Abstract:

It is shown that the surface-enhanced Raman scattering (SERS) technique can be applied to detect organic molecules during in situ experiments. To this purpose, we used trans-1,2-bis(4-pyridyl)ethylene (BPE) as a target molecule. Adsorbed on the SERS chemosensor surface and excited under laser, the vibration modes of the molecules can be identified. SERS chemosensors are based on quartz substrates functionalized by silanization and partially coated with gold nanoparticles. SERS measurements during shipboard experiments were made with a home-made in situ Raman spectrometer connected to a marinized micro-fluidic system. The device was designed to host chemosensors in order to ensure measurements with a flow cell. A theoretical limit of detection was estimated in the range of picomolar (pM) concentrations based on Freundlich isotherm calculations.

Keywords: Surface-enhanced raman scattering; sers; chemosensors; flow cells; shipboard experiments; in situ measurements; freundlich isotherm
1. Introduction

Since the beginning of the 21st century, sea water in situ methods have been developed as in the detection of the organic compounds responsible of the ocean pollution, as in the field of the discovery of new fossil energy. The in situ measurements and the common sampling techniques can be used concomitantly to improve data processing on natural samples. The latter consists in taking water samples for analysis directly aboard the ship or some time later at the laboratory. Some drawbacks arise using such technique: sample contamination, difficulties to maintain the global environment of the sample and variation in time and space of many parameters (Prien, 2007). On the contrary, in situ measurements provide instantaneous data respecting the time and space components. The interests of in situ analytical measurements are: i) a limitation of the modification of unstable species, especially for the deep sea measurements when the time between the sampling and the analysis on board or on shore may be as much as several hours ii) and a better resolution in time and space dues to high frequency measurements and direct analysis (monitoring).

In situ optical and spectroscopic techniques are well adapted to the measurements of chemical parameters in natural seawater because they provide molecular information and are easily marinized. Vibrational spectroscopy techniques allow the identification of a wide variety of chemical compounds. Molecule vibrations give fingerprints that allow their identification. Because of huge absorption by water molecules, infrared spectroscopy is not suitable for in situ measurements. In the opposite, Raman scattering spectroscopy can provide vibrational spectra by exciting molecules in a visible spectral range that ensures in situ measurements. But the major disadvantage is that Raman scattering is very weak. Thereby, in the scope of a molecule detection at low concentration, surface-enhanced Raman scattering (SERS) effect is an alternative to the low efficiency of the Raman scattering. Indeed, SERS is a very sensitive technique with an exaltation factor up to 10^6 fold the normal Raman intensity (Fleischmann et al., 1976; Jeanmaire et al., 1977 and Albrecht et al., 1977). The monochromatic light excites the modes of the surface plasmon of the nanostructured metallic film. Then, an intense local electromagnetic field is created. This field can be relaxed by energy transfer from the surface plasmons to the molecules by evanescent waves. The Raman scattering of the excited molecules can be detected and the adsorbed molecules identified. Actually, molecule detection with an environmental interest can be achieved.

Works were carried out using Raman spectroscopy i) in laboratory and ii) in situ.

i) In the investigation of chemical pollutions, such as PAHs at low (ppm) or traces concentration (ppb) in sea-water, surface-enhanced Raman scattering (SERS) effect reveals an immense potential regarding the marine environment and can be considered as an alternative to the inherent low cross-section of normal Raman scattering. In order to carry out in situ measurements, a SERS optode was developed (Murphy et al., 2000). Within the scope of screening development, a solution of five PAHs in sea-water with LODs of 37.5µM and 0.1µM for naphthalene and pyrene respectively, was analyzed in laboratory (Schmidt et al., 2004). Furthermore, experiments simulating real conditions of pressure and temperature in hydrothermal vent fluids were performed in laboratory for the measurement of sulfate, carbonate and nitrate using Raman spectroscopy (Battaglia et al., 2004). In situ Raman analyses of hydrothermal vent fluids collected into an open-bottomed sampling box were achieved (White et al., 2006a). In situ Raman measurements of synthetic gas hydrates formed in an ocean environment were injected into the cell at ocean depths of 500 to 1000 m (Hester et al., 2006). In situ Raman measurements of natural gas hydrates collected in a cell at the summit of southern Hydrate Ridge were also carried out (Hester et al., 2007).
Other *in situ* spectroscopic and optical techniques were developed. Concerning infrared chemical sensor technology, Beyer *et al.* (2003) presented the development of a mid-infrared spectrometer based on attenuated total reflection (ATR) for detecting chlorinated hydrocarbons. In laboratory, a limit of detection (LOD) of 900 ppb was achieved for tetrachloroethylene thanks to a silver halide fiber sensor coated with polymers. A miniaturized Fourier transform infrared (FT-IR) spectrometer for *in situ* underwater detection of polycyclic aromatic hydrocarbons (PAHs) and chlorinated hydrocarbons was developed by Kraft *et al.* (2003). Methane sensor using surface plasmon resonance (SPR) technique based on refractive index change was developed by Boulart *et al.* (2008).

Here, the purpose of the paper is to present the feasibility of SERS measurements during shipboard experiments with an home-made *in situ* Raman spectrometer connected to a marinized micro-fluidic system. Such measurements were performed with a view to apply this technique to the detection of molecules of environmental interest in sea water. On our knowledge, the present work describes for the first time *in situ* SERS measurements obtained during shipboard experiments. SERS spectra of *trans*-1,2-Bis(4-Pyridyl)Ethylene (BPE) adsorbed on the chemosensor surface were *in situ* recorded down to 20 m depth. The BPE is currently used by the SERS community as molecule probe (Billot *et al.*, 2006; Sun and Grundmeier, 2006; Abell *et al.*, 2009 and Sahoo *et al.*, 2009). These spectra were analyzed using the empirical Freundlich formulation and a LOD was estimated.

## 2. Material and methods

### 2.1. Instrumentation

The marinized system *(Figure 1)* that allows *in situ* Raman measurements of dissolved matter is composed of 4 elements:

- the host structure to ensure safely launching and to implement accessories,
- the main part containing opto-electronic components (instrument body),
- the optode connected by optical fibers (optode) and
- the fluidic system composed of a flow cell and a marinized micro-fluidic system.
2.1.1. The host structure

The square structure is composed of welded aluminum tubes to operate the system in safety conditions (Figure 2 a). A polypropylene base plate is screwed to host equipments. Many holes are made on this plate to avoid any pounding in heavy sea conditions. The host structure is operated by an on-board winch equipped with a cable that ensures data communication by optical fibers and power supplying by electric wires (Figure 2 b).
2.1.2. The instrument body

The Raman spectrometer was firstly developed in collaboration with Jobin-Yvon Horiba SAS during the Sofie, Mispec and Hermes European Projects (Sofie, Mispec and Hermes). It consists in a laser diode (BRM-785, B&WTEK) emitting at 785.4 nm, an axial spectrograph as the system reported by Lehaitre et al. (1997) coupled to a CCD detector (DU440A-FI, Andor) driven by an embedded computer. Since these European Projects, all opto-electronic components have been housed in a single anodized aluminum cylinder (900mm long / 180mm diameter). DC-DC converters are used to supply power from the surface to electric components. An embedded computer Wafer LX-800 allows system monitoring from the surface in real-time. Data are collected on an hard disk drive and also transmitted to the surface computer. The bi-directional communication is ensured through more than 300 meters of cable by Ethernet connection thanks to fiber optic multiplexers. The embedded computer allows not only Raman spectra collection but also provides intrinsic instrument parameters like CCD temperature, system temperature and relative humidity monitoring. This part has been designed and tested to withstand pressure environment for operation down to 3000 meters.

2.1.3. The optode

The optode is based on a probe designed in the frame of the European Projects Sofie and Mispec by Jobin-Yvon Horiba SAS and by Technische Universität Berlin in collaboration with Ifremer teams. It contains lenses, collimator and filters to focus laser on sensing substrates and to collect back-scattered Raman light. The Raman back-scattered light is isolated by a two stage edge filters. The optode is connected to the instrument body by optical fibers composed of a single fiber for laser excitation and a bundle of 7 fibers for Raman scattered signal collection. The optode has been more detailed in Péron et al. (2009a). This part has been designed and tested to withstand pressure environment for operation down to 3000 meters.

2.1.4. The fluidic system

A flow cell of stainless steel AISI 316L is coupled to the in situ Raman spectrometer (Figure 3). It consists in an input/output flowthrough channel, a BK7 window and a mobile part on which the SERS substrate can be mounted. The chemosensor is stuck on a piston whose position can be adjusted with a screw. The laser beam can then be focused precisely onto the substrate. O-rings around the piston and the window ensure the waterproofness of the flow cell.

The flow cell is connected to an Ifremer home-made marine micro-fluidic device (Vuillemin et al., 2009). It allows the injection of different solutions into the flow cell. It is composed of three peristaltic pumps and eight electrovalves. The formers can turn clockwise and anticlockwise and their speed can be adjusted. The latter can be set up in two positions (open or closed). The whole fluidic system is driven by a computer.

The fluidic path is shown in Figure 4. The solutions to be injected in the system are stocked in 250 mL plastic bags purchased from Fisher Bioblock. Two trash bags (250 mL) are placed in the marinized micro-fluidic system to avoid pollution: one behind a marinized peristaltic pump for rinsing the manifolds and another one at the flow cell output. Five solution bags are connected to the electrovalves. Three contain BPE solutions, one ethanol for calibrating the system and one distilled water.

This system allows analyzing directly the seawater, different solutions stocked in plastic bags or a mixture of both.
2.2. Gold nanoparticles, SERS substrates and target solutions

Suspensions of gold nanoparticles were prepared according to the method described by Frens (1973). Such a technique was used in different studies (Lee et al., 1982; Keating et al., 1999 and Seitz et al., 2003) and consists in the reduction of HAuCl₄. After the addition of a reducing agent, the gold particles start to form during a process known as nucleation and subsequently grow. The procedure is detailed elsewhere (Péron et al., 2009b).

The SERS substrates were prepared by immersion. First, clean quartz substrates of 8 mm diameter and 0.8 mm thickness from Heraeus were vertically immersed in methanol solution of 3-MercaptoPropyl triMethoxy Silane (MPMS) according to the results obtained by Seitz et al. (2003). After silanization, they were vertically dipped into the colloidal suspension of gold nanoparticles. Figure 5 presents a scheme of the SERS substrate synthesis procedure.
The target solutions were prepared by dissolving solid *trans*-1,2-Bis(4-Pyridil)Ethylene (BPE) purchased from Fluka in distilled water. Three solutions of concentrations $10^{-4}$ mol.L$^{-1}$ (BPE1), $2.5 \times 10^{-4}$ mol.L$^{-1}$ (BPE2) and $5 \times 10^{-4}$ mol.L$^{-1}$ (BPE3) were prepared.

![Diagram of SERS substrate silanization procedure and immersion in gold colloidal nanoparticles.](image)

**Figure 5:** Schematic representation of the SERS substrate silanization procedure and immersion in gold colloidal nanoparticles.

### 2.3. Experiments

The *in situ* spectrometer instrument was deployed in South Brittany coastal waters at 47°39’N-3°25.7’W (WGS84 geodesic system) from the oceanic vessel “Thalia”. The measurements were performed down to 20 m depth. The system was coupled to a CTD probe (Seabird, SBE 25) which measured the conductivity, temperature and depth of the seawater. At first, the kinetics of the solution injection were studied to ensure that spectroscopic measurements are obtained after the flow cell homogenization. To do so, solution of 96% ethanol was injected into the flow cell, which was then rinsed by distilled water. BPE could not be used for this experiment: its adsorption onto the chemosensor is irreversible. Ethanol was also chosen because of its large Raman scattering cross-section to ensure signal recording with a minimal SERS effect. Then, the three BPE solutions BPE1, BPE2 and BPE3 were analyzed by our system. The solutions were injected by increasing concentration.

### 3. Results and discussion

#### 3.1. Injection kinetics

In order to estimate the time of the flow cell homogenization during *in situ* conditions, the cell was filled with ethanol. Distilled water was then injected and Raman spectra were recorded during the rinse. A kinetic study was then performed to estimate the injection time. Results are presented in **Figure 6**. The distilled water was injected at time $t_0$ and the first recording was done after 20 s which corresponds to the transit time of the solution in the manifold from plastic bags to the flow cell.
Figure 6: a) Raman spectrum of the ethanol solution and decrease of the 880 cm\(^{-1}\) band as a function of the rinsing time with distilled water in inset. Integration time of 20 s. b) The decrease of the 880 cm\(^{-1}\) band intensity versus time and the corresponding fit from 200 s. In inset, the evolution of the band intensity versus time.

Figure 6 a) shows the Raman spectrum of the ethanol solution and the inset figure the evolution of the 880 cm\(^{-1}\) band intensity as distilled water is injected. The strong band located at 880 cm\(^{-1}\) is assigned to the C-C-O stretching band. The stretching vibrations of the alcohol C-O group are located at 1000-1200 cm\(^{-1}\). Hydrogen bonding leads to a slight shift of this band to shorter wavenumber: saturated primary alcohols, like ethanol, absorb strongly in the region 1000-1090 cm\(^{-1}\) (Socrates, 2001). The bands located at 1380 and 1450 cm\(^{-1}\) are assigned to the angular bending of the methylene group. This signal is probably not exalted and corresponds only to the Raman scattering of ethanol molecules in the cell. The strongest band (880 cm\(^{-1}\)) was used for this study and its Raman signal intensity was normalized with the maximum signal obtained at the beginning of the experiment. The evolution of the band intensity versus time is presented Figure 6 b (inset). A slight decline can be observed until 200 s. Then, the signal sharply decreases until it reaches a plateau. Note that the error bar, estimated as the noise, is in the range of symbol size. The decrease of the band intensity from 200 s was fitted with an exponential decay (Figure 6 b). A time constant \(\tau = 28 \pm 3\) s was then calculated. The flow cell is then considered as empty of ethanol after \(t = 200 + 3 \tau \approx 300\) s.

In conclusion, this study demonstrated the ability of our system to inject solutions in the flow cell and to proceed to its analysis within few minutes, all action operated from the surface computer.

3.2. Detection of BPE by SERS measurements

The three solutions BPE1, BPE2 and BPE3 were analyzed by our system. The solutions were injected by increasing concentration. The spectra are shown in Figure 7. A blank spectrum measured with distilled water in the flow cell is also presented. It corresponds to the spectrum of the organic part (MPMS) of the SERS substrate.
Figure 7: SERS spectra of *trans*-1,2-Bis(4-Pyridil)Ethylene (BPE) solutions adsorbed on chemosensor surface from 1 to 5x10^{-4} mol.L^{-1} and the blank. Integration time of 20 s.

Five single bands and one doublet are assigned to the BPE molecule. The observed Raman spectra of the adsorbed BPE are in agreement with the assignment reported in the literature (Sun and Grundmeier, 2006; Zhuang *et al.*, 2007). The band due to the aromatic ring breathing is located at 1015 cm^{-1}. The ring breathing corresponds to the stretching and contracting of the carbon-carbon bonds. The bands at 1195-1240 cm^{-1} are respectively assigned to the ring vibration and the bending mode of C-H. The bands at 1335-1380 cm^{-1} are respectively attributed to the bending mode of C=C and C-H. The bands of the doublet located at 1606-1636 cm^{-1} are respectively assigned to C-N bending and C=C stretching vibrations. The other minor bands are assigned to the organic matter from MPMS silanization.

The previous kinetic study showed that the time to ensure a complete exchange of the solution was about 300 s. The increase of the 1195 cm^{-1} band intensity with regard to time is presented Figure 8. The study was performed on the aromatic ring vibration which presents a well spectral resolution and a high intensity. Moreover, the blank does not reveal any signal at 1195 cm^{-1}. Note that the error bar is estimated as the noise. For each BPE concentration, the band intensity increases with time. As expected after about 300 s the signals do not significantly increase.
Figure 8: Intensity of the SERS band (1195 cm$^{-1}$) versus time for BPE1, BPE2 and BPE3. The BPE injections are indicated with arrows. The classical models that can be used to describe adsorption between the solid phase and aqueous phase at equilibrium are Langmuir (Langmuir, 1918) or Freundlich (Freundlich, 1907a) equations. The Langmuir model is used in the case of homogeneous monolayer adsorption while the empirical Freundlich model is applied for heterogeneous surfaces (Freundlich, 1928b and Meghea et al., 1998). The SEM images of the chemosensors (Péron et al., 2009b) highlight the inhomogeneous spatial distribution of the gold nanoparticles. Furthermore, the nanoparticle size is in the range 40-100 nm including hot spots. Thereby, the empirical model is applied here to describe the adsorption of the BPE molecules onto the chemosensor surface. Figure 9 presents the 1195 cm$^{-1}$ band intensity with regard to the concentration. Assuming that the band intensity was proportional to the amount of molecules onto the surface, the data were fitted with the Freundlich law. It is given by the Eq. 1:

$$I \propto \eta = K_F \times [\text{BPE}]^n$$  \hspace{1cm} \text{Equation 1}

where $\eta$ is the adsorbed quantity (mol.kg$^{-1}$), [BPE] the concentration of adsorbate in the solution (mol.L$^{-1}$), $n$ and $K_F$ two constants depending on the temperature, the type adsorbing and the adsorbed molecules. The Freundlich exponent, $n$ (dimensionless), is indicative of the adsorption intensity; i.e. the distribution and the size of the sites. The Freundlich coefficient, $K_F$ (L$^n$.mol$^{(1-n)}$.kg$^{-1}$), is function of the number of sites and the ionic composition of the liquid phase (Sposito, 1980).

The results of the fit of intensity versus BPE concentration are shown in Figure 9 a). The data are in good agreement with the empirical model according to the $R^2$ coefficient. The $K$ coefficient presented is proportional to the $K_F$ Freundlich coefficient and is homogeneous to count.L$^n$.mol$^{-n}$. Similar experiments were carried out in laboratory to supply more experimental data. The results are shown in Figure 9 b). For shipboard experiments, $n$ is about 0.2 and $K$ about 1300 count.L$^n$.mol$^{-n}$ whereas in the laboratory, $n$ is about 0.1 and $K$ about 700 count.L$^n$.mol$^{-n}$.
Figure 9: a) Signal intensity versus [BPE] for shipboard. b) and laboratory experiments. The error bar is estimated as the spectral noise.

The adsorption liquid-solid is referenced in the classification of Giles et al. (1960) and the obtained fits correspond to the H type. H type is considered as a typical shape for chemisorption: a strong interaction occurs between BPE molecules and the chemosensor surface. Moreover, a value of the Freundlich exponent, with 0<n<1, is a guideline of the favorable adsorption and the surface heterogeneity (Tsai et al., 2003). BPE molecules, probably covalently grafted to the gold nanoparticles by the free nitrogen doublet, can not be desorbed from the chemosensor surface. Thereby, a same chemosensor can not be used both, to make shipboard and laboratory experiments.

For this model of curve, the slope at the origin tends towards the infinity. Indeed, assuming that the smallest detectable peak is three times the noise amplitude, a LOD of 360 pM was estimated during shipboard experiments and a LOD of 0.5 pM was estimated during laboratory experiments.

The surface heterogeneity in terms of adsorption sites leads to a variability of the Freundlich exponent n. The value of the Freundlich coefficient $K_F$ depends on the Freundlich exponent n.

In the literature, $K_F$ and n values of Freundlich equation depends also on temperature and increases positively with temperature (Jain, 2001; Karthikeyan et al., 2005) or decreases with increasing temperature (Gereli et al., 2006 and Qaiser et al., 2009). In our case during shipboard experiments, the CTD probe measured a sea water temperature of 12 °C and the laboratory experiments were performed at room temperature. This variation can be considered as marginal. In our opinion, the major difference obtained between n and K, could be explained owing to the non reproducibility of the chemosensors in nanoparticle size and distribution: the nanoparticle size being in the range of 40-100 nm and randomly distributed (Péron et al., 2009b). Two hypotheses can be suggested to explain the increase of $K_F$ and n in the case of the shipboard experiments:

i) hot spots give rise to intense local surface plasmon and the Raman signal of the excited molecule is more exalted.

ii) BPE adsorbed amount depends on specific surface area of Au nanoparticles. According to the heterogeneity of the nanoparticle size distribution on the chemosensors, the measured intensity is also variable. The same phenomenon has been observed elsewhere concerning adsorption on silica and activated-carbon (McKay et al., 1980; Santhi and Manonmani, 2009).

In order to obviate this non reproducibility of the gold nanoparticles, works are in progress on the SERS sensor fabrication by electron beam lithography using lift-off technique (Grand et al., 2003, 2005). This advanced lithography technique leads to nanostructures of desired size, shape and arrangement with reproducibility.
Conclusion

Shipboard experiments were performed using surface-enhanced Raman scattering (SERS) method. The spectral fingerprint of trans-1,2-Bis(4-Pyridyl)Ethylene (BPE), as target molecule, adsorbed on chemosensor surface was obtained thanks to an home-made in situ Raman spectrometer. The latter was connected to a marinized micro-fluidic system. The chemosensor response can be fitted with the empirical Freundlich equation. The obtained fit corresponds to a model of curve which slope at the origin tends towards the infinity and a limit of detection was notified in the range of the pM concentrations. The underwater/deck communication was confirmed without lost of any data and the marinized micro-fluidic device allowed the injection of the selected solutions into the flow cell. In the immediate future, some SERS experiments are set up on molecules of environmental interest like highly toxic polycyclic aromatic hydrocarbons (PAHs).

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