter; Dynamic light scattering

1. Introduction

Humic matter is a complex mixture, formed by random condensation of degradation products of plants and animals. It plays a central role in environment being one of the major complexing components in soils and natural water systems. Due to its amphipatic nature humic matter binds both to hydrophilic and hydrophobic compounds, including metal ions, organic compounds and particles.

Humic matter in natural waters (pH 4–9) behaves as an anionic heterogeneous ligand with many carboxylic and phenolic groups, and presents polyfunctional and polyelectrostatic effects, respectively, due to different functional sites and net charged groups [1]. However, its composition depends on both the extraction source and the purification procedure.
The characterisation of the physical and chemical properties of humic matter is fundamental to understand its role in the fate, reactivity, and transport of inorganic and organic pollutants.

The determination of the structure and dynamics of humic matter is difficult due to the complexity of the system, which has several components: mixture of several polymers, aggregated or not, with a neutral backbone, polyions, counterions, co-ions and solvent. The multicomponent nature of humic matter is reflected in many types of interactions that should be taken into account: segment–segment and segment–solvent interactions, intermolecular interactions between different macromolecules and interactions between polyions and counterions.

With regard to the physical characterisation of the humic matter the most important parameters that strongly affect the diffusion coefficient are size and size distribution. Size determination has been the subject of many experiments using different techniques: gel permeation chromatography/size exclusion chromatography (GPC/SEC) [2,3], ultrafiltration [4–7], viscometry [8], diffusion through an activated carbon column [9], dynamic and static light scattering [10–13], small angle neutron scattering [14], vapour phase osmometry [15], flow field flow fractionation [16] and voltammetry [17]. Additionally, transmission electron microscopy [4,12] and low angle X-ray scattering [18] have also been performed.

Usually the results obtained by different techniques somewhat disagree from each other. Some of the differences can be attributed to the origin of the samples and/or to the distinct purification procedures used. Even when the same sample is studied by different techniques some of the discrepancies are still present. This can be attributed to the specificity of each technique that responds to a specific signal, e.g., number of particles (viscometry), diffusion coefficient (dynamic light scattering). Different signals might reflect different aspects of the same reality, and so they might lead to dissimilar results. Within this scope DLS would be better to detect large particles in the presence of a greater number of smaller ones, while GPC/SEC would be more sensitive to monomeric units.

Different techniques also require different concentration ranges so that any dependence of aggregation on concentration will lead to divergent results (between techniques). Furthermore, another contribution can result from the molecular size dependence on the ionic strength and pH of the system [19], since each technique poses specific problems regarding the control of these variables.

The differences observed may also be associated with the possibility that humic substances may aggregate to the extent of forming micelle-like structures [16,20]. Micelle forming amphiphilic compounds are characterised by the presence, on the same molecule, of hydrophobic regions (aromatic groups and/or aliphatic chains) and hydrophilic groups (phenolic, carboxylic, alcoholic) [12,21], as is the case for humic matter. Nevertheless, experimental evidence for humic matter does not support a purely micellar structure. Instead, the presence of large aggregates in solution has been shown by several authors [11,12,14], and it is thought that these present regions have a micelle-like behaviour.

The different sizes measured with different techniques may also be related to the dynamic character of the humic aggregates. It was observed in dialysis experiments that aggregates larger than the molar mass cut-off of the membrane were found in the dialysed solution [22]. This led to the conclusion that a humic aggregate could partially disaggregate, crossing the membrane and reaggregate on the other side. If such a phenomenon is real then humic matter should respond readily to changes in pH and/or ionic strength, adopting the most favourable conformation (size) under the experimental conditions used.

In this work the diffusion coefficients of humic matter samples, determined by dynamic light scattering for different pH values and ionic strengths, are analysed. Moreover, some experiments were performed using anodic stripping voltammetry (ASV), under the same experimental conditions of pH, ionic strength and humic matter concentration, in order to compare the diffusion coefficient obtained by each method.

2. Experimental

2.1. Reagents

The humic acid (PPHA) was extracted from an Irish moss peat following the standard IHSS
The elemental composition determined on a freeze-dried sample, was C 52.07, H 5.07, N 2.37, S 0.57 and O 39.93% [23], and the stock solution had a concentration of 6 g l\(^{-1}\), corresponding to 1.5 \(\times\) 10\(^{-2}\) M of deprotonated carboxylic groups at pH 7.0. Dissolved organic carbon analysis yielded 3.0 g l\(^{-1}\) of carbon.

The Fluka humic matter was a commercial sample from Fluka (lot/product number 35069288/53680) and the stock solution had a concentration of 1.7 g l\(^{-1}\), corresponding to 7.1 \(\times\) 10\(^{-3}\) M of deprotonated carboxylic groups at pH 7.0.

To obtain the Fluka stock solution the commercial material was treated with AG 50W-X4 ion exchanger (Bio-Rad) to convert the humics into the acid form, and the solution obtained was dialysed against pure water, using Spectrapor dialysis tubing (molar mass cut-off 1000). The other reagents used were pro analysis.

3. Dynamic light scattering experiments

3.1. Fundamentals

The intensity of the scattered light is a function of both the particle number and size [24]. The intensity of scattered light is proportional to the sixth power of size for a constant number of particles, while a dependence of the third power of size is found if a constant weight of particles is considered. Therefore, dynamic light scattering measurements in polydisperse systems underestimate the mean diffusion coefficient, and consequently the average molecular weight is overestimated.

Dynamic light scattering (DLS) is an effective and a quite straightforward technique for determining the hydrodynamic size of species going from large polymer chains to micelles and to microspheres, among others. The output of this technique is the scattered intensity autocorrelation function, \(C(\tau)\), for correlation time, which is given by

\[
C(\tau) = A \left( 1 + \beta \int_0^\infty P(\Gamma) \exp(-\Gamma \tau) d\tau \right),
\]

where \(A\) is a baseline value, \(\beta\) is an instrumental constant and \(\Gamma\) is the characteristic line width of the distribution function \(P(\Gamma)\). The value contains information regarding the diffusion coefficient \(D\) of the scattering species, these two values being related by the following expression:

\[
\Gamma = Dq^2,
\]

where \(q\) is the scattering vector, which is constant for a given observation angle, incident light wavelength, and so forth.

In addition, if one assumes that the scattering species can roughly be taken as spheres, then the apparent hydrodynamic diameter \(d_h\) of the said species can be calculated through the Stokes–Einstein equation:

\[
d_h = \frac{k_B T}{(3\pi\eta D)},
\]

where \(k_B\) is the Boltzmann constant, \(T\) is the absolute temperature and \(\eta\) is the viscosity. The sizes discussed throughout this work are the Stokes–Einstein diameters, which can be considered as apparent diameters since the aggregates in solution, in most cases, are not hard spheres.

The dynamic light scattering results obtained in the form of Eq. (1) have to be analysed by a fitting procedure. The most straightforward procedure is to assume that only one type of scattering species is present, and to fit the data to a single exponential expanded as a Taylor series (cumulants method). However, due to the limitations of this procedure Eq. (1) should be, in well behaved systems, inverted through a proper Laplace inversion algorithm (e.g. CONTIN). This method is extremely sensitive to noise in the experimental data, the results obtained depending on the inversion limits. This is the situation normally found in natural systems, such as the one under study in this work, where aggregates and dust are difficult to eliminate, undermining noticeably the reproducibility of the results of the procedure for the extraction of humic acid, which involved repeated extraction with KOH/HCl to separate the HA from humin and fulvic acid (FA) and the use of HF to separate silicate impurities. An extra extraction by EDTA solution was added at the end to decrease the Fe content. Finally, the HA in its acid form was extensively dialysed, over six months, in Visking dialysis tubing (molecular weight cut-off 12,000–14,000) to remove free acid.
said inversion. Therefore, in the present work only the cumulants method was used to analyse the DLS measurements.

3.2. Apparatus

Light scattering experiments were performed with a multi-angle apparatus from Brookhaven Instruments, using a BI-2030 AT correlator (136 channels). The coherent light source was a Spectra Physics He-Ne laser (λc=632.8 nm, 35 mW). The refractive index matching bath was composed of filtered decalin and the system was thermostated.

3.3. Procedure

All the solutions were directly filtered through 0.45 μm filters (Millipore Millex HA) into DLS glass cells, in order to reduce the amount of dust. Prior to the measurements, the samples were centrifuged for 45 min at 4500 rpm. The experiments were repeated twice. The observation angle used was 90°, unless otherwise stated.

Two distinct procedures were used for sample preparation, mainly in what concerns pH setting:

Method A: pH was adjusted to the required value by addition of 0.1 M KOH;
Method B: the PPHA solution was set to pH 10 with 1 M KOH, stirred overnight, and then the pH was lowered to the required value by addition of HNO₃ (0.1 or 1 M).

The pH was adjusted after filtration to the DLS cell when studying the variation of the hydrodynamic diameter with concentration at pH 5, with time at pH 2.2, and with the observation angle at pH 4.0 (from 150° to 45°, with 15° intervals). When the influence of pH (pH range from 4 to 10) and ionic strength was studied both parameters were adjusted to the required values, respectively, before and after filtration into DLS cells.

4. Voltammetric experiments

4.1. Fundamentals

In voltammetry the system studied incorporates a metal ion M (charge omitted) which is complexed by the humic acid (HA). The metal ion is electroactive and can be reduced to the metal M⁰ which amalgamates to M⁰(Hg):

\[ M + HA \leftrightarrow M - HA \]

\[ \uparrow \]

\[ M⁰(Hg) \]

Considering semi-infinite planar diffusion from the solution to the electrode the equation for the current in voltammetric techniques is

\[ i_m \propto D'c_{M,i}, \]

where \( i_m \) is the measured current, \( r \) is an exponent which depends on the voltammetric technique and \( D \) is the diffusion coefficient of the species which is being reduced at the electrode [25]. In the absence of ligand, \( D \) is \( D_M \) (diffusion coefficient of the metal). In the presence of a macromolecular ligand the signal is proportional to \( D_{ML} \) (diffusion coefficient of the complexed species), as long as the complex is labile and all the metal is complexed so that \( D_{ML}c_{ML} \geq D_{MCM} \).

Therefore, \( D_L \) can be estimated experimentally dividing the measured currents for the metal in the presence of an excess of ligand and in its absence (Eq. (5)), provided that the value of \( D_M \) is known:

\[ \frac{i_{M+L}}{i_{M}} = \frac{(D_L/D_M)^r}. \]

In this work the value of \( 8 \times 10^{-6} \text{cm}^2 \text{s}^{-1} \) was used for the diffusion coefficient of lead ions [27]. For polydisperse systems \( \bar{D}_L \) should be substituted by \( \bar{D}_L \), which represents a mean diffusion coefficient defined by

\[ \bar{D}_L = \sum_i c_{Li}D_{Li}/\sum_i c_{Li}, \]

where \( i \) stands for each size, with an associated \( c_{Li} \) concentration (in molarity) and a \( D_{Li} \) diffusion coefficient.

4.2. Apparatus

The experiments were carried out with an EG&G PAR 273A voltammeter for the PPHA samples. Data acquisition was performed by an IBM PS2/30-286
computer with the Headstart program from EG&G PAR. For the Fluka HA samples a Metrohm E506 voltammeter coupled to a Metrohm 663 VA stand was used.

A hanging mercury drop electrode (HMDE), a saturated calomel electrode and a counter electrode (Pt for the PAR and graphite for the Metrohm) were used as working, reference and auxiliary electrodes, respectively.

The drop radius was 0.042 cm for the PAR and 0.016 cm for the Metrohm mercury electrode.

The pH was checked with a 91 03 SC semimicro Orion electrode.

4.3. Procedure

Humic matter solutions (1.5x10^-4 and 3.0x10^-4 M) were titrated at pH=5 with lead in the concentration range (0.5-6)x10^-7 M, and the titration was followed by anodic stripping voltammetry. The deposition time with stirring was 120 s followed by 30 s without stirring before scanning the potential to more positive values. The applied potential was -0.75 V during the deposition step.

Dissolved oxygen was removed from solutions in the voltammetric cell by bubbling 99.9995% purity nitrogen. All experiments were carried out at (25.0±0.1)°C.

5. Results and discussion

5.1. Dynamic light scattering measurements

Solutions of PPHA prepared by method A (PPHA/A) (30 mg l^-1) in water at pH 5.0 were passed through a 0.45 μm pore diameter filter. The dynamic light scattering (DLS) measurements of the filtrate shows a distribution of particles with 185 nm average diameter. When the solution was passed through a 0.1 μm pore diameter filter, no light scattering signal was detected. This indicates that no significant population of particles with dimensions lower than 100 nm are present in PPHA/A at pH 5 (see Table 1). The addition of KNO3 to the solution (0.05 or 0.5 M), does not allow filtration through a 0.45 μm filter, which becomes blocked probably due to the coagulation of the PPHA.

Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Diameter (nm)</th>
<th>Intensity (kHz)</th>
<th>Diameter (nm)</th>
<th>Intensity (kHz)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0.45 μm</td>
<td>filter</td>
<td>0.1 μm</td>
<td>filter</td>
</tr>
<tr>
<td>PPHA/A</td>
<td>185±9</td>
<td>0.061</td>
<td>0</td>
<td>0.009</td>
</tr>
<tr>
<td>Fluka</td>
<td>148±9</td>
<td>0.073</td>
<td>49±5</td>
<td>0.009</td>
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</table>

The filtrate of the Fluka sample through a 0.45 μm filter shows a distribution of particles with a lower average diameter of 148 nm. Furthermore, a distribution of small particles of 49 nm average diameter was found in the solution after passing it through a 0.1 μm filter. The light scattering intensity is significant, showing that the solution of the Fluka sample contains a large number of small particles (see Table 1).

Fig. 1 shows the variation of the diameter of particles with concentration for solutions of the PPHA/A and Fluka samples at pH 5 in the absence of added salt. No variation of particle dimensions, within experimental error, was detected. For the PPHA/A at concentrations larger than 300 mg L^-1 the solution showed a small deposit of material after centrifugation. Nevertheless, the appearance of aggregates with increase of concentration, as detected in other polyelectrolyte systems [28], is not observed.
Fig. 2. Hydrodynamic diameter (nm) vs. observation angle for a 120 mg l\(^{-1}\) solution of PPHA/A, at pH 4 (○) and 6 (○).

The dependence of the diameter with the scattering angle presented in Fig. 2 indicates that the particles are not spherical and/or the system is polydisperse.

5.2. Influence of pH

Fig. 3a shows the influence of pH on the dimension of particles of a PPHA/A solution (120 mg l\(^{-1}\)) in the absence of added salt.

The large size of the particles (\(~185\) nm) at pH 5.0, might be attributed to some aggregation occurring during the purification process and/or in the stock solution which has a pH around 3 and is rather concentrated (6 g l\(^{-1}\)). It might be real since no significant disaggregation is observed by dilution to 10 mg l\(^{-1}\) (Fig. 1). Between pH 4 and 6 a noticeable decrease in size was observed, with no further variation until pH 9. Such variation is attributed to the partial disruption of the aggregates which were maintained by hydrophobic interactions, attractive electrostatic interactions and/or hydrogen bonding between carboxylate and phenolic groups of different chains. This is consistent with the assumption of a higher diffusion coefficient for PPHA samples at pH \(\geq 6\), that should be made in order to fit the voltammetric and potentiometric results in terms of speciation [29].

A different behaviour is observed, however, with other humic samples. Indeed, some authors found no variation in particle dimensions in the pH range between 4 and 8 [11,12], while others observed even an increase of dimensions with pH increase [19,22] which was attributed to an increase of hydrogen bonding between deprotonated carboxylic groups and phenolic groups and/or to swelling of the aggregates due to repulsion between negative charges.

It should be pointed out that the pH of the solutions initially set at pH 6, 7 and 8 diminishes after the DLS measurements (see Table 2 row X). These solutions were reset to their original pH values, left stirring overnight, and the pH adjusted before another set of DLS measurements. Even after this the pH of the samples initially at pH 7 and 8 still diminishes (see Table 2 row Y). This is explained by the slow proton

<table>
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<th>Table 2</th>
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<td><strong>Variation of pH with time</strong></td>
</tr>
<tr>
<td>Initial pH</td>
</tr>
<tr>
<td>X</td>
</tr>
<tr>
<td>Y</td>
</tr>
</tbody>
</table>

X – pH measurements after the first set of DLS experiments. The pH values were then reset to the initial ones.

Y – after the second set of DLS experiments.
equilibrium caused by some protonation centers not easily accessible in the macromolecule. When the solution is set initially at pH 9.9, a slow decrease of pH was also observed: pH=9.7 (1 h); 9.5 (2.5 h); 9.16 (15 h). However, as can be seen in Fig. 3a the decrease in size with pH increase is almost instantaneous and independent of the slow proton equilibrium.

For PPHA/B (pH set at 10 and then adjusted in the range between 5 and 9), no variation in size is observed at different pH values (Fig. 3b), these solutions being quite stable in terms of DLS measurements over at least one week. Therefore the process occurring at pH 10 should involve the relatively fast reaction of some groups (e.g. phenolic and carboxylic) that are not recovered by pH lowering.

The same methods A and B were applied to the Fluka sample, similar results being obtained by both procedures, and were related more closely to those obtained for PPHA/B (Fig. 3b). This might be due to differences in the purification procedure and different origin of PPHA and Fluka.

Fig. 4 shows the increase of particle diameter in a PPHA/A solution (60 mg l\textsuperscript{-1}) at pH 2.2, which clearly shows the fast process of particle growing until attaining a diameter of 2 μm after 40 min, then precipitating very quickly. The same trend is observed for PPHA/B. For both PPHA samples this was interpreted as being due to the attenuation of the negative charges through protonation and the consequent enhancement of the intermolecular hydrogen bonds and hydrophobic interactions.

5.3. Ionic strength effect

Fig. 5a shows the variation of particle diameter with ionic strength for the solutions prepared by method A at pH 5.0.

The diameter decreases with increase in ionic strength until it becomes 0.1 M, increasing afterwards to values close to those at zero ionic strength. At low

Fig. 4. Hydrodynamic diameter (nm) vs. time for a 60 mg l\textsuperscript{-1} solution of PPHA/A at pH 2.2.

Fig. 5. Hydrodynamic diameter (nm) vs. ionic strength at pH 5. (a) Samples A, concentration used of 30 mg l\textsuperscript{-1}; (b) Samples B, concentration used of 60 mg l\textsuperscript{-1} (Samples A and B defined in Fig. 3); (c) Fluka HA, concentration used 34 mg l\textsuperscript{-1}. 
ionic strength the repulsion between charges leads to an expanded chain with a rod-like conformation. With the screening of the charges by the counterions, the chain collapses and adopts a coil-like conformation, which explains the results until the minimum is reached. The increase in diameter at higher ionic strengths (the maximum value used is \( I = 0.7 \) M, which is the ionic strength of sea water) is attributed to hydrophobic interactions that become favoured by the modification on the solvent properties. The usual effect found in humic matter is the former \([19,28,30]\). However, the second kind of aggregation was also observed in ionomers formed by a hydrophobic backbone to which are attached ionic polar groups \([31-33]\).

Fig. 5b shows the influence of the ionic strength in samples prepared by method B. The diameter decreases significantly until a plateau is attained at \( I = 0.01 \) M, and then no significant variation in the diameter of the particles is observed until \( I = 0.5 \) M, which suggests that the hydrophobic interactions observed for PPHA/A at \( I > 0.1 \) M are not important in this case. This agrees with the suggestion of irreversible chemical modifications in the polymer occurring at high pH that suppress some groups responsible for hydrophobic interactions.

In the Fluka sample it can be seen that the diameter measured by DLS is not influenced by the ionic strength (Fig. 5c). However this cannot be extrapolated to the total mass because DLS is more sensitive to large aggregates and the Fluka sample has populations of different sizes (Table 1).

### 6. Voltammetric measurements

Table 3 shows the voltammetric measurements and the comparison with the analogous DLS measurements for both PPHA and Fluka humic matter.

When the results between both methods agree, it is a good indication that the sample has only one population with low polydispersity. In some cases a large discrepancy in aggregate dimensions is detected between voltammetry and DLS results indicating the presence of a small amount of large aggregates only clearly seen by DLS. Indeed, the techniques probe in different ways the polydispersity, the DLS measurements being more sensitive to large aggregates even when their contribution to the total mass is small (see Section 2). In voltammetric measurements an average value of the diameter is obtained which is not substantially modified by a small population of large aggregates. This explains the results at \( I = 0.5 \) M for PPHA. Although the increase of ionic strength favours a more coiled structure, reflected by the lower diameter measured by voltammetry, a small part of the total mass is further aggregated which strongly affects the DLS results.

Note that both methods of preparation of PPHA solutions lead to aggregates of different polydisper-

<table>
<thead>
<tr>
<th>Sample</th>
<th>KNO₃ (M)</th>
<th>( D_L ) ( (\text{cm}^2\text{s}^{-1}) \times 10^8 )</th>
<th>Diameter (nm)</th>
<th>( D_L ) ( (\text{cm}^2\text{s}^{-1}) \times 10^8 )</th>
<th>Diameter (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>\begin{tabular}{c} voltammetry \end{tabular}</td>
<td>\begin{tabular}{c} voltammetry \end{tabular}</td>
<td>\begin{tabular}{c} light scattering \end{tabular}</td>
<td>\begin{tabular}{c} light scattering \end{tabular}</td>
</tr>
<tr>
<td>PPHA/A</td>
<td>0.0</td>
<td>5.0</td>
<td>87.5</td>
<td>2.3</td>
<td>185</td>
</tr>
<tr>
<td></td>
<td>0.02</td>
<td>4.4</td>
<td>99</td>
<td>4.4</td>
<td>100</td>
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<tr>
<td></td>
<td>0.02</td>
<td>4.9</td>
<td>90</td>
<td>4.4</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>6.7</td>
<td>65</td>
<td>2.4</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>PPHA/B</td>
<td>0.0</td>
<td>8.4</td>
<td>52</td>
<td>3.6</td>
<td>120</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>12.7</td>
<td>36</td>
<td>4.4</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>—</td>
<td>—</td>
<td>4.9</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>—</td>
<td>—</td>
<td>5.8</td>
<td>75</td>
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<tr>
<td>Fluka</td>
<td>0.01</td>
<td>9.1</td>
<td>48</td>
<td>8.6</td>
<td>51(^a)</td>
</tr>
<tr>
<td></td>
<td>0.01</td>
<td>9.1</td>
<td>48</td>
<td>3.1</td>
<td>142</td>
</tr>
</tbody>
</table>

\(^a\) Sample filtered through a 0.1 m filter.
sity. While method B produces polydisperse samples, method A seems to produce one monodisperse population at $T < 0.1 \text{ M}$.

In the case of the Fluka sample, filtered through a 0.1 $\mu\text{m}$ filter, the solution seems to be composed of almost monodisperse particles that lead to a close agreement between both techniques (voltammetry and DLS). Those particles are much smaller than those detected by DLS in samples filtered through a 0.45 $\mu\text{m}$ filter, which means that large aggregates (>140 nm) should be in much lower concentration than those of 51 nm, otherwise larger diameters would be obtained by voltammetry.

7. Conclusions

The results from DLS and voltammetry show that humic matter present in solution is aggregated in relatively large particles (>30 nm) for PPHA and Fluka samples. Furthermore, only large aggregates ($\approx$ 185 nm), formed during the purification process, are present in PPHA/A. This is not the usual behaviour of polyelectrolytes, where only a small amount of large aggregates contributes to the diffusion mode, and those are usually destroyed by an appreciable dilution of the sample [28].

The aggregates present in solution seem to be of a dynamic nature being influenced by changes of pH and/or ionic strength, although not always in the same way for samples of different origins and/or with different preparation procedures. Humic matter, formed by polymer coils expanded at low ionic strength due to charge repulsion, tends to be coil-like if enough supporting electrolyte is added, which is reflected in the decrease of the hydrodynamic diameter of PPHA samples when the ionic strength increases. By combining DLS and voltammetric measurements, it is found that aggregates with different sizes co-exist for all samples, their relative amount, structure and configuration depending on the sample preparation and on its origin.

The different behaviour presented by PPHA prepared by methods A and B, when the ionic strength and/or the pH are changed, stresses the fact that some properties of humic substances strongly depend on the preparation method.

Symbols and abbreviations

$\Lambda$ baseline value of $C(\tau)$

ASV anodic stripping voltammetry

$c_x$ concentration of the species $x$

$C(\tau)$ scattered intensity autocorrelation function

$d_h$ apparent hydrodynamic diameter

$D_x$ diffusion coefficient of the species $x$

$\bar{D}_L$ mean diffusion coefficient of the ligand

DLS dynamic light scattering

GPC/SEC gel permeation chromatography/size exclusion chromatography

HA humic acid

HMDE hanging mercury drop electrode

$I$ ionic strength

$I_m$ measured current in voltammetry

$k_B$ Boltzmann constant

$M$ free metal ion

$M'(\text{Hg})$ amalgamated metal (in the mercury electrode)

ML complexed metal

$P(\Gamma)$ distribution function

PPHA purified peat humic acid

PPHA/A PPHA prepared by method A

PPHA/B PPHA prepared by method B

$q$ scattering vector

$r$ exponent which depends on the voltammetric technique

$T$ absolute temperature

Greek Symbols

$\beta$ instrumental constant of $C(\tau)$

$\tau$ correlation time

$\Gamma$ characteristic line width of $P(\Gamma)$

$\eta$ viscosity

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