Fluorescence technique for the characterization of natural organic matter in river water

U.K. Ahmad*, Z. Ujang**, Z. Yusop** and T.L. Fong**

* Department of Chemistry, Faculty of Science, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia
** Institute of Environmental & Water Resource Management, Universiti Teknologi Malaysia, 81310 UTM Skudai, Johor, Malaysia

Abstract The complex nature of natural organic matter (NOM), and the impact of this matter on drinking water quality have necessitated the characterization studies of NOM. A fluorescence technique for the characterization of NOM in Malaysian river water is reported. Water samples from several river sampling sites were collected and concentrated using a low-pressure reverse osmosis (LPROM). Solid phase extraction (SPE) using C18 extraction cartridges were used to fractionate the water samples into humic and non-humic fractions. To differentiate and classify various types of humic substances, fluorescence was applied in emission, excitation and in synchronous-scan modes. A synchronous spectral profile was found to be able to differentiate humic and fulvic acids better than the emission or excitation spectra. Synchronous excitation spectra showed different spectral patterns for the water samples due to different origin. All water samples showed the presence of both fulvic and humic acids.

Keywords Fulvic acid; humic acids; natural organic matter; synchronous fluorescence spectra

Introduction

Natural organic matter (NOM) occurs ubiquitously in surface water and consists of humic and non-humic components. NOM is a general term used to describe the complex matrix of organic material that is present in natural waters (Owen et al., 1993). It is derived from external as well as internal sources, i.e. the humates may be formed from phytoplankton in the water or they may be leached into the aquatic environment from terrestrial plants, leaf litter soil or subsurface deposits (Thurman, 1985). Recently, characterization and removal of NOM have been a major research interest in conventional water treatment. This is due to the growing international concern over chlorinated humic substances in drinking water, which are associated with the potential cancer precursor in humans, as well as low drinking water quality (Edzwald et al., 1985).

NOM contributes to odor, taste, colour and acidity problems in water supply. It causes the yellow or brown color in water, which is aesthetically unpleasant to consumers. Colour removal is the very first issue aroused in the drinking water production industry associated with NOM. The presence of NOM also influences the water quality by increasing the transport and distribution of inorganic and organic micro-pollutants such as pesticides and hydrocarbons because of its metal complexation capacity and interactive properties with organic pollutants.

The study of NOM has attracted increased attention in recent years due to its influence in many aspects of water treatment, including the behavior of unit processes. In the coagulation and flocculation process, NOM chelates with aluminium and iron salts for flocculation to the extent that the concentration and character of the NOM present controls the dosage requirements for coagulation. The coloured humic substances also increase the demand of coagulants and also increase the residual aluminium in treated water. They compete with other pollutants for adsorption sites on activated carbon. During the oxidation steps in the water treatment process, toxic organic substances may be produced and distributed.
Furthermore, NOM has been identified as one of the major precursors for potential trihalomethane (THM) formation in the chlorination process. NOM also serves as a substrate for undesirable biological growth in the distribution system. Thus, all these health implications have led to suspicion of the safety of public water supply systems.

At present, limited information about NOM in surface waters in Malaysia has been available, especially its characterization and the implications for water treatment processes. As the characteristics of NOM vary with different origins, the information about NOM gathered from the experience of other countries might not be applicable for local situation. Hence, characterization of NOM is essential in order to provide insights and additional comprehension into water treatment process selection and operations (Vuorio et al., 1998).

As a result of the heterogeneous and ill-defined character of NOM, no analytical techniques are available to measure humic substances and color bodies directly (Aiken, 1985). The present conventional water quality monitoring in Malaysia only focuses on parameters such as turbidity, suspended solids, color and microbial removal which are not suitable for use as analytical parameters to identify the presence of NOM. Without this information, it is difficult to compare treatment efficiencies or to predict what treatment strategies should be carried out in order to tackle NOM problem. Thus, a rapid and efficient monitoring protocol needs to be developed.

Fluorescence techniques have recently been utilized for measuring the content of organic matter in water. Fluorescence spectroscopy has been used to study the molecular and quantitative aspects of the chemistry of fulvic acid and its interactions with metal ions and organic chemicals (Senesi, 1990). Hautala et al. (2000) found that the humic matter content which fluoresces most is fulvic acid and which absorbs most is humic acid. Marhaba (2000) developed a spectral fluorescent signature as a tool for the rapid identification of dissolved organic matter (DOM) fractions in water through a multiple linear regression model. Kalbitz et al. (2000) also conducted similar studies aimed at testing the use of less time-consuming spectroscopic methods applied for original water samples in order to obtain information about DOM composition without any sample preparation.

Synchronous fluorescence spectroscopy (SFS) is a method wherein simultaneous scanning of both excitation and emission spectra is performed at a constant offset value or difference between the emission and excitation wavelengths, \( \Delta \lambda (\Delta \lambda = \lambda_{em} - \lambda_{ex}) \) (Galapate et al., 1998). SFS also provides better structured and resolved peaks compared to other conventional fluorescence methods, allowing one to differentiate the fluorescence spectra of samples of various origins. Furthermore, this method has relatively high sensitivity, which allows studies on natural aquatic samples with low organic matter content without pre-concentration and pre-separation. Synchronous fluorescence spectroscopy has been used for marine DOM and soil fulvic acid after fractionation by reverse-phase high performance liquid chromatography (RP-HPLC) or extraction using C_{18} Sep-Pak cartridges respectively (Lombardi and Jardim, 1999).

A preliminary study was conducted on the characterization of NOM in river water. This paper presents selected results and observations from this study using fluorescence techniques.

**Experimental**

**Sampling**

Water sampling was carried out at Parit Raja Water Treatment Plant, Johor and Sungai Sireh Water Treatment Plant, Tanjung Karang as two case studies for the preliminary research on NOM characterization. Parit Raja Water Treatment Plant occasionally receives colored water with low pH and faces the problem of colour removal, as well as the formation of THMs. Sungai Sireh Water Treatment Plant receives dark coloured water from a
peat swamp forest and the Main Irrigation Canal. River water samples were collected three times between the months of July to September 2000, as shown in Table 1. All of the water samples were taken at the intake point of the water treatment plants except for the peat swamp canal water sample.

The first sampling was conducted at three water treatment plants in Batu Pahat district, Johor. Water samples were taken from three rivers namely Simpang Kiri River, Bekok River and Sembrong River. Rain was not recorded for five days prior to the sampling date. Based on the DOC values of the water samples, the following sampling was focused on the Sembrong River. A 120 L water sample from Parit Raja Water Treatment Plant intake point was taken along with three other bottles of water samples (1 L) for general water quality analysis. It rained every day before the sampling date but the weather was fine on the day of sampling. The 120 L water sample was later concentrated using a Low Pressure Reverse Osmosis Membrane (LROM) unit in the laboratory. Samples were labelled as Parit Raja-Filtered (PR-fil) and Parit Raja Concentrated (PR-conc).

For the third sampling, water samples were taken from sampling points at two irrigation canals in Tanjung Karang, Selangor. The weather was fine and rain was not recorded in that particular area for a week prior to sampling. One sampling point was situated in front of the intake point of Sungai Sireh Water Treatment Plant, which receives raw water from the Main Canal. Another sampling site was situated at the point adjoining a Peat Swamp Canal to the Main Canal in which the water originating from the peat swamp forest was dark in colour. 200 L of dark coloured water from the Peat Swamp Canal was sampled for concentration purposes.

Polyethylene containers (1 L) were used for sampling. The containers were pre-washed by rinsing with successive nitric acid (10%) and then with the river water before sampling. Three bottles of water samples were collected at each sampling points. One bottle of sample was not preserved, while the other two samples were preserved by using HNO₃ and H₂SO₄ respectively. All samples were stored in the freezer at 4°C, and water quality analysis was performed within the storage duration recommended by Standard Methods (APHA, 1995).

Sites description
The sampling sites are situated in Batu Pahat district, Johor. Parit Raja and Yong Peng Water Treatment Plants are managed by the Southern Water Engineerings and Southern Waters Sdn. Bhd., whereas Parit Sulong Water Treatment Plant is under the management of Syarikat Air Johor. All plants in Batu Pahat district are surrounded by peat swamps, palm oil plantations and sago forest. There are two dams nearby namely Empangan Bekok and Empangan Sembrong which were constructed for flood mitigation as well as to provide sufficient water supply for water treatment plant operation. Industrial activities are not found in this area and thus no pollution from industries contributes to the organic matter content in these three rivers.

<table>
<thead>
<tr>
<th>Table 1 Sampling site for the study</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sampling date</strong></td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>August 2000</td>
</tr>
<tr>
<td>September 2000</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
Tanjung Karang Main Canal and Peat Swamp Canal are located near to Sungai Karang Forest Reserves in the northwest of Selangor. Before being constituted as a forest reserve in 1990, logging activity on a rotational basis was active in this area. The land-uses in the adjoining forest reserve are the Tanjung Karang Irrigation Project to the south west and west for paddy production, palm oil estates owned by small holders to the north and northwest. The peat swamp forest is separated from the irrigation scheme in the southwest by the Main Irrigation Canal. The peat swamp forests including Tengi River and Dusun River together with Bernam Catchment are important sources of water for domestic and irrigation uses.

Sungai Sireh Water Treatment Plant operated by the Department of Water Supply, Selangor is situated along the Main Irrigation Canal. The water source comes from the Tengi River Headworks and also from the extraction canals inside the peat swamp forest. During the wet season, the dark coloured water from the Peat Swamp Canals flows into the Main Canal and contributes to the raw water source of the water treatment plant. During the dry season or low flow, the water from the Main Canal flows down from the hilly area. Thus, colour and NOM problems are more critical during high flow or the wet season compared to the dry season.

Reagents and materials
All chemicals used in the experiments are of analytical grade. Humic acid standard was purchased from Fluka, Switzerland. Suwanee River Fulvic Acid (SFA) reference, Suwanee River Natural Organic Matter (S-NOM) reference and Peat Swamp Humic Acid (PS-HA) standard were obtained from the International Humic Substances Society (IHSS), USA. SPE C18 cartridges (6 mL) used in the experiment were supplied by Supelco, USA.

Apparatus
Fluorescence spectroscopy was performed using a Perkin Elmer LS50B Luminescence Spectrometer (USA). Solid phase extraction was carried out using a Baker SPE-10 vacuum manifold (J.T. Baker, UK) and extraction aided using a water aspirator (Eyela A-3S, Tokyo).

Preparation of standard solution
*Fluka Humic Acid standard solution.* 0.05 g of Fluka Humic Acid (FHA) was dissolved in 100 mL distilled water and to remove the undissolved ash in solution, it was further filtered through a membrane filter (cellulose nitrate, 0.45 µm) using a vacuum pump. The filtrate was diluted to 500 mL with distilled water. The resultant stock solution was considered as 100 ppm and its pH was recorded. Standard solutions with concentrations ranging from 1 to 90 ppm were prepared for spectroscopic analysis.

*Suwanee River Fulvic Acid and NOM reference solution.* SFA and S-NOM references do not require any pretreatment before usage (Schlautman and Morgan, 1994). A 100 ppm stock solution was prepared by dissolving 0.01 g of reference sample using deionized water and diluted to the desired concentrations (10–50 ppm) for analysis. Analytical procedures similar to the FHA standard were used for both references.

*Peat Swamp Humic Acid standard solution.* A 100 ppm stock solution of PS-HA was prepared by dissolving 0.01 g PS-HA in a 100 mL volumetric flask using 0.1 M NaOH. The stock solution was then diluted (10–50 ppm) with the same solvent.
Isolation and concentration using low pressure reverse osmosis membrane

A commercial LPROM supplied by Delcol Marketing (M) Sdn. Bhd. was customized as the water concentration unit in this study. The concentration unit using LPROM was designed and modified based on the principle of PROS/2S RO unit developed by Serkiz and Perdue (1990) for NOM isolation and concentration purposes. Specifications of LPROM used are shown in Table 2.

Figure 1 shows the components and processes of the LPROM unit. Two pumps were used in the system. One was used to pump raw water from a water tank (50 L) and the other one was a booster pump used to pump water into the LPROM after being pre-filtered by two fiber filters. The concentrate was recycled into the water tank while the permeate was collected in a permeate tank. The flow-rate of both permeate and concentrate recycled were monitored from time to time. 120 L of PR water and 200 L of PSC water were concentrated to 5 L, and labelled as PR-conc and PSC-conc.

Solid Phase Extraction (SPE)

PR and PSC water samples were concentrated using the LPROM unit before being subjected to SPE. Both filtered and concentrated water samples were acidified to pH < 2 before performing the extraction using SPE C18 cartridges. The SPE cartridge was conditioned using 0.3 × 10^{-3} M HCl (5 mL), methanol (5 mL) and deionized water (5 mL) as described in Lombardi and Jardim (1999). 500 mL of water sample was then passed through the cartridge using a vacuum pump system except for the 50 mL of concentrated PSC. Water samples which passed through the cartridge were collected in a universal vial (28 mL) and labeled as non-extracted fractions of each water sample. Before elution of the adsorbed analytes in water samples, the cartridge was rinsed with 20 mL of deionized water. The fraction of dissolved organic matter (DOM) in water samples was eluted using 20 mL of methanol: water (85:15, v/v) solution followed by 100% methanol and the eluate was collected in a universal vial (28 mL). Two types of elution solvent composition were used and the results were compared using fluorescence spectroscopy.

Table 2 Specification of LPROM unit

<table>
<thead>
<tr>
<th>Items</th>
<th>Specifications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane material</td>
<td>Polyamide</td>
</tr>
<tr>
<td>Membrane configuration</td>
<td>Spiral wound, 10 inch</td>
</tr>
<tr>
<td>Membrane pore size</td>
<td>1 × 10^{-4} µm</td>
</tr>
<tr>
<td>Fiber filter pore size</td>
<td>5 µm</td>
</tr>
<tr>
<td>Booster pump pressure</td>
<td>60–110 psi</td>
</tr>
<tr>
<td>Flow rate</td>
<td>1.5 L/hr</td>
</tr>
</tbody>
</table>

Figure 1 Schematic diagram of the LPROM unit diagram
One procedural blank was performed for every time extraction. Deionized water was used as a blank which underwent the same SPE procedure. Procedural blanks are necessary in order to minimize artifacts due to sample preparation steps. Extracted and non-extracted SPE fractions were characterized by fluorescence spectroscopy using the same instrument setting. The extracted fraction was labelled as ext while the non-extracted fraction was labeled as non-ext.

Fluorescence spectroscopy analysis
Fluorescence properties were studied using a luminescence spectrophotometer. The fluorescence was measured using a 5 cm quartz cell. The scan velocity for fluorescence spectroscopy was adjusted to 200 nm min⁻¹ for both emission and excitation monochromators. All spectra were recorded with a 10 nm slit-width for the emission monochromator and a 5 nm for the excitation monochromator. Distilled water was used as blank except for FHA, PSHA, SPE extracted samples. The solvents used to prepare these solutions were used as blanks.

Three types of spectra were recorded as in Table 3. Fluorescence emission spectra were recorded from 370 nm to 600 nm with a fixed excitation at 350 nm. Excitation spectra were obtained with a fixed emission at 560 nm and a scan range from 300 to 550 nm. Synchronous excitation spectra were recorded from 300 nm to 600 nm with a Δλ = 18 nm. The values of excitation and emission wavelengths used in this investigation are those as used in marine DOM research (Lombardi and Jardim, 1999) while the synchronous scan (Δλ = 18 nm) used was as suggested in a previous work (Senesi, 1990).

Wavelengths and intensity of peaks were recorded and compared with the fluorescence spectra acquired from IHSS (International Humic Substances Society information sheet). Calibration curves were plotted using synchronous spectra intensity and standard solution concentrations.

Results and discussion
Fluorescence spectrophotometric analysis
Fluorescence spectrophotometric studies on standard humic substances have been conducted in order to get three spectrums, namely emission spectra, excitation spectra and synchronous spectra. Humic substances have fluorescent properties mainly because of the presence of aromatic moieties. Figure 2a shows the emission spectra of standard solutions. All the emission spectra gave a broad band with overall intensity and wavelength maxima (λmax) varying for different types of standards. Both SFA and S-NOM have a similar spectra pattern with a λmax at 441.68 nm. while PS-HA and FHA have λmax at 453.7 nm and 459.28 nm respectively. FHA and PS-HA showed lower intensity and a flat spectrum compared to SFA and S-NOM. FHA also shows a shoulder at a wavelength of 533.10 nm. It is very difficult to differentiate between fulvic acid and humic acid through emission spectra because all the fluorescent fractions seem to be overlapping.

The excitation spectra of standard solutions (Figure 2b) mainly showed low intensity profiles, however spectra for fulvic acid and humic acid differ significantly. SFA and S-

<table>
<thead>
<tr>
<th>Table 3 Fluorescence spectroscopy setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spectra</td>
</tr>
<tr>
<td>----------</td>
</tr>
<tr>
<td>Scan range (nm)</td>
</tr>
<tr>
<td>Fixed wavelength (nm)</td>
</tr>
<tr>
<td>Scan speed (nm/sec)</td>
</tr>
<tr>
<td>Slit width (cm)</td>
</tr>
</tbody>
</table>
NOM have a similar spectral pattern with $\lambda_{\text{max}}$ at 347.58 nm and 392.71 nm while FHA showed two peaks at 393.48 nm and 470.57 nm and PS-HA exhibits $\lambda_{\text{max}}$ at 482.49 nm. Humic acid generally shows $\lambda_{\text{max}}$ at a longer wavelength compared to fulvic acid. However, excitation spectra are not suitable for a quantitative study of humic substances due to their low intensity.

Figure 3 shows the synchronous spectra of standards solutions. The synchronous spectral profile produced better resolved peaks to distinguish clearly between humic acid and fulvic acid. Synchronous spectra of S-NOM gave similar profile as that for the SFA. This showed that S-NOM consisted largely of fulvic acid. Both SFA and S-NOM showed $\lambda_{\text{max}}$ at 391.62. FHA showed several peaks at 407 nm, 442 nm, 472 nm and 514 nm while PS-HA gave $\lambda_{\text{max}}$ at 481 nm. It was thought that $\lambda_{\text{max}}$’s of FHA at shorter wavelengths such as 407 nm and 442 nm were primarily due to the presence of fulvic acid. The commercial humic acid used in the study was not 100% pure but contained a mixture of humic acid, fulvic acid and some other impurities. This was supported by the study of Malcolm and MacCarthy (1986) who stated that commercial humic acid also contained a mixture of fulvic acid and ash.

Apart from using synchronous spectra pattern to differentiate different fractions of humic substances, the intensity of synchronous spectral peaks can be used to quantify humic substances. Figure 4 shows the calibration curves plotted between S-NOM synchronous spectra intensity at 441.68 nm and concentrations. The intensity was linear with S-NOM concentration and gave a correlation coefficient, $r^2$ value of 0.9745. Figure 4 is useful in estimating the humic substances in real water samples directly without having to fractionate the samples into fulvic and humic acid.
Synchronous-scan fluorescence was found to be the most suitable technique for the analysis of different aquatic humic solutes. The synchronous-scan technique was capable of increasing the chance of obtaining spectra having a resolved structure by decreasing the adverse effects of two main sources of diffuseness in conventional fluorescence spectra, i.e. the intrinsic broadness and the severe overlapping bands (Senesi, 1990).

Figure 5a shows the synchronous spectra of three different filtered water samples. Different patterns of synchronous spectra were observed for different water sample origins, thus synchronous spectra can be used for distinguishing different origin of water samples. PSC-fil has a right shift in \( \lambda_{\text{max}} \) compared to PR-fil and MC-fil indicating that the water sample was dominated by humic acid. The presence of both fulvic acid and humic acid were confirmed through the synchronous spectra. The presence of fulvic acid was confirmed through the \( \lambda_{\text{max}} \) which ranged from 390 nm to 405 nm while \( \lambda_{\text{max}} \) at 470 nm and shoulders at 500 nm indicated the presence of humic acid. From the \( \lambda_{\text{max}} \), it can be concluded the humic acid content is highest in PSC followed by PR and MC samples.

Figure 5b shows the synchronous spectra of PR water samples. After concentration using LPROM unit, two major peaks were observed at 349.72 nm and 390.5 nm. From the peaks, the dominant fluorophore in the PR-conc sample was fulvic acid. Synchronous spectra of SFA which shows the same maximum peak at 391 nm is evidence to support the conclusion. From the synchronous spectra, \( \lambda_{\text{max}} \) at 350 nm indicated the fraction which passed through the SPE cartridge consisted mainly of the hydrophilic fraction with high polarity. After SPE extraction, one single peak with wavelength at 407 nm was observed. It was believed to be the same peak as the 390.5 nm in the PR-conc which showed the presence of fulvic acid. A shift to longer wavelength was probably due to the molecular structure effect and also the elution solvent.

Based on fluorescence spectra intensity, humic substance solutions can be divided into roughly two groups based on their fluorescent behavior, a highly fluorescent group largely dominated by fulvic acid and low molecular weight fraction, and a weakly fluorescent group mainly consisting of high molecular weight fractions and humic acid. The most fluorescent humic-matter solutions which were relatively low in their molecular size composition are in good agreement with the results of Hautala et al. (2000). This phenomenon has been explained as the particular effect of molecular structure on the fluorophores which will decrease with increasing molecular size of humic solutes (Senesi, 1990). It may be caused by the closed position of hydrophobic subunits (namely aromatic moieties) inside the macromolecular skeleton masked by hydrophilic groups. In general, electron-withdrawing groups (such as OH, NH₂) decrease and the electron-donating groups (e.g. COOH) increase the intensity of fluorescence in aromatic compounds. Carbonyl containing substituents, hydroxyl, alkoxyl and amino groups tend to shift fluorescence to longer wavelengths (Senesi, 1990). Structural factors in samples which fluoresce at long wavelengths

![Figure 5](https://iwaponline.com/wst/article-pdf/46/9/117/426427/117.pdf)

**Figure 5** Synchronous spectra of (a) various water samples and (b) Parit Raja Water Samples
with low intensity (normally humic acid) could be the linearly-condensed aromatic rings and other unsaturated bond systems capable of a high degree of conjugation. Humic acid is the group with higher molecular weight compared to fulvic acid. All water samples showed the presence of both fulvic and humic acids.

**Conclusion**

The characterization of NOM in river water using fluorescence techniques has been developed. Fluorescence was applied in the emission, excitation and in synchronous-scan modes. The synchronous spectral profile was found to be able to differentiate humic and fulvic acids better than the emission or excitation spectra. Synchronous excitation spectra showed different spectral patterns for the water samples due to different origin. All water samples showed the presence of both fulvic and humic acids.

**Acknowledgement**

Thanks are due to Universiti Teknologi Malaysia for financial support under the short term grant (RMC Vote no 71639).

**References**


