Laser diagnostics of humic substances as natural detoxicants and fluorescent indicators of the pollutants in water

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Role of humic substances

In remote laser sensing of water medium there are a lot of problems concerning the investigation of the fluorescence of the humic substances (HS). HS are present in different concentrations in all natural water reservoirs and have many ecological functions – concentration of chemical elements and energy, heat conditions regulation, chemical solutions transfer. One of the most important functions of HS – combining with different solutions and elements harmful for the living organisms.
Main targets of the work

- To define the molecular photophysical parameters of the chosen toxicants and HS fluorophores using the nonlinear and kinetic laser fluorimetry methods.
- To find out the possibility of using HS as a fluorescent indicator of the toxicant presence in natural waters.
- To try to distinguish different types of toxicants, therefore we used the representative of polyaromatic hydrocarbons - pyrene, and the representative of the heavy metal salts – uranyl.
Equipment

- The photophysical parameter’s measurements of HS and toxicants were made on a laser spectrometer. For excitations was used the 4-th harmonic of Nd\(^{3+}\):YAG laser radiation. Wavelength – 266 nm. Duration and frequency of the impulses reiteration are 10 ns and 10 Hz correspondingly. Impulse energy is 0.3 mJ, the photon flux density of the excitation radiation on 266 nm can be changed in the range \(5 \times 10^{23} - 10^{26} \text{ cm}^{-2}\text{s}^{-1}\) not taking into account the distribution of intensity in time and cross-section of the beam.

- For the corrected spectra’s measurements we used the lamp spectrofluorometer FluoroMax 4 (Jobin Yvon).
HS preparations

- CHP, CHPm, LHS – preparations isolated from the coil and the leonardite;
- RF – preparation isolated from the peat;
- SR – preparation isolated from the river water dissolved organic matter;
- POW – commercial preparation «Powhumus»;
- THS - commercial preparation «Tekhnoexport».
Photophysical parameters of HS 
Fluorescence spectra

Gr.1 The corrected fluorescence spectra of HS preparations obtained with FluoroMax4 spectrofluorometer. 1-RF, 2-POW, 3-SR, 4-CHP, 5-CHPm
Photophysical parameters of HS

<table>
<thead>
<tr>
<th>Preparation</th>
<th>$\sigma \cdot 10^{16}$, cm$^2$</th>
<th>$\tau$, ns</th>
<th>$\eta \cdot n/C$, mol/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>RF</td>
<td>$0.65 \pm 0.05$</td>
<td>$2.5 \pm 0.5$</td>
<td>$8 \pm 2$</td>
</tr>
<tr>
<td>POW</td>
<td>$2.7 \pm 0.2$</td>
<td>$2.4 \pm 0.5$</td>
<td>$6 \pm 2$</td>
</tr>
<tr>
<td>SR</td>
<td>$3.5 \pm 0.3$</td>
<td>$2.5 \pm 0.5$</td>
<td>$4 \pm 1$</td>
</tr>
<tr>
<td>CHP</td>
<td>$0.5 \pm 0.04$</td>
<td>$3.0 \pm 0.5$</td>
<td>$37 \pm 5$</td>
</tr>
<tr>
<td>CHPm</td>
<td>$0.35 \pm 0.03$</td>
<td>$2.7 \pm 0.5$</td>
<td>$66 \pm 8$</td>
</tr>
</tbody>
</table>

$\sigma$, $\tau$ – absorption cross-section and the lifetime of the excited state of the HS fluorophore

$\eta$, $n$ – the fluorescence quantum yield and the fluorophores concentration.

C – mass concentration of HS

Measured by nonlinear and kinetic fluorimetry method
Photophysical parameters of pyrene and uranyl

The fluorescence spectra of pyrene and uranyl while excited with 266 nm wavelength

Gr.2 Pyrene fluorescence spectra.

Gr.3 Uranyl fluorescence spectra.
Photophysical parameters of pyrene and uranyl

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pyrene</th>
<th>Uranyl</th>
</tr>
</thead>
<tbody>
<tr>
<td>σ</td>
<td>$(0.7 \pm 0.1) \cdot 10^{-16}$ cm$^2$</td>
<td>$(3.0 \pm 0.3) \cdot 10^{-17}$ cm$^2$</td>
</tr>
<tr>
<td>τ</td>
<td>$85 \pm 3$ ns</td>
<td>$1.8 \pm 0.1$ μs</td>
</tr>
</tbody>
</table>

Obtained with the nonlinear and kinetic fluorimetry methods.

The difference from HS – fluorophore excited state lifetime is much longer than the laser pulse:

$$\tau >> \tau_p$$
HS and pyrene interaction

Chaining constant:

The fluorescence intensity of pyrene decreases (pyrene concentration is 60 ug/l) while the HS concentration rises from 1 mg/l to 10 mg/l. Thus we calculate the chaining constant $K_{oc}$:

$$\frac{F_0}{F} = 1 + K_{oc} \cdot C_{HA}.$$ 

$F_0$ и $F$ – pyrene fluorescence intensity with and without HS.

$$K_{oc} = (3.2 \pm 0.3) \times 10^5 \text{ l/kg for LHS sample;}$$

$$K_{oc} = (3.6 \pm 0.4) \times 10^5 \text{ l/kg for THS sample.}$$
HS and pyrene interaction

Photophysical parameters HS (3mg/l), obtained with the nonlinear and kinetic fluorimetry methods.

\[ \sigma = (3.5 \pm 0.2) \cdot 10^{-16} \text{ cm}^2 \] – without pyrene

\[ \sigma = (2.1 \pm 0.3) \cdot 10^{-16} \text{ cm}^2 \] – with pyrene (60 ug/l)

\[ \tau = 3 \pm 0.5 \text{ ns} \] – without pyrene

\[ \tau = 5 \pm 0.5 \text{ ns} \] – with pyrene (60 ug/l)
HS and uranyl interaction

Content of functional groups in HS

<table>
<thead>
<tr>
<th>Preparation</th>
<th>-COOH, mmol/g</th>
<th>-ArOH, mmol/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHP</td>
<td>4.2 ±0.2</td>
<td>1.1± 0.1</td>
</tr>
<tr>
<td>CHPm</td>
<td>3.6±0.1</td>
<td>4.4± 0.2</td>
</tr>
</tbody>
</table>

Two –COO⁻ groups can bind with the uranyl UO₂²⁺. There for can be calculated the concentrations of uranyl and humic substances at which the interaction between them take place. A good fluorescent signal from uranyl is noticeable at concentrations of about 10⁻⁵ mol/l. So the concentration of –COOH- groups should be about 2 10⁻⁵ mol/l, which means mass concentration of CHP - 5mg/l.
HS and uranyl interaction

Influence of uranyl on fluorescence of HS.

Gr. 4 The samples with concentration of HS 50 mg/l (concentration –COOH-groups 2 $10^{-4}$ mol/l) and concentrations of uranyl 1 $10^{-5}$, 2 $5 \times 10^{-5}$, 3 $10^{-4}$, 4 $5 \times 10^{-4}$ mol/l were used. The ratio of uranyl ions to concentration of –COOH- groups couples was there for 1:10, 1:2, 1:1, 5:1. Type of HS used: CHP.
HS and uranyl interaction

Influence of uranyl on photophysical parameters of HS (concentration of –COOH- groups 2 \(10^{-4}\) mol/l).

<table>
<thead>
<tr>
<th>(UO_2) Concentration</th>
<th>(1 \cdot 10^{-5}) mol/l</th>
<th>(1 \cdot 10^{-4}) mol/l</th>
<th>(5 \cdot 10^{-4}) mol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\sigma_{CHP}, \text{cm}^2)</td>
<td>((0.50 \pm 0.03) \cdot 10^{-16})</td>
<td>((1.0 \pm 0.1) \cdot 10^{-16})</td>
<td>((1.5 \pm 0.2) \cdot 10^{-16})</td>
</tr>
<tr>
<td>(\tau_{CHP}, \text{ns})</td>
<td>(3.0 \pm 0.5)</td>
<td>(3.7 \pm 0.5)</td>
<td>(2.5 \pm 0.5)</td>
</tr>
<tr>
<td>(\sigma_{CHP_m}, \text{cm}^2)</td>
<td>((0.70 \pm 0.04) \cdot 10^{-16})</td>
<td>((1.3 \pm 0.1) \cdot 10^{-16})</td>
<td>((1.4 \pm 0.2) \cdot 10^{-16})</td>
</tr>
<tr>
<td>(\tau_{CHP_m}, \text{ns})</td>
<td>(2.7 \pm 0.5)</td>
<td>(3.7 \pm 0.5)</td>
<td>(3.5 \pm 0.5)</td>
</tr>
</tbody>
</table>

During measuring of the saturation curve signal was registered in detector’s strobe - width 50ns with zero delay from laser pulse.
Conclusion

- With growth of uranyl concentration HS fluorescence intensity is decreasing and simultaneously fluorophor absorption cross section is increasing. Mechanisms are under investigation now.
- Thou not as strong as uranyl, pyrene influences the photophysical parameters of HS too.
- There for another property of HS is proposed – the ability to use it as a fluorescent indicator for definition of toxic substances in water. Difference of behavior in HS fluorescence in cases of pyrene and uranyl is connected with the chemical properties: uranyl exists in solution as an ion, while pyrene isn’t charged (electro neutral). Interaction with uranyl leads to coagulation of HS; in case of pyrene coagulation doesn’t take place.