Surface-Enhanced Raman Scattering (SERS) Surfaces for in-situ trace analysis of PAHs in water by Shifted Excitation Raman Difference Spectroscopy (SERDS)

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der Technischen Universität Berlin
zur Erlangung des akademischen Grades
Doktor der Naturwissenschaften
Dr. rer. nat.

genehmigte Dissertation

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Tag der wissenschaftlichen Aussprache: 29.3.2012

Berlin 2012
D 83


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Abbreviation of some organic compounds

Acknowledgement
Abstract

In-situ monitoring of polycyclic aromatic hydrocarbons (PAHs) in the water body has been of worldwide interest for the last decades because they are known to be toxic to biota even at low concentration in the range of ng/l (ppt). For that purpose, fast response optical sensors based on Raman spectroscopy providing a molecular fingerprint of the analyte are suitable for a rapid identification and quantification of these substances. To achieve the high sensitivity necessary for trace detection surface-enhanced Raman scattering (SERS) was applied. Furthermore, microsystem diode laser modules with two slightly different emission wavelengths allow to introduce shifted excitation Raman difference spectroscopy (SERDS) which considerably reduces the fluorescence based background of the SERS spectra. Based on these techniques, in the project SENSEnet, funded by the European Commission, two types of new high active SERS sensors were developed and adapted for the in-situ detection of PAHs in seawater and fresh water.

To realize an improved SERS sensor suitable for the trace detection of PAHs in seawater 25,27-dimercaptoacetic acid-26,28-dihydroxy-4-tertbutyl calix[4]arene (DMCX) as hydrophobic surfactant was added to sol-gel based SERS substrates. In that way, DMCX functionalized silver colloid based sol-gel films as new type of SERS substrate were developed based on the electromagnetic enhancement due to the nanoparticle aggregation and the preconcentration of PAHs. Using a 671 nm microsystem diode laser excitation source the DMCX functionalized Ag nanoparticle based sol-gel film SERS substrates achieved limits of detection (LODs) of 0.3 nmol/l (60 ppt) for pyrene and 13 nmol/l (1600 ppt) for naphthalene in artificial seawater. In addition, a combination of SERS with SERDS (SERS/SERDS) was realized by applying a microsystem diode laser module with two slightly shifted emission wavelengths (670.8 nm and 671.3 nm) further improving the LODs of selected PAHs (e.g. 100 pmol/l (20 ppt) for pyrene, 310 pmol/l (55 ppt) for anthracene, and 670 pmol/l (135 ppt) for fluoranthene), and thus resulting in a higher sensitivity compared to using only the SERS technique.

The second part of this work focuses on a novel SERS sensor especially suited for the trace detection of PAHs in fresh water applying Ag nanoparticles which are stable under these conditions. Therefore, substrates containing naturally grown Ag nanoparticle ensembles with surface plasmon resonance (SPR) wavelengths around 488 nm were developed in cooperation with University of Kassel and tested using a microsystem diode laser source emitting at 488 nm.
Abstract

A tuning of the SPR wavelength from 453 nm to 548 nm was performed by varying the morphology of the silver nanoparticles. SERS/SERDS investigations of pyrene adsorbed onto a naturally grown silver nanoparticle ensemble applying a microsystem light source with two emission lines (487.61 nm and 487.91 nm) demonstrated that SERS activity, i.e. SERS intensity at a selected concentration and LOD were optimized when the SPR wavelength of the nanoparticles is close to the excitation wavelength of 488 nm. Hence, for pyrene in water a LOD of 2 nmol/l (400 ppt) was achieved using a SERS substrate with a SPR wavelength centered at 491 nm.

The obtained results show the great potential of in-situ optical sensors based on SERS/SERDS for the trace detection of PAHs in selected types of water.
Zusammenfassung


Zusammenfassung

ppt) für Fluoranthen) d. h. eine noch höhere Empfindlichkeit als nur bei Einsatz der SERS-
Technik.

Der zweite Teil der vorliegenden Arbeit befasst sich mit einem neuartigen SERS-Sensor der
speziell für den Spurennachweis von PAKs in Frischwasser geeignet ist. Hierzu fanden Silber-
Nanopartikel basierte Substrate Verwendung, die unter diesen Einsatzbedingungen stabil sind.
In Zusammenarbeit mit der Universität Kassel wurden zu diesem Zweck Substrate mit auf
natürliche Weise gewachsenen Silber-Nanopartikel-Ensembles mit einer Resonanzwellenlänge
der Oberflächenplasmonen (SPR) im Bereich um 488 nm entwickelt und mit einem 488 nm
Mikrosystem-Diodenlaser als Anregungslichtquelle getestet. Durch eine Variation der
Morphologie der Silber-Nanopartikel wurden mehrere Substrate mit SPR-Wellenlängen im
Bereich von 453 nm bis 548 nm realisiert. SERS/SERDS-Untersuchungen von auf diesen
Substraten adsorbiertem Pyren unter Verwendung eines Mikrosystem-Diodenlasers mit zwei
leicht unterschiedlichen Emissionswellenlängen (487,61 nm und 487,91 nm) ergaben, dass für
die SERS-Aktivität, d. h. die SERS-Intensität bei einer festgelegten Konzentration, sowie die
Nachweigrenze die besten Werte erreicht wurden, wenn die SPR-Wellenlänge der
Nanopartikel in der Nähe der Anregungswellenlänge von 488 nm lag. Auf diese Weise konnte
mit einem Substrat mit einer SPR-Wellenlänge bei 491 nm für Pyren in Wasser eine
Nachweigrenze von 2 nmol/l (400 ppt) erreicht werden. Die in dieser Arbeit erzielten Resultate
verdeutlichen das große Potential von auf der SERS/SERDS-Technik basierenden optischen In-
situ-Sensoren zum Spurennachweis von PAKs im Wasser.
Some results of my research work were already published:


Polycyclic aromatic hydrocarbons (PAHs) as environmental pollutant chemicals are produced through the incomplete combustion of organic materials and released into the ecosystem in the form of gases or particles. As the pollutants, they are of global concern because PAHs have been identified as carcinogenic, mutagenic and teratogenic [1]. Specific maximum limits were set by European Union for PAHs not only in water [2] but also in foods such as fish where the environmental pollutants may cause high levels of contamination [3]. From this requirement, analytical methods for the detection of PAHs in food and the environment have been applied [4].

PAHs are dissolved in the sea-water with low concentration in the range of ng/l (ppt) or less and their concentration profiles are changed temporally and spatially [5]. It requires an efficient method to monitor PAHs in water body in real time. Raman spectroscopy as a noninvasive method can be applied for the identification and quantification of toxic chemicals like PAHs because it gives us the fingerprint of the analyte measured. However, its application to the trace analysis of chemical in water body is limited due to low Raman scattering cross section. Surface enhanced Raman scattering (SERS) has become a powerful analytical tool for the trace detection of substances since the significant enhancement of Raman signal from pyridine adsorbed on an electrochemically roughened silver electrode was discovered by Fleischmann, Hendra and McQuillan [6].

In the EU-projects SOFIE (Spectroscopy Using Optical Fibers in the Marine Environment, MAS3-CT97-0157) in the period of 1997-2000 and MISPEC (Multiparametric in-situ Spectroscopic Measuring System for Coastal Monitoring, EVK3-CT-2000-00045) from June 2001 to February 2004, sol-gel based SERS sensors [7-15] have been developed by our group to measure PAHs in seawater resulting in a high sensitivity, e.g. 1.8 nmol/l (335 ppt) as the limit of detection (LOD) for pyrene in seawater [16]. However, LODs for PAHs in seawater using this type of SERS sensor are higher than the mean PAH concentrations in marine environment [5]. Therefore, in the EU-project SENSEnet (International Chemical Sensor Development Network, PITN-GA-2009-237868) our target was to develop SERS sensors with higher activity and lower LODs for PAHs in water body than the previously developed one. For the trace analysis of PAHs in sea-water, SERS sensors such as silver colloid based sol-gel film [7-16],
Introduction

self-assembled gold colloid film [17, 18], have been applied as powerful Raman signal amplifier.

The sensitivity of a SERS substrate for the trace analysis of chemicals depends on two enhancement factors, i.e. electromagnetic field enhancement in the vicinity of metal nanoparticle excited by the incident laser light and chemical effect due to the direct contact of analyte with the metal nanoparticle surface [19]. It has also been reported that the SERS activity is dependent on the distance between the analyte and metal nanoparticle surface [20]. Due to the fact that PAHs as non-polar molecules do not adsorb well to bare metal nanoparticle surface, i.e. small surface coverage of PAHs, a low SERS activity can be achieved. To preconcentrate PAH molecules within the area of high electromagnetic field of nanoparticle, functionalization methods of the metal nanostructures [21, 22] have been applied resulting in high sensitivity (e.g. 700 pmol/l of LOD for pyrene [21]). In addition, SERS investigation of pyrene adsorbed onto laser tailor made gold nanoparticles demonstrated that surface plasmon resonance (SPR) in the vicinity of excitation wavelength is also one among the electromagnetic field enhancement factors for SERS process [23].

Analyte sample luminescence such as fluorescence and phosphorescence has been a prohibiting factor for the wide spread use of Raman spectroscopy as an analytical technique for a broad range of samples [24]. To separate the Raman signal from the background noise, the shifted excitation Raman difference spectroscopy (SERDS) has been applied [25]. In this work for the first time the introduction of SERDS to SERS measurement will drastically improve the sensitivity of the SERS based sensor.

In this thesis, the fabrication of high active SERS substrates for the trace detection of PAHs and their characterization using SERS, and SERS combined with SERDS are presented.

First, the methods for the preparation of not only a SERS substrate in which sol-gel matrix embeds the silver nanoparticles functionalized with 25,27-dimercaptoacetic acid-26,28-dihydroxy-4-tertbutyl calix[4]arene (DMCX) but also a naturally grown silver nanoparticle ensemble on quartz as SERS substrate are described (Chapter 4). Additionally, this chapter also describes the characterization of two SERS substrates, i.e. nanostructure and optical property using the scanning electron microscopy (SEM), atomic force microscopy (AFM) images and extinction spectra.
In order to characterize the SERS substrates which should be operative under the water environment, i.e. sea-water or fresh water, two types of Raman set-ups with different excitation light sources were used: 671 nm microsystem laser diode, a microsystem laser diode with two slightly different emission lines (670.8 nm and 671.3 nm) and a microsystem light source with two emission wavelengths (487.61 nm and 487.91 nm) (Chapter 5). All Raman set-ups involve the flow-through cell system for the continuous measurement.

Chapter 6 describes not only the SERS characterization of DMCX functionalized silver colloid based sol-gel film but also SERS applying SERDS (SERS/SERDS) for PAHs in seawater. In chapter 7, SERS/SERDS investigations of pyrene adsorbed onto the naturally grown silver nanoparticle ensembles with different SPR wavelength are presented. From these research results, it is expected that the metal nanoparticle aggregation, the preconcentration of PAHs to metal surface, and the creation of SPR close to the excitation wavelength are three main factors for high SERS activity in the nanoparticle based chemical sensors.

The experimental demonstrations mentioned above open up the perspective to develop the high sensitive in-situ chemical sensor using SERS combined with SERDS but also in future, to be constructed in Oceanographic mooring for measuring several oceanographic parameters in real time.
2 Environmental pollution and Raman Spectroscopy

2.1 Environmental Pollution due to Chemicals

The discharge of waste water produced from industrial process is a matter of global concern because some compounds like PAHs are known to be toxic to biota [26]. The interest in hydrophobic organic contamination including PAHs has been increased during the last decades. PAHs occur in oil, coal and tar deposit but also are produced as the byproducts of fuel burning in the industrial processes and the domestic human activities such as the power generation by fuels, the production of steel, transportation by cars, trucks and aircrafts, the oil refining and the use of fuels for heating and cooking. Because of high octanol/water partition coefficient, for example, PAHs are dissolved in water body with low concentration and form the suspended particulate matter (SPM) in the surface water with the concentration ranged between 0.15 and 0.9 mg/l [5]. Since PAHs with molecular weight (MW) above 200 g/mol occur in particulate phase, they have a tendency to adsorb to the sediment.

For the purpose of environmental protection from the organic toxic chemicals, the environmental quality standards (EQS) for some pollutants were proposed by not only the Commission of European Community [27] but also the Eastern Europe, Caucasus and Central Asia [28]. As an example, Table 2.1 shows EQS for some priority among PAHs in surface water proposed by European Community.

Table 2.1: EQS for some PAHs in surface water [27]

<table>
<thead>
<tr>
<th>PAH</th>
<th>AA-EQS Inland surface water</th>
<th>AA-EQS Other surface water</th>
<th>MAC-EQS Inland surface water</th>
<th>MAC-EQS Other surface water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anthracene (ppt)</td>
<td>100</td>
<td>100</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>Fluoranthene (ppt)</td>
<td>100</td>
<td>100</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>Naphthalene (ppt)</td>
<td>2400</td>
<td>1200</td>
<td>not applicable</td>
<td>not applicable</td>
</tr>
</tbody>
</table>
In Table 2.1, AA and MAC are the abbreviations of the annual average concentration and maximum allowable concentration, respectively. The EQS are differentiated for inland surface water (rivers and lakes) and other surface waters (transitional, coastal and territorial waters).

Some experimental results [26, 29, 30] showed the way of how PAHs go into the marine organism, and their bad effects. The growing biomass in water body results in high biotransformation of nonpolar substances like PAHs into water-soluble metabolites through the enzymatic process [26]. Fish efficiently metabolize PAHs even after short exposure periods [29] and the organic molecules after biotransformation are bio-accumulated in the body tissue of marine organisms exposed to oil particles [30]. As a result, pacific herring eggs exposed to an initial concentration of 0.7 ppb PAHs for 16 days caused the malformation, genetic damage, mortality, and decreased size and inhibited swimming. For example, the exposure of herring eggs to more weathered oil water sample caused the significantly reduced incubation time, reduced larval swimming ability and morphological abnormalities in larvae. Genetic damage was also induced in the oil-exposed larvae following the exposure to an initial total PAHs concentration of 0.7 ppb.

Therefore International Agency for Research on Cancer (IARC) has evaluated the carcinogenic risks to humans of 34 polynuclear aromatic compounds based on the experimental data and human data [31].

Much effort has been devoted to develop the in-situ chemical sensor and to monitor the aqueous environment. For the trace detection and quantification of PAHs in water body, several methods have been applied including the ultra-violet (UV) fluorescence analysis [32-34], the gas chromatography-mass spectrometry (GC-MS) [5, 35], and the Raman spectroscopy using noble metal nanoparticle [8].

Among the several analytical methods, Raman spectroscopy provides the fingerprint for the identification and classification of chemical and the response time is very fast. In addition, the introduction of laser diode as an excitation source for Raman spectroscopy makes it possible to operate the set-up under the in-situ water condition with a cheap cost; therefore gives us a wonderful prospect to monitor the toxic chemicals in water body directly.
2.2 Raman spectroscopy as analytical tool

Raman scattering is a vibrational spectroscopic technique that allows the substances identification by detecting the inelastic scattered light of molecule excited by laser light. It was discovered by C. V. Raman and K. S. Krishnan in liquids [37] and L. Mandelstam in crystals [38]. When the light is scattered from an atom or molecule, most of photons are elastically scattered (Rayleigh scattering). However, a small fraction of scattered light is inelastically scattered from the molecule (Stokes or anti-Stokes Raman scattering).

Fig. 2.1 shows a schematic representation on the different possibilities of light scattering when the incident photons collide with a scattering molecule.

![Figure 2.1: Schematic representation of 3 types of light scattering from molecules excited by the incident photon.](image)

Raman spectroscopy concerns a change in photon’s frequency (i.e. photon’s energy) when the light is scattered by the molecule or atom. When a photon collides with a scattering molecule,
the collision can be considered either elastic, which causes no change in the scattered light frequency (Rayleigh scattering), or inelastic with small probability which gives rise to a change in the frequency of the scattered light (Raman scattering) reflecting the vibrational information of scattering molecule.

The virtual state in Fig. 2.1 is not necessarily a true quantum state of molecule but can be considered as a very short lived distortion of electron cloud caused by the oscillating electric field of the incident light. The electron cloud of the molecule is also perturbed by the molecular vibration. It is possible for the optical and molecular vibrational oscillations to interact, leading to the Raman scattering. The interaction between the optical electric field and molecular vibration can be understood from the theory of Rayleigh and Raman scattering in which both the electromagnetic radiation and molecular vibration system are treated classically [39].

The frequency-dependent induced electric dipole moment \( P^{(l)} \) is expressed using the following relationship:

\[
P^{(l)} = \alpha \cdot E
\]  

(2.1)

where \( \alpha \) is the polarizability of molecule, \( E \) is the electromagnetic field of incident light.

The variation of the polarizability with the vibrations of molecule can be expressed by expanding the polarizability tensor \( \alpha \) in a Taylor series with respect to the normal coordinates of variation.

\[
\alpha = \alpha_0 + \sum_k \left( \frac{\partial \alpha}{\partial Q_k} \right)_0 Q_k + \frac{1}{2} \sum_{kl} \left( \frac{\partial^2 \alpha}{\partial Q_k \partial Q_l} \right)_0 Q_k Q_l \ldots
\]  

(2.2)

By neglecting the terms which involve powers of the normal coordinate \( Q \) higher than the first, the following expression of the polarizability tensor with respect to the \( k^{th} \) normal coordinate \( Q_k \) is obtained.

\[
\alpha_k = \alpha_0 + \alpha_k' Q_k
\]  

(2.3)
Environmental pollution and Raman Spectroscopy

Here

$$\alpha'_k = \left( \frac{\partial \alpha}{\partial Q_k} \right)_0$$  \hspace{1cm} (2.4)

Assuming that molecular system is in the simple harmonic motion, the time dependence of $Q_k$ is given by

$$Q_k = Q_k^0 \cos(\omega_k t + \delta_k)$$  \hspace{1cm} (2.5)

where $\omega_k$ is the frequency of the molecular vibrational oscillation. Combining Eq. (2.5) with Eq. (2.3), the time dependence of the polarizability tensor resulting from $k^{th}$ normal coordinate of molecular vibration is obtained.

$$\alpha_k = \alpha_0 + \alpha'_k Q_k^0 \cos(\omega_k t + \delta_k)$$  \hspace{1cm} (2.6)

By introducing into Eq. (2.1) the frequency dependences of electric field of incident light $E$ given by

$$E = E_0 \cos \omega_l t$$  \hspace{1cm} (2.7)

and of $\alpha_k$ given by Eq. (2.3), the following expression is obtained.

$$p^{(1)} = \alpha_0 E_0 \cos \omega_l t + \alpha'_k E_0 Q_k^0 \cos(\omega_k t + \delta_k) \cos \omega_l t$$  \hspace{1cm} (2.8)

Using the trigonometric identity

$$\cos A \cos B = \frac{1}{2} \left\{ \cos (A + B) + \cos (A - B) \right\}$$  \hspace{1cm} (2.9)
Eq. (2.8) is reformulated into the following expression.

\[
p^{(1)} = p^{(1)}(\omega_1) + p^{(1)}(\omega_1 - \omega_k) + p^{(1)}(\omega_1 + \omega_k)
\]  

(2.10)

Here

\[
p^{(1)}(\omega_1) = \alpha_0 E_0 \cos(\omega t)
\]

(2.11)

\[
p^{(1)}(\omega_1 \pm \omega_k) = \frac{1}{2} \alpha'_k Q_k \cos((\omega_1 \pm \omega_k) \times t \pm \delta_k)
\]

(2.12)

Eqs. (2.11) and (2.12) present the classical Rayleigh and Raman scattering induced dipole moments, respectively. Especially, Eq. (2.12) shows that Raman scattering is produced from the interaction between the electric field of incident light and the molecular vibrational oscillation.

However, the cross section of a molecule for Raman scattering is about \(10^6\) and \(10^{14}\) times smaller than those of infrared absorption and fluorescent processes [40]. For example, a typical sample for absorption experiment absorbs 90\% of incident light over 1 cm of path length, but only 1 in \(10^{10}\) of incident photons will undergo Raman scattering [41]. Therefore, it was shown that the first major impediment to using Raman spectroscopy is the weakness of the Raman effect. A second problem in applying Raman spectroscopy is another competition effect, i.e. fluorescence. Some visible light sources used for Raman spectroscopy excite the fluorescence of analyte or of impurity. Because the fluorescence process has a significantly higher cross section than Raman scattering, even a weak fluorescence can easily overwhelm the Raman signal. Because of low Raman scattering cross section, the application of Raman spectroscopy to the trace analysis is limited.

To improve the efficiency of Raman scattering process, Resonance Raman spectroscopy (RRS) [42] and Surface Enhanced Raman Spectroscopy (SERS) [6] have been developed. Raman spectroscopy is conventionally performed with green, red or near-infrared lasers. The excitation wavelengths for conventional Raman spectroscopy are below the first electronic transition of most molecules. However, the situation changes if the wavelength of the exciting
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laser is located within the electronic spectrum of a molecule. For example, UV-Raman spectroscopy uses the selective excitation light source in the UV absorption bands of molecule to produce Raman spectra of the particular analytes and chromophoric segments of macromolecule. When the excitation frequency is equal to or close to the molecular electronic absorption band frequency, it is said to be the resonance Raman spectroscopy. This resonance excitation results in an increase in the induced dipole moment of Raman scattering molecule, i.e. an increased scattering efficiency of Raman process. The enhancement factor of RRS compared with that of normal Raman scattering can be as high as \(10^8\) [42]. Therefore, RRS has been applied, e.g. for the trace detection of chemicals in food [25]. First problem in RRS is the significant selectivity of enhancement factor in Raman spectral measurement according to the absorption bands of analyte [42]. Another major disadvantage of RRS is the strong fluorescence caused by the excitation at a frequency coinciding with that of an electronic transition. Therefore some problem in using RRS effect is that RRS to fluorescence ratio is very dependent of the molecule and, as a result, RRS is not a generally useful method.

Another way to improve the Raman scattering efficiency of molecule is the surface enhanced Raman scattering [6]. In 1970’s, Fleischmann et al discovered that the molecules adsorbed onto a specially prepared roughened silver surface gave unusually high intensity Raman spectra that were about a million times greater than expected. This was an immediate breakthrough in applying Raman spectroscopy to study the reaction at the interface between metal and sample solution because it overcame the serious signal to noise ratio problem in Raman spectroscopy. It has been shown that this effect was not simply because of increase in the metal surface area which adsorbs the analyte molecules, and noble metal nanostructures or nanoparticles are responsible for the large enhancement of the Raman scattering cross section for the adsorbed molecule. Single molecule SERS experiments on Ag and Au colloids have dedicated that an enhancement factor amounts to 10-15 orders of magnitude [43]. A large enhancement of Raman signal from the molecule adsorbed onto the noble metal nanostructure opens the door to develop the in-situ Raman chemical sensors which are able to detect and quantify small amount of chemical in water body.

In the following chapter, the principle of surface enhanced Raman scattering (SERS) and the types of SERS substrates for the specific applications, especially for in-situ monitoring of PAHs in aqueous solution will be discussed in a detail.
In this work, Raman spectroscopy was applied for the identification and quantification of toxic chemicals in water body. However, low Raman scattering cross section limits its application. Fleischmann et al prepared the electrochemically roughened Ag electrode which gave the increased number density of adsorbed molecules within a certain surface area irradiated by the incident laser light in attempt to solve this problem [6]. Raman signal from pyridine adsorbed onto the roughened Ag electrode was about a million times greater than expected. This enhancement effect was not simply because of increase in surface area by surface roughening. After that, R. P. Van Duyne labeled a new type of Raman signal enhancement as Surface Enhanced Raman Scattering (SERS) [44]. When one considers the Raman intensity, which is proportional to the square of the induced electric dipole moment $P^{(1)}$, the enhancement effect can be due to either the molecular polarizability $\alpha$ (i.e. molecular effect) or the electric field experienced by the molecule around the metal surface. Several enhancement processes may also occur simultaneously. Theoretical models on Raman signal enhancement involve an electromagnetic effect (i.e. field effect) in which the molecule experiences large local fields caused by the electromagnetic resonance near metal nanostructure and a chemical effect in which the molecular polarizability is affected by an interaction between molecule and the metal surface [45].

3.1 Electromagnetic Enhancement

The electromagnetic enhancement and chemical effect are responsible for SERS application, as expressed by Eq. (3.1) [19, 20].

i. Raman scattering takes place in the enhanced electromagnetic field of the metal nanostructures excited by incident laser light and

ii. A molecule in contact with a metal surface creates a ‘new Raman process’ with a cross section larger than that of a free molecule.

$$S_R(v_S) = N'\sigma_{ads}^{R} |A(v_L)|^2 |A(v_S)|^2 I(v_L)$$  (3.1)
Principle of surface enhanced Raman scattering (SERS)-principles and detection of PAHs

where $S_R(\nu_s)$ is the SERS intensity, $A(\nu_L)$ and $A(\nu_s)$ are the electromagnetic enhancement factors for an excitation source of intensity $I(\nu_L)$ and Raman scattered field, respectively, $
u_L$ and $\nu_s$ are the frequencies of laser light and Raman scattered light, respectively, $\sigma^h_{ads}$ is the increased Raman cross section of the molecule in contact with the metal nanoparticle (chemical effect), and $N'$ is the number of molecules involved in SERS process.

Among two enhancement factors, the electromagnetic interaction between molecule and noble metal nanoparticle surface is crucial for SERS enhancement. Of the different models of the electromagnetic effect, the simplest and best studied one is that of the molecule adsorbed on a single metal nanoparticle (sphere or spheroid) embedded in a homogeneous medium with dielectric constant $\epsilon_0$ [46, 47]. A molecule at distance $d$ from a metal sphere with radius of $r$ and complex dielectric constant $\epsilon(\nu)$ is excited by a field $E_m$, which is the superposition of the incident field $E_0$ and the field of dipole induced in the metal sphere. Therefore, the electromagnetic field enhancement factor $A(\nu_L)$ which is the ratio of the field at the position of the molecule $E_m(\nu_L)$ and the incident field $E_0(\nu_L)$ is expressed by Eq. (3.2).

$$A(\nu_L) = \frac{E_m(\nu_L)}{E_0(\nu_L)} = \frac{\epsilon(\nu_L) - \epsilon_0}{\epsilon(\nu_L) + 2\epsilon_0} \left( \frac{r}{r + d} \right)^3$$ \hspace{1cm} (3.2)

The Raman scattered field is also enhanced in the same manner, but with $\nu_s$ instead of $\nu_L$; therefore the electromagnetic enhancement contribution can be written into Eq. (3.3).

$$|A(\nu_L)|^2 A(\nu_s) = \frac{\epsilon(\nu_L) - \epsilon_0}{\epsilon(\nu_L) + 2\epsilon_0} \left( \frac{r}{r + d} \right)^{12}$$ \hspace{1cm} (3.3)

Eq. (3.3) shows several of the important features of the electromagnetic enhancement for SERS. First of all, the enhancement depends on the fourth power of the localized electric field at the nanoparticle surface. Second, the enhancement is maximized when both the incident laser and Raman scattered fields are in resonance with the surface Plasmon – the collective oscillation of the conduction electrons at the metal surface by the excitation of incident light, i.e.
when $\epsilon(\nu_L) + 2\epsilon_0 = 0$. Third, although the enhancement does not require the direct contact of the metal and molecule, the dependence of distance between metal surface and the analyte is strong, i.e. twelfth power of $r$ in $r + d$. Therefore, the electromagnetic enhancement for isolated single nanoparticle ranged in $10^6 - 10^7$ [48]. This theoretical result can be used to explain the SERS in sol-gel based film containing the isolated metal nanoparticles. Especially, the dependence of not only the distance between metal surface and the analyte (see Eq. (3.3)) but also the surface coverage of the analytes $N'$ (see Eq. (3.1)) on the SERS intensity reveals that the preconcentration of PAHs to nanoparticle surface will contribute to high SERS activity. SERS enhancement of a single spheroid metal nanoparticle is strongly dependent on the shape of nanoparticle, i.e. when the prolate spheroidal metal nanoparticle with a certain axial ratio $(b/a)$ is in SPR with the excitation wavelength the electromagnetic enhancement factor is theoretically predicted to be greatly increased [46, 47]. For example, in the case of highly prolate silver spheroids, i.e. $b/a = 4:1$, field enhancement at the tip is very large, 10-25 times larger than the average enhancement. G. C. Schatz and R. P. Van Duyne [49] predicted that the peak Raman enhancement at the tip of spheroidal particle should be $10^2$-$10^3$ times larger than the average Raman enhancement, so if the average enhancement is $10^6$, the peak enhancement would be $10^9$. Raman signal enhancement of pyrene adsorbed to laser tailored nanoparticles as metal spheroids has experimentally demonstrated that SERS intensity strongly depends on the particle shape [23].

Stronger enhancements come from the sharp features and surface area of large curvature in the noble metal nanoparticle, i.e. colloidal aggregates [50 - 52] or nanocrystal junctions or edges [53]. In such metal particle system with complex geometry, each nanoparticle is electromagnetically coupled to many other particles [54]. Collective theory for SERS reveals that not only the electromagnetic enhancement between neighboring metal nanoparticles is maximized when the distance between them is equal to the diameter of metal particle but also the excitation wavelength which gives SERS maximum is shifted to longer one with decreasing the distance between metallic objects. This electromagnetic coupling is essential for producing the Raman enhancements as large as $10^5$-$10^6$. This theory also explains the field enhancements on the metal surfaces with different roughness, i.e. the maximum is blue-shifted and its enhancement is strongly reduced with decreasing the roughness of metal surface. Based on this theory, partition-layered Ag film over nanosphere was demonstrated to have high SERS.
Principle of surface enhanced Raman scattering (SERS)-principles and detection of PAHs

sensitivity (e.g. LOD of 700 pmol/l for pyrene) [21]. As well this effect reflects that the nanoparticle aggregation is one of the enhancement factors.

3.2 Chemical Enhancement

Except for the field enhancement, Raman signal enhancement of molecule adsorbed on the metal surface can be explained by chemical or electronic enhancement because of the increase in Raman cross-section in contact with a metal nanostructure. An important process that produces an increase in apparent value of the molecular polarizability, $\alpha$, involves the charge transfer mechanisms or chemical bond formation between the metal and adsorbate [45]. This chemical effect is associated with the overlap of metal and molecule electronic wavefunctions, which leads to the light-induced charge transfer process [55].

In the charge-transfer model, an electron of the metal which is excited by the incident laser photon tunnels into a charge-transfer excited state of the adsorbed molecule. The resulting negative ion, i.e. the complex of the adsorbate molecule and an electron, has the different equilibrium geometry with the original neutral adsorbate molecule. Therefore, the charge transfer process induces a nuclear relaxation in the adsorbate molecule which, after return of the electron to the metal, leads to a vibrationally excited neutral molecule but also to the emission of a Raman shifted photon.

The chemical enhancement effect is particularly effective for molecules adsorbed at defect sites on the metal surface. The adatom model predicted resonant Raman scattering by the adsorbate vibrations through a photon excited charge transfer transition from localized electronic state at sites of atomic scale roughness on the silver surface to the affinity level of the adsorbate [56].

The major difference between the electromagnetic and chemical effects is the fact that the chemical effect contribution to SERS is nearly short-ranged (0.1 nm – 0.5 nm), which depends on the adsorption site, the geometry of the bonding, and the energy levels of the adsorbate molecule [45].

The contribution of charge-transfer processes to SERS has been estimated to be approximately $10^{-10}$ [55-57]. Chemical enhancement can provide the useful information on the chemisorption interactions between metal and adsorbate.
3.3 SERS for trace detection of PAHs

Analytical chemists continually develop new type of sensor to achieve a required sensitivity on the specific toxic chemicals in ecosystem. Social need and global concern on the environmental problems drive this work increasingly. Especially, SERS technique developed by our group gave a powerful potential to enable the trace detection of toxic chemicals like PAHs in seawater [8, 13].

As can be seen in above section, theoretical models on the electromagnetic and chemical enhancements reveal that the main factors for high SERS activity are the creation of surface plasmon resonance (SPR) close to the emission wavelength of incident laser light, metal colloidal aggregation, hot spot effect (or localized resonant plasmon modes) and the improvement of surface coverage of analyte molecules to metal nanoparticle.

Much effort has been devoted to develop the high active SERS sensor for the trace detection of PAHs in aqueous solution by several research groups [8, 18, 21-23]. Fig. 3.1 shows various types of SERS active nanostructures for the trace detection of PAHs in aqueous solution.

Metal colloid based sol-gel film as SERS substrate was developed by our group to detect the PAH molecules in seawater [7-16]. In this substrate, metal colloids are encapsulated in sol-gel matrix as the homogeneous medium (see Fig. 3.1 a)).

This approach is based on the combination of the high SERS activity of metal colloids with the chemical and mechanical stability of sol-gel based xerogel film. Because bare silver colloid is very unstable in seawater, SERS activity is reduced to zero within a few minutes because of the adsorption of chloric ions or the formation of AgCl on the silver surface.

In the following, PAHs as non-polar molecules do not adsorb well to bare metal nanoparticle surfaces resulting in the small surface coverage, i.e. low SERS activity. Therefore, sol-gel film with the hydrophobicity contributes to not only high SERS activity by the improvement of adsorption of PAH molecules to the metal surface but also chemical and mechanical stability of SERS substrate by the protection of silver colloids from the attack of chloride ions dissolved in seawater. It has been shown that sol-gel process is one of the efficient methods for fabricating a high sensitive SERS substrate suitable for the trace detection of PAHs in seawater.
High SERS activity for PAH molecules has been achieved by using the special types of metal nanostructures such as spheroid metal nanoparticle ensemble on quartz substrate (see Fig. 3.1 b)) and metal film over nanosphere (see Fig. 3.1 c)). The optical spectrum of metal nanoparticle is dominated by SPR, which is attributed to a coherent excitation of the conduction band electrons [58]. Plasmon resonance on the nanoparticle surface i. e. surface plasmon resonance (SPR) provides a major contribution to electromagnetic enhancement with non-selectivity [45]. Incident light irradiated to metal surface excites the conduction electrons of metal and induces the excitation of surface plasmon leading to Raman signal enhancement. At the plasmon resonance frequency, the metal nanostructure becomes strongly polarized, resulting in the large local field enhancement on the surface. Polarizability of the metal nanoparticle is dependent on the dielectric constants of metal nanoparticle and surrounding medium, the size and shape of nanoparticle [58]; therefore SPR
wavelength of nanoparticle is shifted according to its size and shape. Especially, the polarizability of non-spherical metal nanoparticle such as oblate or prolate one is a tensor, i.e. the tensor elements which depend on the shape of nanoparticle but also can be diagonalized corresponding to the three main axes of a nanoparticle [59]. This means that for the oblate nanoparticle not only the surface plasmon splits into two dipole modes, a high-energetic (1,0) mode, due to the collective oscillation of electrons through a short axis and a low-energetic (1,1) mode, due to the collective oscillation of electrons through a long axis but also SPR mode can be tuned by adjusting the size and shape of metal nanoparticle. Spheroid gold nanoparticle ensemble on quartz substrate was prepared by using the naturally grown technique and laser-assisted growth technique under ultrahigh vacuum condition [23].

Tuning of the SPR frequency in SERS substrate was achieved by controlling the morphology of metal nanoparticles and the SERS activity has been optimized by stabilizing the surface plasmon resonance of gold nanoparticles in the vicinity of the laser wavelength of $\lambda = 647\, \text{nm}$ and $\lambda = 785\, \text{nm}$ used for SERS excitation. However, this type of SERS substrate has not been demonstrated with respect to the some issues which should be characterized as the nanoparticle based chemical sensor, i.e. first, the dependence of SPR wavelength on the SERS activity, second, the concentration dependence calibration curve and LOD for selected PAHs and third, what is the operating condition for this substrate.

In addition, high SERS activity of periodically patterned nanorhomb arrays for biomolecule analysis was also achieved by adjusting the shape of nanoparticle [60]. The hydrophobicity of substrate has been demonstrated experimentally to improve the SERS activity for PAH molecules by self-assembling gold colloids onto mercaptopropyl-trimethoxysilane (MPMS) functional layer (see Fig. 3.1 d)), i.e. MPMS monolayer with hydrophobicity contributes to the high surface coverage of PAH molecules to metal colloid surface. Gold colloid as a source of electromagnetic enhancement is very stable in sea-water compared to silver colloid. Therefore, the self-assembled gold colloid film has been applied as SERS substrate for the trace detection of PAHs in artificial seawater.

Based on the collective theory for SERS [54], silver film over nanosphere was employed as a nanostructure for high electromagnetic enhancement [21]. However, the analytes which are able to be detected by a certain type of SERS substrate are largely limited to species that have a natural affinity for either the noble metal surface or something that binds to the noble metal surface, where the large electromagnetic fields are localized. Therefore, it has been attempted to
develop the efficient method that facilitate the use of SERS for nontraditional SERS analytes like Glucose and PAHs by employing a substrate (or metal colloid)-bound partition layer to preconcentrate the analyte of interest within the zone of electromagnetic enhancement [21, 22, 61-63]. Decanethiol monolayer-modified silver film over nanosphere (see Fig. 3.1 c)) and dithiocarbamate calixa[4]arene (DTCX) functionalized silver nanoparticles (see Fig. 3.1 e)) are typical examples for preconcentrating the PAH molecules within the localized area of high intensity electric field of nanoparticle excited by an incident laser light.

For the trace detection of chemicals, the limit of detection (LOD) is the most important quantity. LOD is a minimum concentration which the sensor yields the analyte signal 3 times greater than the standard deviation of the blank signal. Table 3.1 shows the LODs for selected PAHs determined by the previously developed SERS sensors [8, 17, 21-23].

<table>
<thead>
<tr>
<th>Substrate type</th>
<th>Integration time</th>
<th>Laser power ,Excitation wavelength</th>
<th>PAH (solvent)</th>
<th>LOD (ppt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ag colloid based sol-gel film [8]</td>
<td>5 s</td>
<td>25 mW, 785 nm</td>
<td>Pyrene (sea water)</td>
<td>335</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Fluoranthene (sea water)</td>
<td>600</td>
</tr>
<tr>
<td>Self-assembled Au colloid film [18]</td>
<td>10 s</td>
<td>0.1 mW, 632.8 nm</td>
<td>Naphthalene (seawater)</td>
<td>10000</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pyrene (seawater)</td>
<td>10000</td>
</tr>
<tr>
<td>Partition-layered Ag film over nanosphere [21]</td>
<td>30 s</td>
<td>2.84 mW, 532 nm</td>
<td>Anthracene (methanol)</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Pyrene (methanol)</td>
<td>140</td>
</tr>
<tr>
<td>DTCX functionalized Ag colloid [22]</td>
<td>/</td>
<td>785 nm</td>
<td>Pyrene (water)</td>
<td>2020</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Coronene (water)</td>
<td>30</td>
</tr>
<tr>
<td>Laser tailored Ag nanoparticle ensemble [23]</td>
<td>/</td>
<td>785 nm</td>
<td>Pyrene (methanol)</td>
<td>not determined</td>
</tr>
</tbody>
</table>

By comparing EQS proposed by European Union (See Table 2.1.) with LODs for PAHs obtained by above substrates (Table 3.1), we can say that higher active SERS sensor is necessary for the trace detection in real water condition.
Summarizing the above, it is concluded that the following points are critical for developing the high active SERS sensor suitable for the trace detection of PAHs in water body, i.e. seawater and/or fresh water.

i. Silver nanoparticles are required to be protected from the attack of chloride ions in seawater by encapsulating them into the sol-gel matrix for mechanical and chemical stability of the SERS substrate in seawater.

ii. Because of the strong dependence of the distance between analyte and metal surface on SERS activity but also low natural affinity (surface coverage) of PAHs to the bare metal surface, it is required to employ the efficient method to functionalize the metal surface with partition layer (or hydrophobic layer) that can preconcentrate PAH molecules within the local area of high electric field induced by incident laser light.

iii. The combination of sol-gel process (Fig. 3.1 a)) with the calixarene functionalization method (Fig. 3.1 e)) will contribute to improve the sensitivity of the SERS substrate for the trace detection of PAHs in seawater.

iv. By adjusting the shape of Ag nanoparticle, SERS substrate with SPR close to the emission wavelength of laser light (Fig. 3.1 b)) can be obtained to achieve high sensitivity for the trace detection of PAHs in water. However, because Ag nanoparticle is unstable in seawater, Ag nanoparticle substrate without a protecting material is suitable for the trace detection of PAHs only in fresh water.

Especially, SERS substrate with the schematic structure like Fig. 3.2 is aimed to prepare for the trace detection of PAHs in seawater according the above point (iii).

Figure 3.2: Aimed scheme of calixarene functionalized Ag colloid sol-gel film
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In the following chapter, the fabrication methods of high sensitive SERS substrates which are suitable for the trace detection of PAHs in water body (artificial sea-water and distilled water) are presented in a detail.

In addition, the nanostructures of the SERS substrates are also characterized with scanning electron microscopy (SEM), atomic force microscopy (AFM) and the optical extinction spectrum.
4 Preparation of SERS substrates and sample solutions

The main purpose of my research work is to develop the high sensitive SERS substrates which are suitable for the trace detection of PAH molecule as non-traditional SERS analytes dissolved in water body. This chapter describes the preparation of two SERS substrates and the characterization of their nanostructures, i.e. the calixarene functionalized Ag colloid based sol-gel film (section 4.1) and the naturally grown Ag nanoparticle ensemble (section 4.2), and the preparation of the analyte sample solutions (section 4.3).

4.1 Calixarene functionalized Ag colloid based sol-gel film

In all experiments on SERS investigation, specially prepared SERS substrates are always required depending on the specific applications. For the \textit{in-situ} SERS measurement under the marine environment, it is necessary to prepare the SERS substrate which metal nanoparticle as an amplifier of Raman signal should be prevented from the attack of chloride ions in sea-water. Sol-gel process was adapted by our group to encapsulate the metal colloid in sol-gel matrix [11]. In addition, as mentioned above, the introduction of a hydrophobic functionalization to nanoparticle system will improve the sensitivity of the substrate for PAHs, which the calixarene derivatives were recognized to be promising. To improve the preconcentration of PAHs to nanoparticle surface, a calixarene derivative which has a specific selectivity to PAHs was developed and applied as a dopant material for the preparation of high sensitive sol-gel SERS substrate for trace detection of PAHs dissolved in seawater. New type of calixarene derivative was developed by us for the first time with a simple procedure.

4.1.1 Preparation of Calixarene derivative

4-tert-butyl-calix[4]arene (C_{44}H_{58}O_{4} : CX) has the hydrophobic property that is able to attract non-polar molecules to its cavity. Because of its special chemical structure, it has been recognized to have the high selective attraction of PAH molecule to its cavity through the formation of the host-guest complex resulting in the significant surface coverage of PAH.
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molecules around the metal nanoparticle surface for high SERS activity [64]. In addition, CX has 4 hydroxyl groups in the inner rim (see Fig. 4.1.1 a)).


To attach CX to the silver nanoparticle surface, two hydroxyl groups should be replaced with other functional groups which are able to chemically adsorb to the metal surface. For this purpose, mercaptoacetic acid (HSCH$_2$COOH) is very promising because it is chemisorbed to the metal nanoparticles and therefore has been successfully used as a metal colloid stabilizing agent [65, 66].

To modify CX with the mercaptoacetic acid groups, the following procedure was used. At the first stage, CX solution was prepared by mixing 300 mg each of CX and K$_2$CO$_3$ with 10 ml of tetrahydrofuran (C$_4$H$_8$O) followed by heating the mixture at 65 ºC for 12 hours [67].

In the following, mercaptoacetic acid chloride (HSCH$_2$COCl) was prepared by slowly adding 2.45 ml of thionyl chloride (SOCl$_2$) to 1.9 ml of mercaptoacetic acid. During the chemical reaction, gases such as sulfur dioxide (SO$_2$) and hydrogen chloride (HCl) were produced to evaporate. Therefore, it should be handled carefully.
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In the next step, 102 mg of mercaptoacetic acid chloride were added to 10 ml of 46 mM 4-tert-butyl calix[4]arene in tetrahydrofuran, thus forming 25,27-dimercaptoacetic acid-26,28-dihydroxyl 4-tert-butyl calix[4]arene (C_{44}H_{52}(OH)_{2}(HSCH_{2}COO)_{2}: DMCX).

Molecular structure of DMCX is shown in Fig. 4.1.1 b). As can be seen in Fig. 4.1.1, two hydroxyl groups in CX (Fig. 4.1.1 a)) are replaced with two mercaptoacetic acid groups to form DMCX (Fig. 4.1.1 b)).

After that, DMCX was dissolved in ethanol to have a molar concentration of 10^{-2} mol/l as a stock solution. To obtain the optimized concentration of DMCX for high SERS activity, stock solution was diluted with ethanol to have the selected concentrations of 10^{-6} mol/l, 10^{-5} mol/l, 10^{-4} mol/l, 10^{-3} mol/l and 10^{-2} mol/l, respectively.

4.1.2 Preparation of SERS substrate

To prepare the SERS substrates silicon slides with a thickness of 0.8 mm were cut into 10×15 mm² sections cleaned using piranha solution (70 % H_{2}SO_{4}, 30 % H_{2}O_{2}) heated at 70 ºC for 30 minutes. After that treatment the slides were rinsed with methanol several times and stored in methanol until use.

The SERS substrates were prepared using a sol-gel procedure previously developed by our group [15]. At first, 17.4 mg of AgNO_{3} were dissolved in 60 µl of pH 0.6 H_{2}O by stirring with a magnetic bob for 2 minutes. To this solution, 149 µl of DMCX in ethanol with selected concentration as a dopant material but also 163 µl of methyltriethoxysilane (CH_{3}Si(OC_{2}H_{5})_{3}: MTEOS) were added to form the sol-gel solution. The mixture was stirred with a magnetic bob for 5 minutes and after that every silicon section was spin-coated with the sol solution at 3000 rpm for 10 second. The spin-coated substrates were baked for 17 hours at 70 ºC, after which AgNO_{3} was thermally reduced by heating the substrate to 230 ºC for 2 hours forming the silver nanoparticles in the sol-gel matrix. During heating process, AgNO_{3} in sol-gel film is converted into silver colloids due to the heat reduction and DMCX which is involved in sol-gel matrix will influence the SERS activity of the substrate.

This substrate will be named according to the metal nanoparticles, calixarene derivative and precursor type used. For example, a Ag,DMCX:MTEOS SERS substrate refers to a MTEOS-derived sol-gel film containing Ag nanoparticle and DMCX molecule which is aimed to functionalize the Ag nanoparticle. Finally, the SERS substrates were stored in distilled water.
Preparation of SERS substrates and sample solutions

until use. The aimed schematic representation of the adsorption of a pyrene molecule to the DMCX functionalized silver nanoparticle in sol-gel matrix is shown in Fig. 4.1.2.

![Schematic representation of the adsorption of a pyrene molecule to the silver colloid functionalized with DMCX.](image)

Figure 4.1.2: Schematic representation of the adsorption of a pyrene molecule to the silver colloid functionalized with DMCX.

4.1.3 Influence of DMCX concentration on the SERS activity

SERS activity of a Ag,DMCX:MTEOS film might be affected by some parameters for fabricating SERS substrate, i.e. the value of AgNO₃, DMCX concentration, the value of MTEOS and pH value of water contained in the sol solution. The important factor for optimizing the SERS substrate is the concentration of DMCX in ethanol, because DMCX improves the natural affinity of PAH molecules onto the silver colloid surface. To investigate the influence of DMCX concentration on the SERS activity, three preparation parameters such as the value of AgNO₃, the value of MTEOS and pH value of water contained were fixed at every SERS substrate [15]. DMCX concentration involved in the sol solution was selected to be $10^{-6}$ mol/l, $10^{-5}$ mol/l, $10^{-4}$ mol/l, $10^{-3}$ mol/l and $10^{-2}$ mol/l, respectively.
Preparation of SERS substrates and sample solutions

Sol solution was made to have a selected DMCX concentration and after that spin-coated onto every silicon slide. 5 spin-coated substrates with different DMCX concentrations were baked according to the heating procedure described above (see section 4.1.2).

The influence of DMCX concentration on the SERS activity of Ag,DMCX:MTEOS film was carefully investigated with 5 SERS substrates at different concentration. To do this, 1 droplet of 40 \( \mu \text{mol/l} \) pyrene in methanol was dropped onto every Ag,DMCX:MTEOS film and 15 minutes after putting it in air condition SERS spectra were recorded from the pyrene adsorbed substrate at the sample position of 671 nm Raman set-up. Experimental parameters, i.e. laser power at the sample position and integration time were selected to be 6 mW and 10 s, respectively. SERS activity of every Ag,DMCX:MTEOS film was evaluated using 1234 cm\(^{-1}\) Raman band of pyrene adsorbed. Fig. 4.1.3 shows the influence of DMCX concentration on SERS activity of Ag,DMCX:MTEOS film.

As can be seen in Fig. 4.1.3, SERS activity is dependent on the DMCX concentration in sol solution. The best SERS activity was obtained when using 1 mmol/l DMCX in ethanol for the preparation of Ag,DMCX:MTEOS film.

![Figure 4.1.3: Influence of DMCX concentration on SERS activity of Ag,DMCX:MTEOS film evaluated using 1234 cm\(^{-1}\) Raman band of pyrene with its concentration 40 \( \mu \text{mol/l} \) in methanol; excitation wavelength: 671 nm, laser power at the sample position: 6 mW, integration time: 10 s.](image-url)
Preparation of SERS substrates and sample solutions

In a detail, with increasing DMCX concentration, $1234 \text{ cm}^{-1}$ pyrene Raman band was increased until 1 mmol/l of DMCX concentration was reached. Especially, when 10 mmol/l DMCX in ethanol was added in sol solution, the precipitant was formed to make the blur film after spin-coating. SERS spectra from Ag$_x$DMCX:MTEOS film with 10 mmol/l DMCX contained high fluorescence and the analyte Raman band was suddenly decreased. This is because with increasing the concentration, DMCX with hydrophobicity is difficult to be mixed with pH 0.6 water containing AgNO$_3$. Ag$_x$DMCX:MTEOS film from which a sol solution contains 1 mmol/l DMCX in ethanol was used to investigate the nanostructure of SERS substrate using the scanning electronic microscopy (SEM) image.

4.1.4 Nanostructure of SERS substrate

Noble metal nanoparticle acts as a strong amplifier of Raman signal when the analyte is adsorbed to the metal surface. High SERS activity can be achieved by improving the natural affinity of silver colloid based sol-gel SERS substrate surface previously developed by our group [8]. In attempt to modify the affinity of nanoparticle surface for PAHs, sol-gel film contains DMCX with two functionalities, i.e. not only the selective enrichment of PAH molecules toward calixarene cavity through the formation of host-guest complex but also the attachment of calixarene to the nanoparticle surface through the chelate reaction of mercaptoacetic acid group (see sections 4.1.1 and 4.1.2). The addition of DMCX as a dopant material into MTEOS derived sol solution will influence the nanostructure of SERS substrate. The SERS substrate was characterized using the scanning electronic microscopy (SEM). SEM images of the substrates were taken from ZELMI, Technical University Berlin.

Fig. 4.1.4 shows the SEM images of a Ag$_x$DMCX:MTEOS SERS substrate at different magnifications. As can be seen in Fig. 4.1.4 (a), silver colloid blocks are formed in the sol-gel matrix containing many pores. A closer view in Fig. 4.1.4 (b) shows that the silver colloid blocks are not only formed in the pores of the sol-gel matrix but are also encapsulated in the sol-gel matrix. Especially, silver colloid blocks with small size are embedded in sol-gel matrix but also those with large size are placed in the pores of sol-gel matrix. MTEOS which was used for the formation of sol-gel matrix has a hydrophobic property due to the methyl group and also dissolves the silver nitrate with high solubility. Methyl group in MTEOS has a poor affinity for water and render the sol-gel surface hydrophobic [68].
Figure 4.1.4: Scanning electron microscopy images of the Ag,DMCX:MTEOS SERS substrate at different magnifications. (a) Sol-gel matrix containing the silver colloid blocks, (b) Silver colloid blocks in the sol-gel matrix, (c) Aggregation of silver nanoparticles (taken in ZELMI, TU Berlin).
When MTEOS was added to the silver nitrate solution containing DMCX, MTEOS molecules have the sufficient interparticle forces to cause the aggregation and/or flocculation prior to their growth. In the following, the formation of a more open continuous network containing a liquid phase was done to generate the silicate polymer which forms the ceramic components in 2 and 3 dimensions.

After the liquid phase from the sol-gel solution is removed by the spin-coating and heating process at 70 °C, micro-porous amorphous gel with hydrophobicity is produced. Therefore, when a Ag,DMCX:MTEOS SERS substrate is in the seawater, the hydrophobicity of MTEOS based sol-gel matrix rejects the chloride ions of seawater with hydrophilic property but also contributes to the attraction of PAH molecule as non-polar one to SERS substrate surface. This means that silver nanoparticles are protected from the attack of seawater due to the action of sol-gel matrix to provide long-term chemical stability.

From Fig. 4.1.4 (c), it becomes clear that each silver colloid block is a collective of separate silver colloidal particles. Fig. 4.1.4 (c) also shows the silver colloidal particle distribution which ranges from 50 nm to 120 nm in diameter. The aggregation of the silver colloid [51] and the adsorption of PAH molecules to the silver colloid [22] are critical for improving the SERS activity of the substrate.

It has been shown that zeta potential of the gold nanoparticles functionalized with benzylmercaptan is lowered compared to those without functionalization; therefore the metal colloid is aggregated and the surface plasmon resonance (SPR) is red-shifted [69]. This means that the formation of hydrophobic surfactant monolayer onto the metal nanoparticle surface contributes to the reduction of surface charge of metal nanoparticle, i.e. the attraction between neighbor particles is stronger than the repulsion force. The formation of DMCX monolayer onto Ag colloid surface will be experimentally proved by the presence of DMCX Raman bands from the SERS spectrum of Ag,DMCX:MTEOS substrate in the Chapter 6.

In the Ag,DMCX:MTEOS SERS substrate, high aggregation of the silver nanoparticles was obtained by the addition of DMCX in sol-gel matrix, thus forming a monolayer of CX onto the silver nanoparticle surface. A. Wei demonstrated that although the nanoparticles are presumably separated by two surfactant monolayers such as resocinarene, the interparticle separation is in order of 1 nm or less [70].

In Ag,DMCX:MTEOS SERS substrate, silver nanoparticles might be separated by the calixarene monolayer on the metal surface. As can be seen in Fig. 4.1.4 (c), silver colloid blocks
Preparation of SERS substrates and sample solutions

with high aggregation are due to the formation of calixarene monolayer onto the silver nanoparticle surface after the heat reduction of AgNO$_3$ in sol-gel film and the chelate reaction between mercaptoacetic groups in DMCX and silver surface. Silver nitrate and DMCX remaining in sol-gel matrix after spin-coating and heating process at 70 °C are converted into the silver nanoparticles blocks through the heat reduction process at 230 °C. The silver nanoparticles might be aggregated due to the addition of DMCX to the sol-gel matrix; thus being closely packed into the silver colloid block, at which SERS activity is produced basically by an electromagnetic coupling between neighbor silver colloids.

Comparison of the SEM image of the Ag,DMCX:MTEOS SERS substrate (see Fig. 4.1.4) with that of a Ag:MTEOS SERS substrate [8] reveals that the addition of DMCX to the sol solution causes a higher aggregation of the silver nanoparticles than without DMCX. This type of SERS substrate was used to investigate not only the SERS but also SERS/SERDS for PAHs in artificial seawater.

SERS characterization of this substrate using 671 nm Raman set-up was performed by some experimental results, i.e. the presence of DMCX Raman bands for demonstrating the DMCX functionalization of Ag nanoparticle, the influence of DMCX on the SERS activity, the SERS analysis of PAH-DMCX interaction, the LODs of selected PAHs (e. g. pyrene and naphthalene) in artificial seawater, and signal response time according to pyrene concentration change. This SERS substrate was also used to investigate SERS/SERDS of 3 selected PAHs (e. g. pyrene, anthracene and fluoranthen) in artificial seawater using Raman set-up with a microsystem laser diode module with two emission lines (670.8 nm and 671.3 nm) (see chapter 6).
4.2 Naturally grown Ag nanoparticle ensemble

One factor for the high electromagnetic enhancement in SERS substrate has been shown to be the surface plasmon resonance (SPR) of the nanoparticle in the vicinity of the excitation wavelength [23]. To demonstrate the influence of SPR wavelength on the SERS activity of substrate, it is necessary to tune the SPR wavelength of SERS substrate.

4.2.1 Preparation of naturally grown Ag nanoparticle ensemble

The naturally grown Ag nanoparticle ensembles as SERS substrates with different SPR wavelengths were successfully fabricated in cooperation with Institut für Physik and Center for Interdisciplinary Nanostructure Science and Technology-CINSaT, Universität Kassel. All substrates were prepared under the ultrahigh vacuum (UHV) condition at room temperature. Experimental set-up for the preparation of SERS substrate (Fig. 4.2.1 (a)) contains UHV system with a load lock chamber, a transfer system and a chamber for SERS substrate preparation operating at a base pressure of $p = 5 \times 10^{-9} \text{mbar}$ [71]. An electron beam evaporator (Fig. 4.2.1 (b)) is used for generating a beam of silver atoms, and a Xenon arc lamp (Osram, XBO 450 W/l) combined with a monochromator (Amko, 600 lines/mm, blaze: 400 nm) for measuring the optical spectra of substrate in a chamber (Fig. 4.2.1 (b)). The size of quartz substrate purchased from CrysTec GmbH is 10 x 10 mm$^2$ with a thickness of 0.5 mm. The surface coverage of silver atoms on quartz substrate was determined by measuring the beam flux of neutral silver atoms using a water-cooled quartz crystal microbalance (Fig. 4.2.1 (b)).

After the deposition of silver atoms with a certain monolayers, the optical spectra were measured in-situ using p-polarized light with the wavelength range from 400 nm to 954 nm. The incidence angle of p-polarized light was set to be 45 ° with respect to the surface normal of the substrate. Tuning of SPR wavelength of SERS substrate was monitored by measuring the extinction spectra of the substrate after the deposition of silver atoms with selected monolayers.

After completing the silver deposition for SERS substrate with a required SPR wavelength, it remained in UHV chamber for 17 hours until the substrate temperature was reached to room temperature. At room temperature, extinction spectrum of every substrate was measured again. SPR wavelengths of 5 naturally grown Ag nanoparticle ensembles were measured to be 453 nm, 468 nm, 491 nm, 518 nm and 548 nm, respectively.
Preparation of SERS substrates and sample solutions

(a)

![Image of experimental set-up](image1)

(b)

![Image of chamber setup](image2)

Figure 4.2.1: (a) Experimental set-up for preparing the naturally grown Ag nanoparticle ensemble; (b) the chamber for the substrate preparation with an electron beam evaporator, quartz crystal microvalence and monochrometer (obtained from CINSaT, Universität Kassel).

The Ag nanoparticle ensembles were also characterized by ex situ atomic force microscopy (Park Scientific, Autoprobe-CP) under ambient conditions and in non-contact mode. This type of SERS substrate was further characterized using a Raman set-up containing a microsystem light source with two emission wavelengths (487.61 nm and 487.91 nm) (chapter 7).
4.2.2 Characterization of naturally grown Ag nanoparticle ensemble

To characterize the naturally grown Ag nanoparticle ensemble on quartz substrate, their extinction spectra were measured under in-situ condition. The applied silver atom coverage which was measured by a water-cooled quartz crystal microbalance ranges from $25.8 \times 10^{15}$ atoms/cm$^2$ to $62.2 \times 10^{15}$ atoms/cm$^2$. Extinction spectra which was measured by CINSaT, Universität Kassel show that two dipole plasmon modes, i.e. (1,0) and (1,1) modes can be excited through a short axis and a long axis in the oblate Ag nanoparticle. Extinction spectra also revealed that the dominating (1,1) mode was shifted toward the long wavelength and increases in amplitude with the increasing amount of deposited material.

To demonstrate that with increasing the Ag atom coverage the naturally grown Ag nanoparticles become more and more oblate, the SERS substrates with different SPR wavelength were also characterized with the atomic force microscopy (AFM) images (taken in CINSaT, Universität Kassel).

As an example, AFM images of the naturally grown silver nanoparticle ensemble on a quartz substrate with a SPR wavelength of 491 nm are shown in Fig. 4.2.2 at different magnifications. After the Ag atom deposition of $45.5 \times 10^{15}$ atoms/cm$^2$ corresponding to 491 nm of SPR wavelength, the naturally grown silver nanoparticles have a mean equivalent radius of $\langle R_{eq} \rangle = 15 \pm 4$ nm which is the radius of a sphere with the same volume as the actual particles and mean axial ratio of $a/b = 0.21$ which $a$ denotes the short axis perpendicular to and $b$ the long axis parallel to the substrate surface. AFM images of the naturally grown silver nanoparticle ensemble on quartz substrate in Fig. 4.2.2 show the broad size and shape distribution of silver nanoparticles. Silver nanoparticles were produced by the deposition with subsequent diffusion and nucleation of thermal silver atoms on quartz, i.e. Volmer-Weber growth [72].

It has been experimentally demonstrated that in Volmer-Weber growth the shape of silver nanoparticles is sphere at 1 nm of equivalent radius while axial ratio is decreased if they increase in the equivalent radius. Due to the growth process, larger nanoparticles have a pronounced oblate shape [73, 74].
Figure 4.2.2: AFM images of naturally grown silver nanoparticles with a mean equivalent radius of $15 \pm 4$ nm and a SPR wavelength at 491 nm (taken in CINSaT, Universität Kassel).

Fig. 4.2.3 shows the influence of Ag atom coverage on the SPR wavelength and the mean equivalent radius of Ag nanoparticle in the substrate obtained from the analysis of AFM images and extinction spectra.
As can be seen in Fig. 4.2.3, SPR wavelength and equivalent radius of Ag nanoparticles increase with the Ag atom coverage. Therefore, it can be seen that with increasing the surface coverage the size of Ag nanoparticles become larger; therefore resulting in the shift of SPR toward longer wavelengths. This type of SERS substrates were used not only to characterize the SERS spectra and SERS/SERDS spectra of pyrene and the concentration dependent calibration curves but also to demonstrate the influence of SPR wavelength on SERS activity, i.e. the SERS intensities at a certain analyte concentration and LOD for pyrene in water (Chapter 7). As can be seen in Fig. 4.2.3, a naturally grown silver nanoparticle ensemble with SPR wavelength close to 488 nm of the excitation wavelength was obtained at the Ag atom coverage of 45.5 x 10^{15} atoms/cm^2.
4.3 Preparation of sample solution

The sample solutions containing selected PAH were prepared with the following procedures:

1) **Preparation of artificial sea-water samples:**
   For the measurements in laboratory we have prepared the artificial seawater containing synthetic sea salt Instant Ocean® (Aquarium System) with the salinity 35 ‰ and the density 1.022 kg/l at 20 ºC [16]. 300 g of Instant Ocean® salt was dissolved in 10 liters of distilled water. The solution was contained in an open vessel during three days in order to fully saturate with the oxygen and carbon dioxide, and followed it was filtered.

2) **Preparation of stock solutions**
   PAHs in solid phase with the highest available purity and phenylacetylene in liquid phase were purchased from Fluka and Merck. The chemical structures of these chemicals and their properties are shown in Appendix I. Stock solutions of PAHs were prepared by dissolving them in methanol with a purity of 99.8 % to have the selected concentrations. Every PAH concentration in the stock solution was chosen to be 100 times higher than the saturation concentration of PAH in water body (artificial seawater or distilled water), i.e. 18 µmol/l for anthracene, 63 µmol/l for fluoranthene, 18 mmol/l for naphthalene and 44 µmol/l for pyrene. Because the PAHs are known to be extremely toxic to human, they must be handled with a great care.

3) **Preparation of water sample solutions containing selected PAH**
   SERS intensity of selected PAHs at different concentration of methanol showed that when the methanol concentration in seawater is 1 % the SERS intensities decreased by 5 %, while they strongly decreased up to 21 % at the methanol concentration of 2 % in seawater [16]. Therefore the concentration of methanol to be used to dissolve every PAH was selected to be 1 % in the water sample. The water samples containing the selected PAH were prepared at different concentration from the saturation concentration (178 nmol/l for anthracene, 627 nmol/l for fluoranthene, 180 µmol/l for naphthalene, and 435 nmol/l for pyrene) down to the selected minimum concentrations (0.75 nmol/l for anthracene, 1 nmol/l for fluoranthene, 40 nmol/l for naphthalene, and 0.5 nmol/l for pyrene).

Additionally, 1 mmol/l phenylacetylene in water was made by diluting phenylacetylene in liquid phase into pure distilled water.
4.4 Discussion and Conclusion

The preparation of a newly developed SERS substrate which sol-gel film contains silver colloids functionalized with 25,27-dimercaptoacetic acid-26,28-dihydroxyl 4-tertbutyl calix[4]arene (DMCX) in sol solution was presented. The nanostructure of the substrate by SEM images was shown. The influence of DMCX concentration on SERS activity reveals that the best substrate was obtained when 1 mmol/l DMCX in ethanol was added in sol solution. This substrate was used to investigate the nanostructure by SEM images. SEM image of Ag,DMCX:MTEOS SERS substrate showed that the silver colloid blocks with high aggregation were formed in the sol-gel matrix due to the DMCX effect.

Furthermore, SERS substrates with naturally grown silver nanoparticles which were fabricated by the deposition of neutral silver atoms on quartz under the ultrahigh vacuum (UHV) condition were investigated with respect to the nanostructure and optical properties. Naturally grown silver nanoparticle ensembles with different surface plasmon resonance (SPR) wavelengths were prepared by varying the morphology of spheroid silver nanoparticle.

The influence of Ag atom coverage on the SPR wavelength and the mean equivalent radius of Ag nanoparticles was analyzed by using the optical extinction spectra and AFM images. It was shown that with increasing surface coverage the size of silver nanoparticles becomes larger, resulting in the shift of SPR toward long wavelength.
5 Experimental Raman set-ups

In this work, the SERS spectra of selected PAHs in water body were measured with the use of Raman set-ups containing microsystem laser diode modules with different emission lines and a flow-through cell system with a selected SERS substrate.

5.1 671 nm Raman set-up

To obtain the Raman signal of analyte at low concentrations using the developed SERS substrate, the experimental Raman set-up should have high sensitivity in the wavelength range of Raman scattering. The quantum efficiency (QE) starts to decrease at above 700 nm, because the penetration length for the red photons is longer than the thickness of the depletion layer for the conventional back-illuminated CCD [75]. A back-illuminated deep depletion CCD detector (EHRB 1340 x 400, ROPER Scientific) in spectrograph (Chromex 250IS) has a higher QE at 671 nm than that at 785 nm [76].

For the SERS investigation of PAHs in artificial sea-water using a Ag,DMCX:MTEOS film, a Raman set-up with 671 nm microsystem laser diode was used.

A laboratory measurement system applying a microsystem diode laser (FBH Berlin) emitting at 671 nm (or 488 nm) as an excitation light source is shown in Fig. 5.1.

To protect the SERS substrate from damage the optical power of the collimated beam from a microsystem laser diode at 671 nm (1) is reduced to 6 mW (or 12 mW) at the sample position by a gray filter (2). The laser beam is spectrally cleaned up by a band pass filter (Semrock, Inc.) (3), reflected by two dielectric mirrors (Thorlabs GmbH) (4) and a Raman edge filter (LOT Oriel group) (5) and focused by a lens (Thorlabs GmbH) (6) with a focal length of 30 mm onto the SERS substrate located in a flow-through cell (7). The backscattered radiation is collected by the same lens (6) and filtered by two Raman edge filters (5) which are blocking the Rayleigh scattered radiation as well as the anti-Stokes Raman scattering. Only the Raman Stokes radiation is transmitted, passes a spatial filter containing 2 lenses (6) and a pinhole (8) and is then focused inside the spectrograph (Chromex 250IS) (9). Raman spectra were recorded using a back-illuminated deep depletion CCD detector (EHRB 1340 x 400, ROPER Scientific) (10) cooled down to -70 °C and at last analyzed by a computer (11) running Winspec software.
Experimental Raman set-ups

(Roper Scientific). A PilotPC laser driver from Sacher Lasertechnik was used to control the injection current and the temperature of the microsystem diode laser.

Figure 5.1: Scheme of the experimental Raman set-up: (1) microsystem laser diode at 671 nm (or 488 nm), (2) gray filter in 671 nm (or glass color filter in 488 nm), (3) bandpass filter, (4) dielectric mirrors, (5) Raman edge filters, (6) lenses, (7) flow-through cell with SERS substrate, (8) pinhole, (9) spectrograph, (10) CCD detector, (11) computer

The wavelength stability of excitation laser beam less than 1 cm\(^{-1}\) is important to assure Raman shift precision and to avoid the Raman band broadening [41]. A microsystem laser diode at 671 nm is possible to generate laser beam with a spectral width of 70 pm corresponding to 1.55 cm\(^{-1}\) and an optical power of 52 mW at 25 °C of laser diode temperature and 400 mA of injection current.

SERS spectrum of PAHs in seawater contains fluorescence based background and this renders the results useless at low concentration. To separate Raman signal from the fluorescence based background, shifted excitation Raman difference spectroscopy (SERDS) was applied. Detailed examples of the SERDS treatment using a microsystem laser diode module with two slightly different emission wavelengths will be shown in the chapter 6.

For Raman and SERS applying SERDS (Raman/SERDS and SERS/SERDS), a microsystem laser diode was replaced with a microsystem laser diode module with two emission lines (670.8 nm and 671.3 nm). 0.5 nm of the spectral distance between both central wavelengths
Experimental Raman set-ups

corresponds to a wavenumber shift of 11.1 cm\(^{-1}\) which is well suited for SERDS. The introduction of laser diode module with two emission lines into experimental Raman set-up makes it possible to remove the fluorescence based noise from SERS spectra of PAHs in water body at low concentration; therefore improving signal to noise ratio and reducing the LOD for analyte.

5.2 488 nm Raman set-up

A naturally grown Ag nanoparticle ensemble has the SPR wavelength in the range of 453 nm to 548 nm. In addition, because the weak Raman signals can be enhanced by several orders of magnitude using the resonance-Raman effect that enables a contactless detection of chemicals at low concentration a microsystem light source at 488 nm was applied for the shifted excitation resonance Raman difference spectroscopy (SERRDS) [77]. Therefore, a microsystem light source with two emission lines (487.61 nm and 487.91 nm) was also introduced for the excitation of SERS substrate adsorbing pyrene in distilled water.

488 nm Raman set-up (see Fig. 5.1) was used to characterize the naturally grown Ag nanoparticle ensemble on quartz substrate. Raman set-up contains a microsystem light source with two emission lines of 487.61 nm and 487.91 nm [25]. A microsystem light source (1) generates 976 nm fundamental emission line from the distributed feedback (DFB) ridge waveguide (RW) diode laser as a pump source but also 488 nm blue light by the second harmonic generation (SHG) of a periodically poled MgO:LiNbO\(_3\) (PPMgLN) crystal [78]. The collimated blue light passes through a colored glass filter (BG 38, LOT Oriel Group) (2) to remove the fundamental emission line at 976 nm. A blue light at 488 nm is spectrally cleaned up by a band pass filter (Semlock, Inc.) (3), reflected by two broadband dielectric mirrors (Thorlabs, GmbH) (4) and a Raman edge filter (Semlock, Inc.) (5), and focused by a lens with a focal length of 30 mm (Thorlabs, GmbH) (6) to a SERS substrate in the flow-through cell (7). The backscattered light from the sample is collected by the same lens (6).

The only Raman Stokes signal passes through two Raman edge filters (5) which are blocking the Rayleigh scattering light and anti-Stokes Raman scattered light. The Raman signal passes a spatial filter containing 2 lenses (6) and a pinhole (8) and is then focused inside spectrograph (Horiba Jobin Yvon, iHR 320) (9) equipped with a 1800 lines/mm grating and a back-illuminated (UV) anti-reflection (AR) coated charge-coupled device (2048 x 512 BIUV-1LS)
Experimental Raman set-ups

with the liquid nitrogen cooling (10). The selection of different excitation wavelengths was done by adjusting the temperature and injection current of a microsystem light source with laser diode driver (LDC 1000) e.g. laser light with an emission wavelength of 487.61 nm and the laser power of 6 mW at the sample position was generated from a microsystem light source by selecting the temperature and injection current to be 30 °C and 350 mA, respectively, while laser light of 487.91 nm and 8 mW at the sample position was obtained by choosing them to be 38 °C and 361 mA, respectively.

5.3 Flow-through cell system

As can be seen in Fig. 5.1, a self-constructed flow-through cell was positioned at the sample position of experimental Raman set-up for the continuous measurement of Raman signal of PAHs in water body under the experimental condition similar to real field. The flow-through cell was designed in order that laser beam is focused onto the SERS substrate surface after passing the sample solution.

Fig. 5.2 shows the scheme of the flow-through cell. The flow-through cell was made from a quartz window and a seawater resistant aluminum alloy. The thickness of sample solution in the cell is 3 mm and the effective volume is 113 µl.

Figure 5.2: Scheme of flow-through cell for continuous detection of PAHs in water body.
Experimental Raman set-ups

A peristaltic pump (PP1-05, ISMATEC) was positioned at the outlet side of flow-through cell system to pump the sample solution from the reservoir to the cell without any contamination from other chemicals. High precision liquid chromatograph (HPLC) metal capillary tubes were linked to the cell to protect the sample solution from contamination. A peristaltic pump draws the solution from the sample reservoir through the flow-through cell with the speed in the interval from 20 µl/s to 115 µl/s by adjusting the rotation rate.

Fig. 5.3 shows the relation between the rotation rate of peristaltic pump and the flow-rate of artificial seawater sample in our flow-through cell system.

![Graph showing the relation between rotation rate of peristaltic pump and flow-rate of artificial seawater sample. The equation is y = 1.8823x + 2.0251 with R² = 0.9849.]

During the SERS measurement, the flow-rate of artificial seawater containing a selected PAH remained to be 30 µl/s.

Fig. 5.4 is a picture of the experimental Raman set-up containing a 671 nm microsystem laser diode and flow-through cell system for characterizing a Ag,DMCX:MTEOS SERS substrate. This Raman set-up was used for the continuous SERS measurements of artificial seawater samples containing pyrene and naphthalene, respectively.

For SERS/SERDS investigation of 3 selected PAHs e.g. pyrene, fluoranthene and anthracene in artificial seawater, excitation source was replaced into a microsystem laser diode module which is able to generate two emission lines of 670.8 nm and 671.3 nm.
Figure 5.4: A picture of the experimental Raman set-up containing a microsystem laser diode with an emission line of 671 nm (or with two emission lines of 670.8 nm and 671.3 nm) and flow-through cell with SERS substrate. The Raman stray light marked with yellow color was focused in the spectrograph.

Fig. 5.5 shows the experimental Raman set-up for SERS/SERDS investigation which contains a microsystem light source with two emission lines (487.61 nm and 487.91 nm) and also the flow-through cell with a naturally grown Ag nanoparticle ensemble as SERS substrate.

For SERS measurements of PAH in artificial seawater at the different concentration, sample solution in reservoir drew to the flow-through cell containing a SERS substrate for 15 minutes by a peristaltic pump to reach the perfect adsorption equilibrium of PAH molecules in the interface between SERS substrate and sample solution, and followed the SERS spectra were recorded.
Experimental Raman set-ups

Figure 5.5: A picture of the experimental Raman set-up (excitation source: microsystem light source with two emission lines of 487.61 nm and 487.91 nm) for characterizing a naturally grown Ag nanoparticle ensemble suitable for trace detection of PAHs in distilled water.
6 Trace analysis with DMCX functionalized Ag colloid based sol-gel film

In this chapter, we discuss the main factors of high SERS activity in the Ag,DMCX:MTEOS substrate regarding with the electromagnetic enhancement but also the role of DMCX as a dopant material on the SERS sensitivity of the substrate for PAHs in artificial seawater. The concentration dependent SERS intensities of two selected PAHs e.g. pyrene and naphthalene but also signal response of this substrate according to the concentration change of pyrene in artificial seawater are discussed (section 6.1). Raman and SERS measurements applying SERDS which result in Raman/SERDS spectra and SERS/SERDS spectra of three selected PAHs (pyrene, anthracene and fluoranthene) in seawater, and the improved sensitivity by SERDS are presented (section 6.2).

6.1 SERS of DMCX functionalized Ag colloid based sol-gel film

Pyrene is dissolved in seawater with high concentration [5], and naphthalene is an environmental pollutant stated by European Union [27]; these PAHs were selected as the analytes for characterizing this SERS substrate.

6.1.1 Influence of DMCX on SERS activity

6.1.1.1 Metal colloid aggregation and SERS activity

At first, the SERS activity, especially due to the electromagnetic enhancement, is discussed regarding with the existence of DMCX in the sol-gel matrix. To demonstrate experimentally the influence of the DMCX addition into the sol-gel matrix on the electromagnetic enhancement factor for the analyte with one benzene ring, two types of SERS substrates, i.e. Ag,DMCX:MTEOS SERS substrate and Ag:MTEOS SERS substrate, were characterized with 1 mM phenylacetylene in water. SERS spectra of 1 mM phenylacetylene in water were recorded using a 671 nm Raman set-up with a laser power of 7 mW and an integration time of 1 s. The structure of phenylacetylene and its property are shown in Appendix I.
Fig. 6.1.1 shows the SERS spectra of 1 mM phenylacetylene in water from two substrates, i.e. Ag:MTEOS film and Ag,DMCX:MTEOS film. As can be seen in Fig. 6.1.1, the Ag,DMCX:MTEOS film (a) has higher SERS activity than Ag:MTEOS film (b), i.e. the Raman intensities are more than ten times higher than those from the latter one. This means that LOD for phenylacetylene in water can be reduced by one order of magnitude.

In general, the aggregation of metal nanoparticles is a key factor for a strong SERS effect i.e. electromagnetic enhancement [66]. Silver nanoparticle aggregation results in a stronger SERS effect compared to isolated silver nanoparticles because of the coupling effect between the silver colloids [54]. H. Xu et al have theoretically demonstrated that the electromagnetic contribution at the midpoint between two spherical silver particles with the same radius is increased with decreasing the distance between two particles [51]. In addition, electromagnetic enhancement
Trace analysis with DMCX functionalized Ag colloid based sol-gel film

was proven experimentally to be dependent on the distance between the analyte molecule and metal surface [79]. The comparison of SERS spectrum with conventional Raman spectrum of phenylacetylene showed that some of Raman bands after the adsorption of the analyte to the SERS substrate are modified because of the interaction between the analyte and the metal [45, 80, 81]. Two SERS spectra (Fig. 6.1.1 (a) and (b)) reveal that high aggregation of Ag nanoparticles due to the addition of DMCX in sol solution (compare the SEM image in Fig. 4.1.4 with that of Ag:MTEOS film [9].) is one factor for high SERS activity of the Ag,DMCX:MTEOS film adsorbing phenylacetylene.

To show not only the influence of Ag colloid aggregation on SERS activity for PAH but also the reason why the Ag colloid aggregation is caused by the DMCX addition, Fig. 6.1.2 shows (a) the Raman spectrum of DMCX in solid phase, (b) the SERS spectrum of pure artificial seawater using a Ag:MTEOS substrate, and the SERS spectra of (c) pure artificial seawater using a Ag,DMCX:MTEOS substrate and (d) 0.5 nmol/l pyrene in artificial seawater using a Ag,DMCX:MTEOS substrate. To record the SERS spectra of the two substrates, the laser power and integration time were selected to be 12 mW and 10 s, respectively. The flow-rate of the analyte sample solution was fixed to be 30 µl/s.

Raman spectrum of DMCX in solid phase (Fig. 6.1.2 (a)) shows characteristic Raman bands in the region of 500-1650 cm$^{-1}$ (569, 696, 792, 910, 1122, 1201, 1295, 1449, and 1605 cm$^{-1}$). The SERS spectrum of pure artificial seawater (blank sample) using Ag:MTEOS substrate (Fig. 6.1.2 (b)) shows that MTEOS Raman bands appears at 620 cm$^{-1}$, 816 cm$^{-1}$, 956 cm$^{-1}$, 1039 cm$^{-1}$ and 1290 cm$^{-1}$ (very weak), respectively, while silicon Raman band is at 520 cm$^{-1}$.

During the Ag,DMCX:MTEOS substrate preparation, DMCX in the sol solution can be subjected to have a molecular change due to a high temperature. To demonstrate experimentally the presence of DMCX in the sol-gel matrix after the heating process to 230 °C for 2 hours, Ag,DMCX:MTEOS SERS substrate (Fig. 6.1.2 (c)) was compared with a Ag:MTEOS one (Fig. 6.1.2 (b)) using the SERS spectra of pure artificial seawater. Because the Ag,DMCX:MTEOS SERS substrate was made by the mixture of AgNO$_3$, DMCX in ethanol and MTEOS, the Raman bands of DMCX are more contained in the SERS spectrum of blank sample using Ag,DMCX:MTEOS substrate (see Fig. 6.1.2 (c)) compared to that using Ag:MTEOS substrate (see Fig. 6.1.2 (b)).
Trace analysis with DMCX functionalized Ag colloid based sol-gel film

The DMCX in our work can be seen to be chemisorbed to the silver nanoparticle surface due to the thiol interaction process. This process has already been observed and corresponds to the S-H cleavages with the formation of the S-Ag bond [82].

Figure 6.1.2: (a) Raman spectrum of DMCX in solid phase, SERS spectra of (b) pure artificial seawater using Ag:MTEOS substrate, (c) pure artificial seawater using Ag,DMCX:MTEOS substrate and (d) 0.5 nmol/l pyrene in artificial using Ag,DMCX:MTEOS substrate; excitation wavelength: 671 nm, laser power at the sample: 12 mW, and integration time: 10 s.
Trace analysis with DMCX functionalized Ag colloid based sol-gel film

The S-H bond in DMCX is replaced by the Ag-S bond during the substrate preparation procedure by combining the thermally reduced silver colloid with the sulfur atom of the mercaptoacetic acid group. Because only a small amount of DMCX as a dopant material is added to the sol solution for improving the SERS activity, the SERS spectrum of pure artificial seawater using Ag,DMCX:MTEOS film (see Fig. 6.1.2 (c)) shows that the strong Raman bands from the SERS substrate are from the METEOS derived Ormosil [83-85] and from silicon but also the broad and weak Raman bands are from the DMCX adsorbed to silver colloid surface and from the overlapping of the vibration modes of DMCX and MTEOS derived Ormosil. In detail, the 520 cm\(^{-1}\) Raman band is from silicon and the Raman bands at 620, 816, 956 and 1039 cm\(^{-1}\) are from the vibrations of CH\(_3\)-Si and Si-O-Si groups in the MTEOS derived sol-gel matrix but also a broad Raman band at 1290 cm\(^{-1}\) is due to the overlapping of the DMCX and sol-gel Raman bands. Additionally, the broad and weak Raman bands at 1200 cm\(^{-1}\), 1449 cm\(^{-1}\) and 1600 cm\(^{-1}\) are also from the DMCX contained in the SERS substrate. It is also demonstrated by the DMCX Raman band shift that DMCX molecules in Ag,DMCX:MTEOS film are functionalized onto the silver colloid surface by the heat reduction process forming the calixarene monolayer.

SERS spectra of 0.5 nmol/l pyrene in seawater using Ag,DMCX:MTEOS substrate (Fig. 6.1.2 (d)) and Ag:MTEOS substrate (not shown but the same with Fig. 6.1.2 (b)) also reveal that Ag colloid aggregation due to the DMCX addition into sol solution also contributes to a high SERS activity for PAH molecule.

Therefore, one of the main reason for high SERS activity of Ag,DMCX:MTEOS film i.e. electromagnetic enhancement is explained to be a high aggregation degree of silver nanoparticles (see Fig. 4.1.4) due to the formation of calixarene monolayer onto the silver surface compared to that of Ag:MTEOS film [9]. It can be stated that the electromagnetic enhancement factor, i.e. \(\left| A(v_2) \right|^2\left| A(v_3) \right|^2\) in formula (3.1), of the SERS substrate is obtained by the silver colloid aggregation by the addition of DMCX in sol solution.

To investigate the influence of DMCX on the SERS characteristics of two selected PAHs in artificial seawater i.e. SERS analysis of PAH-DMCX interaction, Raman spectra of pyrene and naphthalene adsorbed to the Ag,DMCX:MTEOS SERS substrate surface were recorded under the continuous flow conditions of the sample solution.
6.1.1.2 Raman band shift of pyrene

To investigate the influence of DMCX on the analyte Raman band shift, SERS spectrum of 100 nmol/l pyrene in artificial seawater (Fig. 6.1.3 (b)) is compared with the conventional Raman spectrum of pyrene in solid state (Fig. 6.1.3 (a)). For all SERS spectra the integration time was set to be 10 s and 10 single spectra were averaged each.

![Raman Spectra](image)

Figure 6.1.3: (a) Conventional Raman spectrum of pyrene in solid phase and (b) SERS spectrum of 100 nmol/l pyrene in artificial seawater; excitation wavelength: 671 nm, laser power at the sample position: 6 mW and integration time: 10 s, average of 10 spectra.

The comparison of SERS spectrum of 100 nmol/l pyrene in artificial seawater with Raman spectrum of pyrene in solid state reveals that all Raman bands of pyrene adsorbed onto the Ag,DMCX:MTEOS SERS substrate are slightly shifted e.g. 1239 cm\(^{-1}\) pyrene Raman band in solid phase was shifted by 5 cm\(^{-1}\) in SERS spectrum of 100 nmol/l pyrene in artificial seawater.
Similarly, a pyrene Raman band position shift of 6 cm\(^{-1}\) has also been reported in a DTCX/DCEC functionalized Ag colloid system adsorbing pyrene [22, 86]. There are two possibilities for the molecular adsorption on the metal surface, namely physisorption and chemisorption. The spectrum of physisorbed molecules is practically the same as that of free molecules; the difference being observed affects only the Raman bandwidth [85]. In the silver colloid based sol-gel SERS substrate for in-situ detection of PAHs [8], the analyte Raman band positions remain unchanged compared to the conventional Raman spectrum since the adsorption of pyrene to the silver nanoparticle surface is based on the physisorption. When the molecules are chemisorbed, the overlap of the electron wave functions between the adsorbate and the metal nanoparticle produces a modification of the molecular structure of the adsorbate resulting in a Raman peak position shift [80, 87]. However, it has been shown that pyrene SERS bands reveal the interesting difference with respect to solid compound due to the complexation with the host calixarene [22].

In our case the pyrene molecule as the guest forms a complex with DMCX (host molecule). The pyrene Raman band shift in the SERS spectra can be regarded as being due to the formation of the host-guest complex with DMCX which is chemically adsorbed to the silver nanoparticle surface. This means that DMCX as host chemical forming the monolayer on the silver nanoparticle surface contributes to the preconcentration of PAH molecule within the zone of high electromagnetic enhancement of silver nanoparticle through the interaction with PAH molecules.

To evaluate the reproducibility of SERS characterization, i.e. the Raman band shift, pyrene molecules were removed from SERS substrate by cleaning with methanol and water. SERS measurement after another exposure of the substrate to pyrene in artificial seawater revealed that the pyrene Raman bands have the same feature with former exposure.

### 6.1.1.3 Raman band shift of naphthalene

This Raman band shift after the adsorption of the analyte to the SERS substrate surface is also shown in the SERS spectra of naphthalene in artificial seawater at a concentration of 30 µM (Fig. 6.1.4 (b)) compared to the conventional Raman spectrum of naphthalene in solid phase (Fig. 6.1.4 (a)). In detail, 757 cm\(^{-1}\) Raman band of naphthalene in SERS spectrum (Fig. 6.1.4 (b)) corresponds to the shift from 762 cm\(^{-1}\) band in conventional Raman spectrum (Fig. 6.1.4 (a)).
(a), while 1377 cm\(^{-1}\) band in SERS spectrum corresponds to that from 1379 cm\(^{-1}\) one in the conventional Raman spectrum.

![Graph showing Raman and SERS spectra](image)

**Figure 6.1.4:** (a) Raman spectrum of naphthalene in solid phase and (b) SERS spectrum of 30 µM naphthalene in artificial seawater; 671 nm of excitation wavelength, 6 mW of laser power at the sample position and 10 s of integration time, average of 10 spectra.

Appendix II shows all Raman band positions of pyrene and naphthalene in Raman spectrum and SERS spectrum.

Generally, the detectable analytes using SERS are largely limited by their natural affinities for either the noble metal surface or something that binds to the noble metal surface, where the large electromagnetic fields are localized [21]. For example, PAHs as a non-traditional SERS analytes have the poor affinity to the bare metal surface, i.e. small surface coverage of PAHs to the nanoparticle surface.

Therefore, there has been the significant effort to develop strategies that facilitate the use of SERS for nontraditional SERS analytes by employing a substrate-bound partition layer to
preconcentrate the analyte of interest within the zone of electromagnetic enhancement [61-63, 79]. It has been shown that the host-guest complex formed in a calix[4]arene-functionalized silver colloidal surface e.g. DCEC/pyrene complex formed in the metal colloidal solution caused higher SERS activity than in the absence of calixarene [22, 86].

The formation of calixarene monolayer onto the nanoparticle surface might contribute to the selective enrichment of PAH molecules to SERS substrate surface through the host-guest complex between PAH molecule and calixarene forming the monolayer on the silver nanoparticle surface through the chelate reaction with mercaptoacetic acid group. Table 6.1 shows the shift of Raman bands of two PAHs, e.g. pyrene and naphthalene, between Raman spectra in solid phase and SERS spectra due to the host-guest complexation between PAH and DMCX in SERS substrate, which was observed by our SERS measurements.

<table>
<thead>
<tr>
<th>PAHs</th>
<th>Raman (cm$^{-1}$)</th>
<th>SERS (cm$^{-1}$)</th>
<th>Shift (cm$^{-1}$)</th>
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</tr>
<tr>
<td></td>
<td>1626</td>
<td>1622</td>
<td>5</td>
</tr>
<tr>
<td>Naphthalene</td>
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<td></td>
</tr>
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<td></td>
<td>762</td>
<td>757</td>
<td>5</td>
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<tr>
<td></td>
<td>1379</td>
<td>1377</td>
<td>2</td>
</tr>
</tbody>
</table>

It is explained that the formation of host-guest complex between PAH molecule and DMCX onto silver nanoparticle surface may cause the preconcentration of PAH molecule within the zone of high electromagnetic enhancement around the metal nanoparticles excited by laser light and is considered as other one of the main factors for high SERS activity of Ag,DMCX:MTEOS film with respect to PAH detection. In detail, the formation of PAH-DMCX complex contributes to the increase in the surface coverage of PAH molecules on the silver nanoparticle surface, i.e. $N'$ in Eq. (3.1) is increased by this complexation.

Therefore, it can be manifested that the metal colloid aggregation by the formation of DMCX layer on Ag surface but also the preconcentration of PAH molecule around Ag surface through the formation of host-guest complex between the analyte and DMCX are two main factors for high activity of the SERS substrate.
6.1.2 Concentration dependent SERS intensity

To evaluate the concentration dependence of the Raman signal intensity of the analyte, SERS spectra of 2 selected PAHs e.g. pyrene and naphthalene in artificial seawater were collected from the SERS substrate in the flow-through cell.

At every measurement, flow-rate of the sample solution in the flow-through cell system was fixed to be 30 µl/s. After assembling the SERS substrate in the flow-through cell, at first SERS spectra of pure artificial seawater were recorded to evaluate the standard deviation of the blank signal.

After that, every artificial seawater sample containing pyrene at different concentration from 0.5 nmol/l to 400 nmol/l was pumped to the flow-through cell for 15 minutes to reach the concentration equilibrium in the interface between SERS substrate surface and the analyte solution. 15 minutes after the sample solution flow SERS spectra were recorded from the SERS substrate in flow-through cell.

Fig. 6.1.5 shows SERS spectra of (a) pure artificial seawater, pyrene in artificial seawater at the concentrations of (b) 0.5 nmol/l, (c) 1 nmol/l, (d) 12 nmol/l, (e) 100 nmol/l and (f) 400 nmol/l, respectively. As can be seen in Fig. 6.1.5, SERS spectra at different concentrations of pyrene in artificial seawater have different backgrounds. For example, SERS spectrum in the wavenumber range from 1350 cm\(^{-1}\) to 1650 cm\(^{-1}\) is changed according to the change of pyrene concentration in seawater.

In addition, the changes in several parameters such as turbidity and flow-rate of the sample solution can influence the net intensity of the analyte Raman bands. Therefore, a Raman band intensity normalization using an internal standard was applied.

In that case, non-overlapping bands of the internal standard material can be used to correct for any Raman intensity variation of the sample [88].

Wehling et al. reported that the Raman band intensity of p-tert-butyl calix[4]arene-tetrathiol coated onto silver surface was used as an internal standard to normalize the Raman band intensities of aromatics in the gaseous state [89].

The DMCX and MTEOS molecules in our SERS substrate remain unchanged after the preparation. Therefore, all pyrene Raman bands were normalized to the 1290 cm\(^{-1}\) Raman band as an internal standard to compensate for the intensity variation.
Figure 6.1.5: (a) SERS spectrum of pure artificial seawater, SERS spectra of pyrene in artificial seawater with the concentration of (b) 0.5 nmol/l, (c) 1 nmol/l, (d) 12 nmol/l, (e) 100 nmol/l and (f) 400 nmol/l, respectively; excitation wavelength: 671 nm, laser power at the sample position: 6 mW and integration time: 10 s. A 1290 cm\(^{-1}\) Raman band due to the overlapping of DMCX and MTEOS vibration modes as an internal standard is marked by (*).

Because the wavenumber range which involves the strong MTEOS Raman bands is not necessary for the normalization of the analyte Raman band, this range was omitted in Fig. 6.1.5.
Among several pyrene Raman bands, the intensive Raman bands are at 1234 cm$^{-1}$ and 1401 cm$^{-1}$. Therefore, 2 Raman bands were used to obtain the concentration dependent calibration curves.

As an example, Fig. 6.1.6 shows the normalization of SERS spectra of pyrene in artificial seawater in the wavenumber range of 1200-1400 cm$^{-1}$ at different concentrations of 0 nmol/l, 0.5 nmol/l, 1 nmol/l, 12 nmol/l and 100 nmol/l, respectively. In this case, a Raman band at 1290 cm$^{-1}$ as an internal standard was considered as a unit, i.e. $I_{1290} \equiv 1$.

![Normalized Raman Intensity](image)

Figure 6.1.6: Normalized SERS spectra of pyrene in artificial seawater at different concentrations (0 nmol/l (pure seawater), 0.5 nmol/l, 1 nmol/l, 12 nmol/l and 100 nmol/l). The pyrene Raman band intensity at 1234 cm$^{-1}$ increases with the concentration of pyrene. The broad Raman band at 1290 cm$^{-1}$ was used for the normalization of SERS spectra.

The adsorption of molecules to the SERS surface is a phenomenon which can be explained according to the physical adsorption (physisorption) and chemical adsorption (chemisorption). The basis for this destination is the nature of the bonding between the molecule and the
substrate surface [16]. Each adsorption model is characterized by an adsorption isotherm which the analyte surface coverage is expressed as a function of the pressure, or of the concentration. Since the pyrene molecules are adsorbed on the silver colloid surface through the formation of host-guest complex between pyrene and DMCX which is based on the non-covalent bonding such as van der Waals force and hydrophobic interaction, the concentration dependent calibration curve (6.1) can be explained by a Langmuir adsorption isotherm [16], where $I$ is the normalized Raman intensity of pyrene, $I_{\text{max}}$ its maximum value, $c$ the concentration of pyrene in seawater, and $K$ the adsorption constant.

$$I = I_{\text{max}} \frac{Kc}{(1 + Kc)} \quad (6.1)$$

The calibration procedure for pyrene in artificial seawater was performed using the normalized Raman intensity of the bands at 1234 cm$^{-1}$ and 1401 cm$^{-1}$ (Fig. 6.1.7). In Fig. 6.1.7, the Raman intensity of 1401 cm$^{-1}$ band was also normalized with the standard Raman band at 1290 cm$^{-1}$. Applying the formula (6.1) to fit the experimental points over the concentration range from 0.5 nmol/l to 400 nmol/l (Fig. 6.1.7), at first the adsorption constant and SERS maximum value $I_{\text{max}}$ of pyrene were obtained. The adsorption constant $K$ and SERS maximum value $I_{\text{max}}$ for pyrene in artificial seawater were calculated to be $0.353 \times 10^9$ l/mol and 0.6595, respectively. At low concentration range with $Kc$ much smaller than 1, Eq. (6.1) is of linear form and its slope is $KI_{\text{max}}$, i.e. the calibration curve at low concentration is expressed like Eq. (6.2).

$$I \approx KI_{\text{max}} c \quad (6.2)$$

In the following, LODs for pyrene in artificial seawater using two intensive Raman bands of pyrene were calculated based on the concentration dependent calibration curves and the standard deviation of blank signal.

As an example, a standard approach to LOD calculation for pyrene in seawater using 1234 cm$^{-1}$ Raman band was applied. Because LOD is the minimum concentration which the signal intensity is 3 times greater than the standard deviation of blank signal, its value was determined by dividing $3 \sigma$ criterion by its slope $KI_{\text{max}}$ (see Eq. (6.3)) [15, 16, 90].
Figure 6.1.7: Concentration dependent calibration curves of pyrene in artificial seawater using two Raman bands at 1234 cm\(^{-1}\), and 1401 cm\(^{-1}\). The Raman bands were normalized with an internal standard at 1290 cm\(^{-1}\).

\[ LOD = \frac{3\sigma}{K I_{\text{max}}} \]  

(6.3)

Fig. 6.1.8 shows the standard approach to calculate the LOD based on the Eq. (6.3). At first, a concentration dependent calibration curve (line 1) was drawn based on the method mentioned above. This curve is of the nonlinear form. A mean blank signal (line 2) and 3 \( \sigma \) criterion (line 3) were drawn using the averaged value of blank signals at 1234 cm\(^{-1}\) and their standard deviation. To the end, by projecting the intersection between the line 1 and line 3 to x-axis (line 4), LOD for pyrene in artificial seawater using 1234 cm\(^{-1}\) Raman band was calculated and amounts to 0.30 nmol/l (60 ppt).

In addition, applying this approach LOD for pyrene in artificial seawater using 1401 cm\(^{-1}\) Raman band amounts to 0.29 nmol/l (58 ppt).
Figure 6.1.8: Standard approach to LOD calculation for pyrene in seawater based on the concentration dependent calibration curve for the 1234 cm⁻¹ Raman band. The Raman band at 1234 cm⁻¹ was used for creating the nonlinear calibration curve (1). A long dashed line (2) and dashed line (3) mark a mean blank signal and the 3 σ criterion line of blank signal, respectively. A solid line (4) marks the LOD line which was drawn by projecting the intersection point between line 1 and line 3.

For naphthalene the result of the concentration dependent calibration procedure using the intensive Raman bands at 757 cm⁻¹ and 1377 cm⁻¹ is presented in Fig. 6.1.9. Because a 1377 cm⁻¹ Raman band is the most intensive, this band was used for determining the adsorption constant and LOD for naphthalene in artificial seawater. The determination of the adsorption constant \( K \) as well as of the LOD was performed in the same way like for pyrene. Using our SERS substrate the adsorption constant amounts to 8.2 \( \times 10^6 \) l/mol and the LOD was calculated to be 13 nmol/l (1600ppt) for naphthalene in artificial seawater. The achieved LOD for naphthalene is lower than the AA-EQS in inland surface water proposed by European Union (see Table 2.1).
Figure 6.1.9: Calibration curves of naphthalene in artificial seawater using the Raman bands at 757 cm$^{-1}$ and 1377 cm$^{-1}$.

6.1.3 Raman signal response

Marine environmental monitoring of several chemicals contained in real seawater requires in-situ chemical sensors with fast signal response, because the analyte concentration profiles vary temporally and spatially according to the influence of temperature, pressure and wind in the real field. The in-situ chemical detection applying the SERS technique is based on the principle that the Raman signal can be used for the determination of the surface coverage of the analyte adsorbed onto the SERS substrate surface.

In the case of Ag,DMCX:MTEOS films, the adsorption isotherm of pyrene as non-polar molecule to the SERS sensor is characterized by the non-covalent bonding and its surface coverage to the sensor is expressed as a function of the analyte concentration in aqueous solution. The adsorption and desorption rates of the analyte on the SERS sensor is proportional to the analyte concentration. Therefore, the SERS sensor requires a certain time period to reach
an equilibrium state between adsorption and desorption on the sensor surface after changing the analyte concentration.

To evaluate how the in-situ SERS sensor responds to a concentration change of pyrene in artificial seawater, at first a SERS spectrum at the saturation concentration of 400 nmol/l pyrene in artificial seawater was recorded under continuous flow conditions for 15 minutes. After that, the sample solution was changed into pure artificial seawater and continuously pumped into the flow-through cell system and SERS spectra were recorded each 5 minutes.

Fig. 6.1.10 exemplary shows the SERS spectra of a) 400 nmol/l pyrene in artificial seawater, b) 10 minutes and c) 15 minutes after changing the sample solution to the pure artificial seawater.

Figure 6.1.10: Raman signal response according to a concentration change of pyrene in artificial seawater from 400 nmol/l to 0 nmol/l. SERS spectra of 400 nmol/l pyrene in artificial seawater (a), 10 minutes (b) and 15 minutes (c) after continuous flow of pure artificial seawater; excitation wavelength: 671 nm, laser power: 6 mW and integration time: 10 s.

As can be seen, with the time passed after changing the sample solution the Raman intensity of every band became decreased. All pyrene Raman bands in the SERS spectrum disappeared 15
minutes after the 400 nmol/l pyrene in artificial seawater sample was changed to pure artificial seawater applying a flow rate of 30 µl/s.

Therefore, it is manifested that Raman signal response of this SERS sensor to the analyte concentration change is influenced by the adsorption and desorption of PAH molecules to a substrate surface.

In the real seawater, several physical and chemical processes are continuously performed; thus resulting in the undesired experimental factors such as the turbidity of seawater and the formation of gas bubbles from the sediment. Especially, optical monitoring of the environmental toxic chemicals using SERS technique will be influenced by the formation of air bubble from the seafloor. Fig. 6.1.11 shows the influence of gas bubbles, in that case air bubbles, in the flow-through cell on the SERS measurement of pyrene in artificial seawater.

Figure 6.1.11: SERS spectra of 20 nmol/l pyrene in artificial seawater a) without air bubbles and b) with air bubbles of 1.5 mm in diameter formed in the flow-through cell. (*), (**) and (***) remark the Raman bands of silicon, MTEOS and methanol, respectively.

As can be seen in Fig. 6.1.11, the formation of air bubbles in the cell during the pumping of sample solution made it impossible to record the SERS spectra of analyte in seawater. Fig. 6.1.11 (a) shows the SERS spectrum of 20 nmol/l pyrene in artificial seawater. However, when
the sample solution with air bubbles was pumped and air bubbles are formed in the flow-through cell, the incident laser light and Raman scattered light caused the great loss in intensity due to the light reflection in the interface between air bubbles and solution. As a result, only one Raman band of methanol in artificial seawater with 1\% of concentration was recorded after the formation of air bubbles (see Fig. 6.1.11 (b)).

6.2 SERS/SERDS of DMCX functionalized Ag colloid based sol-gel film

In this section, SERS applying SERDS (SERS/SERDS) of 3 selected PAHs adsorbed to a Ag,DMCX:MTEOS film is presented.

6.2.1 SERDS of polystyrene, DMCX and PAHs

The fluorescence based background from the sample is a prohibiting factor for the widespread use of Raman spectroscopy [24]. It often overwhelms the weaker Raman signal and renders the results useless. Especially, SERS spectrum contains high fluorescence based background; therefore this masks the weak Raman bands of analyte. The useful way to remove the fluorescence from the Raman spectrum is to obtain the derivative-like spectrum by subtracting one Raman spectrum from another one with little Raman band shift since the fluorescence common to both spectra is very smooth compared to Raman band. Another Raman spectrum was obtained by the excitation with another emission wavelength of laser diode.

As an example, Fig. 6.2.1 shows two Raman spectra of polystyrene in solid phase excited by two emission laser lines with slightly different wavelengths (670.8 nm and 671.3 nm) generated from a microsystem laser diode module. For the Raman measurements, laser powers at 670.8 nm and 671.3 nm were selected to be 12 mW and 13 mW at the sample position, respectively, while the integration time is 1 s. As can be seen in Fig. 6.2.1, two Raman spectra with little band shift contain the fluorescence based background which can be considered as noise in Raman spectroscopy. This background can be removed by obtaining the derivative-like spectrum (Fig. 6.2.2 a)). The derivative-like difference spectrum is then fitted with the difference of suitable peak shapes, and the result is used to reconstruct the true Raman spectrum free of fluorescence based background noise (Fig. 6.2.2 b)).
Figure 6.2.1: Raman spectra of polystyrene in solid phase excited at two different emission lines; laser power of 12 mW at 670.8 nm and 13 mW at 671.3 nm, integration time of 1 s.

Figure 6.2.2: (a) Derivative like spectrum and (b) SERDS spectrum of polystyrene obtained by the excitation at two emission lines (670.8 nm and 671.3 nm)
Trace analysis with DMCX functionalized Ag colloid based sol-gel film

This method is called as shifted excitation Raman difference spectroscopy (SERDS) which was firstly proposed by A. P. Shreve et al [91]. In order to calculate the SERDS spectrum using two corresponding Raman spectra, a self-developed Matlab program was applied [92].

To separate the Raman bands from the fluorescence based background, SERDS was also applied with respect to DMCX in solid phase. DMCX in solid phase caused the high fluorescence and weak Raman bands; therefore being difficult to identify them from the original Raman spectrum (Fig. 6.2.3 a)). During the measurement, DMCX in solid phase as a sample was sandwiched between two microscope slides and installed in the sample position of Raman set-up.

![Figure 6.2.3: (a) Raman and (b) SERDS spectra of DMCX in solid phase; laser power of 6 mW at 670.8 nm and 7 mW at 671.3 nm, integration time of 10 s. Raman spectrum was obtained at 670.8 nm of emission wavelength.]

By applying SERDS, all Raman bands are clearly visible (Fig. 6.2.3 b)), which were used for identifying the DMCX Raman bands in SERS applying SERDS (SERS/SERDS) spectrum of pure artificial seawater using Ag,DMCX:MTEOS substrate in the next part (see section 6.2.2).
Pyrene is being widely used for SERS characterization of the developed substrate [8, 17, 21-23] and dissolved in seawater with high concentration [5]. And European Union stated the EQS for anthracene and fluoranthene in surface water [27]. Therefore; 3 PAHs were selected as the analytes for the Raman and SERS measurements.

SERDS spectrum of every PAH in solid phase, e.g. pyrene, anthracene and fluoranthene, was recorded in order to investigate the Raman band shift in SERS/SERDS spectrum of artificial seawater sample containing PAH (see section 6.2.2).

Fig. 6.2.4 shows the conventional Raman spectrum (a) and Raman/SERDS spectrum (b) of anthracene in solid phase as an example of SERDS removing the fluorescence based noise from the conventional Raman spectrum.

![Figure 6.2.4: (a) Raman spectrum and (b) Raman/SERDS spectrum of anthracene in solid phase; laser power of 6 mW at 670.8 nm and 7 mW at 671.3 nm, integration time of 1 s. Raman spectrum was obtained at 670.8 nm of emission wavelength.](image-url)
Raman and Raman/SERDS spectra of pyrene in solid phase are also shown in Fig. 6.2.5 respectively demonstrating the powerful potential to remove the fluorescence based background noise in Raman spectroscopy.

Figure 6.2.5: (a) Raman spectrum and (b) Raman/SERDS spectrum of pyrene in solid phase; laser power of 6 mW at 670.8 nm and 7 mW at 671.3 nm, integration time of 1 s. Raman spectrum was obtained at 670.8 nm of emission wavelength.

Additionally, Raman and Raman/SERDS spectra of fluoranthene in solid phase are shown in Fig. 6.2.6. SERDS technique using a microsystem laser diode module with two emission lines at 670.8 nm and 671.3 nm can be efficiently applied to remove the fluorescence from SERS spectrum.
6.2.2 SERS/SERDS of several PAHs and Raman band position shift

In the SERS measurement, the fluorescence based background also makes it impossible to identify the weak Raman bands of analyte and renders the result useless. For example, SERS spectrum of 0.5 nmol/l pyrene in artificial seawater which has high fluorescence was observed by selecting an integration time to be 10 s [93].

For our SERS measurement, an emission line with 670.8 nm of excitation wavelength and 12 mW of laser power at the sample position was generated from a microsystem laser diode in the module by selecting a temperature and injection current of laser diode to be 25 ºC and 390 mA, respectively.
Trace analysis with DMCX functionalized Ag colloid based sol-gel film

Fig. 6.2.7 (a) shows a SERS spectrum of 0.5 nmol/l pyrene in artificial seawater which contains a high fluorescence based background.

Figure 6.2.7: (a) SERS spectrum and (b) SERS/SERDS spectrum of 0.5 nmol/l pyrene in artificial seawater; 12 mW of laser power at the sample position, 10 s of integration time, Raman bands of pyrene marked by arrows. 670.8 nm of excitation wavelength was used for SERS measurement (a).

As can be seen in Fig. 6.2.7 (a), the weak Raman bands at 408 cm\(^{-1}\), 1066 cm\(^{-1}\) and 1619 cm\(^{-1}\) are screened by the fluorescence based background noise.

In order to solve this problem, SERDS was applied in the SERS investigation. A SERS applying SERDS (SERS/SERDS) spectrum of 0.5 nmol/l pyrene in artificial seawater is shown in Figure 6.2.7 (b). Since the fluorescence based background was effectively removed by the SERDS treatment [91-96], the Raman bands of pyrene were identified also at very low concentrations down to the pmol/l range. For example, the weak Raman bands at 408 cm\(^{-1}\), 1066 cm\(^{-1}\) and 1619 cm\(^{-1}\) are clearly visible in SERS/SERDS spectrum of 0.5 nmol/l pyrene in artificial seawater.
Trace analysis with DMCX functionalized Ag colloid based sol-gel film

This result demonstrates that the SERDS technique using a microsystem laser diode module with two slightly different emission lines is very promising to improve the SERS sensitivity of the nanoparticle based chemical sensor which should be operative at very low concentration of PAH in seawater.

In the following, to evaluate the Raman band position shift after the adsorption of pyrene to SERS substrate surface, Raman/SERDS spectrum of pyrene in solid phase (Fig. 6.2.8 (a)) was compared with SERS/SERDS spectra of 12 nmol/l (Fig. 6.2.8 (b)) and 1 nmol/l pyrene in artificial seawater (Fig. 6.2.8 (c)). The SERS/SERDS spectrum of pure artificial seawater is also shown in Fig. 6.2.8 (d).

Pyrene molecule causes the Raman band position shift after being adsorbed to SERS substrate. For example, 1626 cm$^{-1}$ Raman band of pyrene in solid phase (Fig. 6.2.8 (a)) is shifted by 4 cm$^{-1}$ in SERS/SERDS spectrum obtained by the adsorption of analyte to the substrate surface (Fig. 6.2.8 (b) and (c)). This phenomenon was also observed in SERS measurement of pyrene adsorbed to a Ag,DMCX:MTEOS SERS substrate (see Fig. 6.1.4). All Raman bands in the SERS/SERDS spectrum of pure artificial seawater (Fig. 6.2.8 (d)) are 1039 cm$^{-1}$ Raman band from the MTEOS based sol-gel matrix and 520 cm$^{-1}$ Raman one from silicon but also the weak DMCX Raman bands at 670 cm$^{-1}$ and 789 cm$^{-1}$. This result reveals that DMCX molecules in the SERS substrate which were functionalized to Ag nanoparticles contribute to the preconcentration of pyrene within the zone of highly localized electric field around Ag nanoparticle surface excited by laser light. This phenomenon was already demonstrated in the SERS investigation (see section 6.1). This Raman band shift in SERS/SERDS spectrum compared with the Raman/SERDS spectrum was also observed by other PAH in seawater sample.

SERS/SERDS investigation of anthracene in artificial seawater was performed by a Ag,DMCX:MTEOS SERS substrate excited by a 671 nm microsystem laser diode module. Fig. 6.2.9 shows (a) Raman/SERDS spectrum of anthracene in solid phase and SERS/SERDS spectrum of (b) 15 nmol/l, and (c) 0.75 nmol/l anthracene in artificial seawater and (d) pure artificial seawater. As can be seen in Fig. 6.2.9, the anthracene Raman bands in SERS/SERDS spectra are little shifted compared with the Raman/SERDS spectrum of anthracene in solid phase. This is because anthracene molecule also forms the host-guest complex with DMCX functionalized to silver nanoparticle surface after the adsorption of the analytes to the Ag,DMCX:MTEOS SERS substrate.
Figure 6.2.8: (a) Raman/SERS spectrum of pyrene in solid phase, SERS/SERDS spectrum of (b) 12 nmol/l and (c) 1 nmol/l pyrene in artificial seawater and (d) pure artificial seawater; 670.8 nm and 671.3 nm of excitation wavelengths, 12 mW of laser power at the sample and 10 s of integration time.
Figure 6.2.9: (a) SERDS spectrum of anthracene in solid phase, SERS/SERDS spectrum of (b) 15 nmol/l anthracene in artificial seawater, (c) 0.75 nmol/l anthracene in artificial seawater and (d) pure artificial seawater, 12 mW of laser power at the sample position, 10 s of integration time; anthracene Raman bands marked in spectra (a), (b) and (c) but also MTEOS Raman band and silicon Raman band also marked in spectrum (d).

The Raman band shift after the adsorption of the analyte to SERS substrate surface has been observed from the SERS/SERDS spectra of fluoranthene in artificial seawater. Raman/SERDS
Trace analysis with DMCX functionalized Ag colloid based sol-gel film

spectrum of fluoranthene in solid phase (a), SERS/SERDS spectra of 200 nmol/l (b) and 1
nmol/l (c) of fluoranthene in artificial seawater as well as pure artificial seawater (d) are shown
in Fig. 6.2.10.

Figure 6.2.10: (a) SERDS spectrum of fluoranthene in solid phase and SERS/SERDS spectrum of (b) 200 nmol/l fluoranthene in artificial seawater, (c) 1 nmol/l fluoranthene in artificial seawater and (d) pure artificial seawater, 12
mW of laser power at the sample position, 10 s of integration time, fluoranthene Raman bands marked in (a), (b), (c),
silicon and MTEOS Raman bands marked in (d).
Trace analysis with DMCX functionalized Ag colloid based sol-gel film

For fluoranthene, the shift of every Raman band was also observed by comparing the SERS/SERDS spectrum with the Raman/SERDS spectrum.

Table 6.2 shows the shift of Raman bands of 3 selected PAHs, e.g. pyrene, anthracene and fluoranthen, obtained by the comparison of Raman/SERDS spectrum with SERS/SERDS one.

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<td>1606</td>
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Therefore, the Raman band shift in SERS/SERDS spectrum of every selected PAH in artificial seawater can be considered as being caused by the formation of the host-guest complex with DMCX which is chemisorbed to the silver nanoparticle surface.
Additionally, the introduction of SERDS to SERS measurement, i.e., SERS/SERDS measurements improves the ratio of Raman signal to background noise compared to SERS measurement. For example, the weak Raman bands in SERS/SERDS spectrum are clearly visible at very low concentration, e.g., 0.5 nmol/l pyrene in artificial seawater using the Ag,DMCX:MTEOS SERS substrate excited by the emission lines at 670.8 nm and at 671.3 nm for SERDS.

The SERS/SERDS spectra of 3 selected PAHs in artificial seawater at the saturation concentration (e.g., 435 nmol/l for pyrene, 627 nmol/l for fluoranthene and 178 nmol/l anthracene) are shown in Fig. 6.2.11-13.

In addition, Appendix II shows the Raman bands in Raman spectra and SERS spectra of five PAHs, i.e., pyrene, naphthalene, anthracene and fluoranthene, obtained by using SERS and/or SERS/SERDS technique.

Figure 6.2.11: SERS/SERDS spectrum of 435 nmol/l pyrene in artificial seawater using a Ag,DMCX:MTEOS substrate, 12 mW of laser power, integration time 10 s. The pyrene Raman bands are marked in the spectrum.
Figure 6.2.12: SERS/SERDS spectrum of 427 nmol/l fluoranthene in artificial seawater using a Ag,DMCX:MTEOS substrate, 12 mW of laser power, integration time 10 s. The fluoranthene Raman bands are marked in the spectrum.

Figure 6.2.13: SERS/SERDS spectrum of 178 nmol/l anthracene in artificial seawater using a Ag,DMCX:MTEOS substrate, 12 mW of laser power, integration time 10 s. The fluoranthene Raman bands are marked in the spectrum.
6.2.3 Calibration curves of PAHs using SERS/SERDS

To evaluate the concentration dependence of the Raman signal intensity of every selected PAH in artificial seawater sample using SERS/SERDS, SERS spectra of every PAH in artificial seawater excited by two emission lines at 670.8 nm and 671.3 nm were recorded at different concentrations. Artificial seawater samples containing the PAHs were prepared at different concentrations starting from the saturation concentration (e.g. 435 nmol/l for pyrene, 178 nmol/l for anthracene and 627 nmol/l for fluoranthene) down to the selected minimum concentration of 0.5 nmol/l for pyrene, 0.75 nmol/l for anthracene and 1 nmol/l for fluoranthene, respectively.

A peristaltic pump (PP1-05, ISMATEC) at the outlet side of the flow-through cell was used to keep a defined flow-rate of 30 µl/s. For the continuous in-situ measurement, SERS substrate was located in the flow-through cell and this cell is at the sample position of experimental Raman set-up. During the SERS measurement laser power at the sample position and integration time were selected to be 12 mW and 10 s at different excitation wavelengths. The in-situ detection of chemicals in seawater requires the continuous flow of the sample solution towards the SERS sensor.

At first, to evaluate the standard deviation of the blank signal, 5 SERS/SERDS spectra of pure artificial seawater were obtained by flowing pure artificial seawater into the cell. Every time when the sample solution was changed into one with different concentration, SERS spectra were recorded 15 minutes after the continuous flow of artificial seawater sample in order to reach the adsorption equilibrium in the interface between substrate surface and sample solution at given concentration.

In that way, the changes in several parameters such as turbidity and flow rate of the sample solution can influence the net intensity of the analyte Raman bands. On the other hand, the MTEOS molecules in SERS substrate remain unchanged after preparation. Therefore, all analyte Raman bands were normalized to the 1039 cm\(^{-1}\) MTEOS Raman band as an internal standard to compensate for the intensity variations [89]. After the normalization of SERS/SERDS spectrum, Raman intensity of 1039 cm\(^{-1}\) MTEOS band was considered as unit (Normalized Raman intensity \(\equiv 1\)).

The SERS/SERDS spectra of artificial seawater sample containing pyrene reveal that the normalized Raman intensity of 1234 cm\(^{-1}\) band is higher than that of the 1402 cm\(^{-1}\) one at low concentration and vice versa at high concentration. The relationship between the normalized...
Raman intensity at these two Raman bands and the corresponding pyrene concentration in artificial seawater can be fitted with a Langmuir isotherm [97] and is shown in Fig. 6.2.14. The most intensive 1234 cm$^{-1}$ pyrene Raman band at low concentration range was used to determine the adsorption constant and limit of detection (LOD) for pyrene in artificial seawater. The adsorption constant of pyrene on the surface of the SERS substrate and the normalized SERS maximum value can be fitted with a Langmuir isotherm and amounts to $8.46 \times 10^7$ l/mol and 7.1, respectively, using a Raman band at 1234 cm$^{-1}$.

![Figure 6.2.14: Calibration curves of pyrene in artificial seawater using two selected Raman bands at 1234 cm$^{-1}$ and 1402 cm$^{-1}$](image)

In the range of small value of the analyte concentration, Langmuir isotherm is of the form of linear function as shown in Fig. 6.2.15. Therefore, the LOD of pyrene in artificial seawater is calculated by using the slope of a linear fit of the experimental points in the low concentration range and the $3\sigma$ criterion (see Fig. 6.2.15).
Trace analysis with DMCX functionalized Ag colloid based sol-gel film

To determine the LOD for pyrene using 1234 cm$^{-1}$, a linear regression line of Raman intensity (line 1 of Fig. 6.2.15) was drawn at the concentration range up to 5 nmol/l. Using an intersection point between line 1 and y-axis, a mean blank signal line (line 2 of Fig. 6.2.15) was drawn. The blank signal standard deviation line which follows the 3 $\sigma$ criterion was also drawn (line 3 of Fig. 6.2.15). By projecting the intersection point between line 1 and line 3 to x-axis, LOD for pyrene in artificial seawater was determined to be 100 pmol/l (20 ppt).

![Figure 6.2.15: Standard approach for LOD determination using a regression line of 1234 cm$^{-1}$ Raman intensity at very low concentration (line 1). The long dashed (2), dash dot (3) and dashed (4) lines mark the mean blank signal, the blank signal standard deviation and LOD determination line, respectively.](image)

The concentration dependence of the normalized Raman intensity of anthracene dissolved in artificial seawater was obtained using the most intensive Raman bands at 752 cm$^{-1}$ and 1183 cm$^{-1}$ to determine the LOD (see Fig. 6.2.16). The normalized intensity of the 1183 cm$^{-1}$ Raman band is higher than that of the 752 cm$^{-1}$ one in the low concentration range. Therefore, the 1183 cm$^{-1}$ Raman band was used to determine the adsorption constant and LOD for anthracene in seawater.
Figure 6.2.16: (a) Calibration curves of anthracene in artificial seawater derived from the Raman bands at 752 cm$^{-1}$ and 1183 cm$^{-1}$, and (b) Standard approach for LOD determination using a regression line of 1183 cm$^{-1}$ Raman intensity at very low concentration (line 1). The long dashed (2), dash dot (3) and dashed (4) lines mark the mean blank signal, the blank signal standard deviation and LOD determination line, respectively.
Trace analysis with DMCX functionalized Ag colloid based sol-gel film

From Fig. 6.2.16 (a), the adsorption constant for anthracene in artificial seawater using 1183 cm\(^{-1}\) Raman band was determined to be \(0.115 \times 10^9\) l/mol.

LOD for anthracene in seawater was calculated based on the same way like Fig. 6.2.15. Fig. 6.2.16 (b) shows the standard approach for LOD calculation using 1183 cm\(^{-1}\) anthracene Raman band. In that way, the LOD for anthracene in artificial seawater using the Raman band at 1183 cm\(^{-1}\) amounts to 310 pmol/l (55 ppt).

LOD for anthracene in seawater is lower than the AA-EQS in surface water proposed by European Union (see Table 2.1).

As can be seen in Fig. 6.2.10 (c), the characteristic Raman bands of fluoranthene in artificial seawater can be seen at 351 cm\(^{-1}\), 830 cm\(^{-1}\), 1102 cm\(^{-1}\), 1405 cm\(^{-1}\) and 1606 cm\(^{-1}\), which are clearly visible even at the concentration of 1 nmol/l. Two of the intensive Raman bands at 1102 cm\(^{-1}\) and at 1606 cm\(^{-1}\) were used for creating the concentration dependent calibration curves of fluoranthene in artificial seawater in order to determine the LOD (see Fig. 6.2.17 (a)).

The LOD of fluoranthene in artificial seawater using the 1102 cm\(^{-1}\) Raman band was calculated by using the slope of a linear fit of the experimental points in the low concentration region and 3\(\sigma\) criterion (Fig. 6.2.17 (b)); therefore amounts to 670 pmol/l (135 ppt).

The determined LOD for fluoranthene is much lower than the MAC-EQS, while slightly higher than the AA-EQS in surface water (see Table 2.1).

Table 6.3 shows the comparison of LOD for selected PAHs with the EQS in surface water proposed by the European Community. In addition, this table involves the applied method for the trace detection of PAHs in artificial seawater.

As well, the comparison of the obtained LODs for PAHs with the results shown in Table 3.1 reveals that the Ag,DMCX:MTEOS SERS substrate in combination with a microsystem laser diode module for SERS (or for SERS/SERDS) is very sensitive for the trace detection of PAHs in water body.
Figure 6.2.14: (a) Calibration curves of fluoranthene in artificial seawater obtained from two Raman bands at 1102 cm$^{-1}$ and 1606 cm$^{-1}$, and (b) Standard approach for LOD determination using a regression line of 102 cm$^{-1}$ Raman intensity at very low concentration (line 1). The long dashed (2), dash dot (3) and dashed (4) lines mark the mean blank signal, the blank signal standard deviation and LOD determination line, respectively.
Trace analysis with DMCX functionalized Ag colloid based sol-gel film

Table 6.3: Comparison of LOD with EQS for some PAHs in surface water

<table>
<thead>
<tr>
<th>PAH</th>
<th>Applied method</th>
<th>LOD (ppt)</th>
<th>EQS [27] (concentration, type)</th>
</tr>
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<td>Pyrene</td>
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<td></td>
</tr>
<tr>
<td>Naphthalene</td>
<td>SERS</td>
<td>1600</td>
<td>2400 ppt, AA-EQS</td>
</tr>
<tr>
<td>Pyrene</td>
<td>SERS/SERDS</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Anthracene</td>
<td>SERS/SERDS</td>
<td>55</td>
<td>100 ppt, AA-EQS</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>SERS/SERDS</td>
<td>135</td>
<td>100 ppt, AA-EQS or 1000 ppt, MAC-EQS</td>
</tr>
</tbody>
</table>
6.3 Discussion and Conclusion

In a series of experiments I have studied the SERS and SERS/SERDS characterizations using a newly developed substrate (Ag,DMCX:MTEOS one) which has been prepared by a MTEOS based sol-gel film encapsulating silver colloid functionalized with 25,27-dimercaptoacetic acid-26,28-dihydroxyl-4-tertbutyl calix[4]arene (DMCX).

High SERS activity of Ag,DMCX:MTEOS substrate was obtained due to the silver nanoparticle aggregation and the preconcentration of PAHs around the nanoparticle surface by the addition of DMCX in sol-gel matrix. The comparison of SERS spectrum with Raman spectrum of every selected PAH revealed that Raman band shift was caused by the host-guest complexation between DMCX and PAH.

Using the calibration curve of the selected Raman band versus PAH concentration and a standard deviation of blank signal, limit of detections (LODs) for pyrene and naphthalene in seawater were determined to be 0.29 nmol/l (58 ppt) and 13 nmol/l (1600 ppt), respectively. The introduction of SERDS to the SERS investigation improved the signal to background noise ratio of SERS spectrum; therefore, the weak Raman bands of every PAH were clearly visible even at low concentration in the range of pmol/l. The standard approach to LOD determination for every PAH showed that LODs are 100 pmol/l (20 ppt) for pyrene, 310 pmol/l (55 ppt) for anthracene and 670 pmol/l (135 ppt) for fluoranthene, respectively. The comparison of these values with some parameters, e.g. EQS in surface water and LODs of other type of SERS substrate demonstrates that this chemical sensor which contains the Ag,DMCX:MTEOS SERS substrate and a microsystem laser diode at 671 nm of emission wavelength is very sensitive.
7 Trace analysis with naturally grown Ag nanoparticle ensemble

We discuss main factor of high SERS activity in this substrate regarding to electromagnetic enhancement, i.e. the influence of surface plasmon resonance (SPR) wavelength on the SERS activity of the naturally grown Ag nanoparticle ensemble is discussed.

7.1 SERS/SERDS of pyrene in water

As can be seen in Fig. 4.2.3 (page 39), the SPR wavelength of naturally grown silver nanoparticle ensemble is in the range from 453 nm to 548 nm. Therefore, in order to demonstrate the influence of SPR wavelength on SERS activity, the experimental Raman set-up containing a microsystem light source at 488 nm [25] was selected. For the SERS measurement, a continuous flow of the distilled water sample containing pyrene for 15 minutes was done to adsorb the pyrene molecules to a SERS substrate in the flow-through cell. Laser light with 487.61 nm of an emission wavelength and 6 mW of the laser power at the sample position was generated from a microsystem light source by selecting the temperature and injection current to be 30 °C and 350 mA, respectively. At 4 different randomly chosen points of the substrate, 10 SERS spectra were recorded with an integration time of 10 s each. SERS spectrum of pyrene adsorbed to the naturally grown silver nanoparticle ensemble contains a fluorescence based background noise which masks some of the Raman feature at low concentration. As an example, Fig. 7.1.1 (a) shows an average of 10 SERS spectra of 200 nmol/l pyrene in distilled water obtained from a naturally grown silver nanoparticle ensemble with 468 nm of SPR wavelength.

As can be seen in Fig. 7.1.1 (a), several Raman bands at 409 cm\(^{-1}\), 592 cm\(^{-1}\), 1066 cm\(^{-1}\) and 1403 cm\(^{-1}\) were screened by high fluorescence based background in SERS spectrum. Especially, the fluorescence based background is more critical in the SERS investigation of pyrene at low concentration because even the intensive Raman band is also wrapped into the background. As mention above, SERDS treatment was experimentally demonstrated to be useful to improve the signal to background noise in SERS spectrum of PAH adsorbed to Ag,DMCX:MTEOS substrate (see section 6.2).
Figure 7.1.1: (a) SERS spectrum and (b) SERS/SERDS spectrum of 200 nmol/l pyrene in distilled water obtained from a naturally grown silver nanoparticle ensemble with 468 nm of SPR wavelength, 6 mW at 487.61 nm and 8 mW at 487.91 nm of optical laser power at the sample position, 10 s of integration time, Raman bands of pyrene and quartz marked by arrows.

To separate the Raman bands from a high fluorescence based background, the shifted excitation Raman difference spectroscopy (SERDS) was introduced using a microsystem laser diode module with two emission lines (487.61 nm and 487.91 nm)[25]. For SERS spectrum applying SERDS (SERS/SERDS), a naturally grown silver nanoparticle ensemble adsorbing the pyrene molecules was excited by two emission lines with 487.61 nm of excitation wavelength and 6 mW of laser power and with 487.91 nm and 8 mW. First emission line at 487.61 nm was obtained by controlling the temperature and injection current of laser diode to be 38 °C and 361 mA, respectively. Another emission line at 487.91 nm was generated from a microsystem light source by adjusting the temperature and injection current of laser diode to be 38 °C and 361 mA, respectively. An example for Raman band shift in SERS spectra which were obtained by
two excitation lines is shown in Fig. 7.1.2, which around 12 cm\(^{-1}\) of Raman band position shift was obtained for both emission wavelengths (487.61 nm and 487.91 nm).

![Raman band position shift in SERS spectra](image)

Figure 7.1.2: Raman band position shift in SERS spectra of 200 nmol/l pyrene in distilled water, laser power 6 mW at 487.61 nm and 8 mW at 487.91 nm, integration time 10 s. The SERS spectra were obtained from the pyrene molecules adsorbed to a naturally grown silver nanoparticle ensemble with SPR wavelength of 468 nm.

In order to calculate the SERS/SERDS spectrum using two corresponding SERS spectra, a self-developed Matlab program was also applied [92].

Fig. 7.1.1 (b) shows the SERS/SERDS spectrum of 200 nmol/l pyrene in distilled water obtained from a naturally grown silver nanoparticle ensemble with 468 nm of SPR wavelength. SERS/SERDS spectrum reveals that SERDS technique using a microsystem light source with two slightly different emission wavelengths removes the fluorescence based background from the SERS spectrum so that weak Raman bands at 409 cm\(^{-1}\), 592 cm\(^{-1}\), 1066 cm\(^{-1}\) and 1403 cm\(^{-1}\) are clearly visible.
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Additionally, SERS/SERDS spectra of pyrene at different concentrations have been demonstrated to have the same Raman feature with the corresponding SERS spectra obtained by applying the background subtracted method (not shown).

To evaluate how the adsorption of pyrene molecule to Ag nanoparticle ensemble influences the Raman band position, Raman/SERDS spectrum of pyrene in solid phase (Fig. 7.1.3 (a)) was compared with SERS/SERDS spectra of 400 nmol/l (Fig. 7.1.3 (b)) and of 60 nmol/l (Fig. 7.1.3 (c)) pyrene in distilled water which were obtained from a naturally grown silver nanoparticle with 491 nm of SPR wavelength.

SERS/SERDS spectrum of blank sample (Fig. 7.1.3 (d)) was also obtained from this SERS substrate by pumping the pure distilled water into the flow-through cell. Fig. 7.1.3 (d) shows that the 489 cm$^{-1}$ Raman band is produced from the quartz substrate, while no Raman bands due to librational and OH bending modes of water [98] occurs because of weak Raman scattering cross section. In addition, SERS/SERDS spectrum in Fig. 7.1.3 (d) contains the undesirable Raman bands which are expected to be produced from small amount of unknown substances adsorbed to substrate before installing.

As can be seen in Fig. 7.1.3, there is no shift of Raman bands of pyrene in SERS/SERDS spectra compared to those in Raman/SERDS spectrum of the analyte in solid phase.

Generally, there are two options for the analyte molecules adsorption on the metal nanoparticle surface; i.e. physisorption and chemisorption. Especially, all Raman bands of physisorbed molecule in SERS spectrum have the same positions with those of free molecule [87]. Therefore; it is explained that pyrene molecules in water sample are physisorbed to a naturally grown silver nanoparticle ensemble because of the weak bonding.

In the following, SERS/SERDS spectra of pyrene in distilled water were recorded at the different concentrations to make the concentration dependent calibration curve of every SERS substrate. To prepare the water sample, at first pyrene in solid phase was dissolved in methanol to make the stock solution with a concentration of 40 µmol/l. After that, the stock solution was diluted with distilled water to have the concentration ranging from 400 nmol/l down to 6 nmol/l of selected minimum concentration.

During the SERS measurement, the changes in several parameters such as flow rate and turbidity can influence the absolute net intensity of the Raman bands of pyrene adsorbed on a SERS substrate.
Figure 7.1.3: (a) Raman/SERDS spectrum of pyrene in solid phase, SERS/SERDS spectra of (b) 400 nmol/l and (c) 60 nmol/l pyrene in distilled water, and (d) a SERS/SERDS spectrum of distilled water. The latter spectra have been obtained with a naturally grown silver nanoparticle ensemble with a plasmon wavelength at 491 nm, an optical laser power 6 mW at 487.61 nm and 8 mW at 487.91 nm on the sample position, and an integration time of 10 s. The Raman bands of pyrene (a, b, c) and also of quartz (d) are marked in spectra.
Trace analysis with naturally grown Ag nanoparticle ensemble

In Fig. 7.1.3 (d), 489 cm\(^{-1}\) Raman band is from the quartz and does not overlap with pyrene Raman bands. Therefore, it can be used as an internal standard for normalization. For calibration process, all Raman bands of pyrene adsorbed to SERS substrate were normalized with a quartz Raman one to compensate for intensity variation during the measurement.

As an example, Fig. 7.1.4 shows the concentration dependent calibration curves using pyrene Raman bands at 409 cm\(^{-1}\), 1239 cm\(^{-1}\), 1403 cm\(^{-1}\) and 1624 cm\(^{-1}\) obtained from a SERS substrate with 491 nm of SPR wavelength.

Among several pyrene Raman bands, 1239 cm\(^{-1}\) Raman band is the highest one at low concentration range from 6 nmol/l to 60 nmol/l. Therefore, the most intensive Raman band at 1239 cm\(^{-1}\) was used to determine the LOD of pyrene in distilled water.

![Figure 7.1.4: Concentration dependent calibration curves of pyrene in distilled water using Raman bands at 409 cm\(^{-1}\), 1239 cm\(^{-1}\), 1403 cm\(^{-1}\) and 1624 cm\(^{-1}\).](image)

LOD was calculated by obtaining the slope of a linear calibration curve at low concentration range (from 6 nmol/l to 60 nmol/l) and 3 \(\sigma\) criterion of the blank signal \([99]\).
Trace analysis with naturally grown Ag nanoparticle ensemble

As an example, Fig. 7.1.5 shows the standard approach to LOD calculation for pyrene in water using SERS substrate with 491 nm of plasmon resonance wavelength. As can be seen, the linear fit of concentration dependent normalized intensity of 1239 cm\(^{-1}\) Raman band was drawn using several experimental points in the concentration range from 6 nmol/l of selected minimum concentration to 60 nmol/l (line 1 in Fig. 7.1.5) and expressed as Eq. 7.1.

\[
I_{1239} = 4.25 \times 10^7 \times C_{\text{pyr}} + 1.7874
\]

(7.1)

where \(I_{1239}\) is the normalized pyrene Raman intensity at 1239 cm\(^{-1}\), \(C_{\text{pyr}}\) is the concentration of pyrene in water with a unit of mol/l.

Figure 7.1.5: Standard approach to LOD calculation for pyrene in water of SERS substrate with 491 nm of plasmon resonance wavelength. The Raman band at 1239 cm\(^{-1}\) was used for creating the calibration curve (1). A dashed line (2) and solid line (3) mark a mean blank signal and the blank signal standard deviation, respectively.

And then a mean blank signal line (line 2 in Fig. 7.1.5) was drawn from the intersection point between y-axis and a linear fit of concentration dependent Raman intensity \(I_{1239}\). Another line,
Trace analysis with naturally grown Ag nanoparticle ensemble

i.e. the blank signal standard deviation which corresponds to the 3σ criterion was also drawn (line 3 in Fig. 7.1.5). By projecting an intersection point between linear fit (line 1) and the blank signal standard deviation (line 3) to x-axis, LOD for pyrene in distilled water using 1239 cm$^{-1}$ Raman band was determined by a SERS substrate with 491 nm of the plasmon resonance wavelength and amounts to 2 nmol/l.

7.2 Influence of SPR wavelength on SERS sensitivity

In this section, the influence of SPR wavelength on the SERS characteristics, i.e. SERS intensity and LOD, is evaluated using the naturally grown silver nanoparticle ensembles with different SPR wavelengths.

To evaluate the SERS intensity of the naturally grown silver nanoparticle ensemble with respect to SPR wavelength, the concentration of pyrene in distilled water was fixed to be 100 nmol/l. Because 1239 cm$^{-1}$ Raman band is one of the most intensive Raman bands at 100 nmol/l of pyrene concentration in distilled water, this band is used for evaluating the SERS intensity.

The normalized Raman intensity of 1239 cm$^{-1}$ pyrene band is shown with respect to SPR wavelength of the naturally grown silver nanoparticle ensemble (Fig. 7.2.1).

As can be seen in Fig. 7.2.1, the pyrene Raman intensity at 1239 cm$^{-1}$ is optimized at the SPR wavelength close to 488 nm of the excitation one of a microsystem light source. From Fig. 7.2.1, we can see two important facts. First fact is that the highest Raman enhancement for silver nanoparticle was obtained at 491 nm of SPR wavelength close to the emission line of a microsystem light source. This enhancement is attributed to the excitation of surface plasmon from the spheroid Ag nanoparticles with an equivalent radius of 15 ± 4 nm and axial ratio of 0.21. As second fact, even though SERS substrate with SPR wavelength of 518 nm is in the resonance with Raman scattering wavelength corresponding to 1239 cm$^{-1}$ pyrene Raman band, a substrate with 491 nm of SPR wavelength has the highest intensity at this Raman band. It has been reported that the best Raman signal enhancement is obtained for the localized surface plasmon resonance position located exactly between the excitation wavelength and the Raman wavelength [100]. In our case, the SERS substrates which satisfy this requirement are those with SPR wavelengths at 491 nm and 518 nm because Raman wavelength range corresponding to Raman shift of pyrene bands (from 409 cm$^{-1}$ to 1624 cm$^{-1}$) is from 497 nm to 530 nm. From
these experimental facts, it can be said that the plasmon resonance by a laser electric field has higher contribution to SERS activity than that by the Raman scattering field.

![Graph showing the influence of SPR wavelengths on the SERS intensity of pyrene Raman band at 1239 cm⁻¹. SERS intensity was taken as the normalized Raman intensity of 100 nmol/l pyrene in distilled water. λ_L marks the laser wavelength, λ_R1, λ_R2, and λ_R3 mark the Raman scattering wavelengths corresponding to 409 cm⁻¹, 1239 cm⁻¹ and 1624 cm⁻¹ of pyrene Raman bands, respectively.]

Raman signal enhancement for the isolated silver spheroidal nanoparticles is dependent on the size and shape of nanoparticle [49]. For example, this enhancement for a silver spheroid with an axial ratio of 4:1 becomes larger with increasing particle size ranging from 10 nm to 40 nm and decreased in the range of 40 nm to 70 nm. In that case, larger spheroid produces the red-shifted plasmon resonance. Therefore, it is explained that the metal spheroidal nanoparticle ensemble will produce the highest SERS activity by the irradiation of laser light which has the wavelength close to its own SPR one.
Theoretical calculation of the extinction cross-section and field enhancement for various axial ratio of Ag spheroids has shown that for more oblate spheroid both peaks of extinction and field enhancement move to longer wavelength while a detuned nanoparticle ensemble with a plasmon resonance at longer wavelengths yields a higher SERS activity than an ensemble, whose plasmon resonance is detuned by the same amount to shorter wavelengths [101]. In our case, with the silver atom coverage increased, nanoparticles are more oblate while their number density drops off. In a detail, a SERS substrate at 453 nm of plasmon resonance wavelength has 3 times more nanoparticles than at 548 nm (see Fig. 4.2.3). Hence, the surface area where the molecules can attach to is significantly higher for the former ensemble and thus more molecules contribute to Raman signal. It reflects that among the detuned nanoparticle ensembles, larger one exhibit higher field enhancements. Therefore, it is possible to explain that a large Ag spheroid with equivalent radius of 20.3 nm yields higher Raman intensity, i.e. local electric field, above 3 times higher than a small one of 10.8 nm in radii.

In the following, the concentration dependent calibration curve of every SERS substrate was obtained in order to evaluate the influence of SPR wavelength on LOD for pyrene in distilled water. To do this, SERS/SERDS spectra were obtained at different concentration ranging from 6 nmol/l to 400 nmol/l and normalized by the quartz Raman band at every SERS substrate. As can be seen in Fig. 7.1.4, 1239 cm\(^{-1}\) Raman band is the most intensive among the several Raman bands of pyrene in distilled water at low concentration range. Therefore, the normalized Raman intensity of 1239 cm\(^{-1}\) band was used for creating the concentration dependent calibration curves of pyrene in distilled water with respect to the SPR wavelength of the naturally grown silver nanoparticle ensemble.

Fig. 7.2.2 shows the concentration dependent calibration curves of 1239 cm\(^{-1}\) pyrene Raman band using several SERS substrates with different SPR wavelength. As can be seen in Fig. 7.2.2, a naturally grown silver nanoparticle ensemble with SPR wavelength close to an excitation wavelength of microsystem light source produced the 1239 cm\(^{-1}\) Raman band even at low concentration of pyrene in water sample. For example, a 1239 cm\(^{-1}\) pyrene Raman band at the concentration of 6 nmol/l is visible using a naturally grown silver nanoparticle ensemble with a SPR wavelength of 491 nm.

The LOD for pyrene in distilled water was determined by using the slope of linear fit of the concentration dependent Raman intensity at low concentration and the standard deviation of blank sample (see Fig. 7.1.5).
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Figure 7.2.2: Calibration dependences of normalized Raman intensity of pyrene in distilled water using SERS substrates with different SPR wavelengths of 453, 468, 491, 518 and 548 nm, respectively. 1239 cm\(^{-1}\) Raman band was used for creating the calibration curve.

The influence of SPR wavelength of a naturally grown Ag nanoparticle ensemble on the LOD for pyrene in distilled water is shown in Fig. 7.2.3. As can be seen in Fig. 7.2.3, LOD for pyrene in distilled water determined using the naturally grown silver nanoparticle ensemble is very dependent on the SPR wavelength of nanoparticle. Especially, a naturally grown silver nanoparticle ensemble with 491 nm of SPR wavelength yielded the lowest LOD for pyrene in distilled water.

In the following, when SPR wavelength of SERS substrate is away from the emission wavelength of a microsystem light source at 488 nm, the value of LOD for pyrene in distilled water becomes increased. It shows that the LOD for pyrene in water are strongly dependent on the plasmon wavelength. While the LOD is several tens of nmol/l for the substrates that are off-resonant with the laser light, it amounts to as little as 2 nmol/l for a substrate with on-resonance.
The influence of plasmon resonance wavelength on LOD for pyrene also reveals that the plasmon resonance close to the laser wavelength is more contributed to the sensitivity of SERS substrate, i.e. LOD for pyrene, than that close to Raman scattering wavelength.

The reason for this is that only if the plasmon resonance is in the vicinity of the excitation wavelength for the SERS measurements, the plasmon will be strongly excited, resulting in high local field enhancements.

In addition, Fig. 7.2.1 reveals that the SERS intensity of a substrate with 548 nm of plasmon resonance wavelength is higher than with 453 nm while Fig. 7.2.3 shows that LOD of a substrate with 548 nm is lower than that with 453 nm.

In conclusion, it is demonstrated that the SERS sensitivity of silver spheroid strongly depends on their morphology, i.e. on the size and shape and, thus, on the optical properties of the metal nanoparticles.

Figure 7.2.3: Relationship between SPR wavelength of the naturally grown silver nanoparticle ensembles and detection limit for pyrene in distilled water. $\lambda_L$ and $\lambda_{R2}$ mark the laser wavelength and Raman scattering wavelength corresponding to 1239 cm$^{-1}$ Raman band.
7.3 Discussion and conclusion

In this research work I have studied SERS activity of the naturally grown silver nanoparticle ensemble which was fabricated by the deposition of neutral silver atoms on quartz under the ultrahigh vacumm (UHV) condition in cooperation with CINSaT, Kassel Universität. The naturally grown silver nanoparticle ensemble as SERS substrate has been characterized by the experimental Raman setup containing a microsystem light source with two emission lines of 487.61 nm and 487.91 nm for SERDS which is able to separate the Raman bands from a high fluorescence based background. The comparison of SERS/SERDS spectra of pyrene in distilled water with Raman/SERDS spectrum of pyrene in solid phase shows that there is no Raman band shift after adsorption of pyrene molecule to silver nanoparticle surface meaning that the adsorption of the analyte to metal surface is based on the physisorption. SERS/SERDS spectra of pyrene in distilled water at different concentrations were obtained to make the concentration dependent calibration curve. Limit of detection (LOD) for pyrene in distilled water was determined at every SERS substrate by introducing the standard approach for LOD calculation for pyrene in water using 1239 cm$^{-1}$ Raman band. The influence of SPR wavelength on SERS intensity and LOD for pyrene in distilled water reveals that when the surface plasmon of metal nanoparticles is in resonance with the excitation wavelength SERS intensity and LOD for analyte are optimized, i.e. for on-resonance substrate with laser field LOD for pyrene in water amounts to 2 nmol/l, while for slightly off-resonance substrates, especially even for on-resonance with Raman scattering field, its value goes up in the range of several tens of nmol/l.
Summary and Outlook

8 Summary and Outlook

Summary
The identification and quantification of PAHs in water body has been of an increasing interest during the last decades because some compounds such as PAHs are known to be toxic to biota even at low concentrations. For that purpose, the high sensitive nanoparticle surfaces suitable for the trace detection of PAHs in the water body were fabricated based on the surface enhanced Raman scattering (SERS) technique and were experimentally characterized with the experimental Raman setups containing microsystem laser diode module and flow-through cell. The SERS technique has been recognized as a powerful analytical method because metal nanoparticles act as amplifier which exceedingly enhances the incident electromagnetic field of laser light but also Raman scattering intensity. In addition, microsystem laser diode module with two slightly different emission wavelengths provides us a great potential to introduce the shifted excitation Raman difference spectroscopy (SERDS) being free from the fluorescence based background from SERS spectrum.

After brief reviews on firstly environment pollution and Raman spectroscopy in Chapter 2 and secondly SERS mechanism and its application to PAHs detection in Chapter 3, the methods for the fabrication of the SERS substrates to be used in the experiments and the nanostructures of the substrates were presented in Chapter 4. To improve the SERS activity of silver colloid based sol-gel film, 25,27-dimercaptacetic acid-26,28-dihydroxyl 4-tert-butylcalix[4]arene ($\text{C}_{44}\text{H}_{52}(\text{OH})_2(\text{HSCH}_2\text{COO})_2$: DMCX) was newly developed. New type of SERS substrate, i.e. DMCX functionalized silver colloid based sol-gel film was prepared by adding DMCX in sol-gel matrix. A SERS substrate which 1 mmol/l DMCX in ethanol was used for sol solution was demonstrated to be the best one. Therefore, this substrate was used for the characterization of SEM image and SERS of PAHs in seawater. This type of SERS substrate is named as Ag,DMCX:MTEOS SERS substrate. SEM image of Ag,DMCX:MTEOS substrate showed that sol-gel matrix contains the silver colloid blocks; every block is a collective of separate silver colloidal particles ranging from 50 nm to 120 nm in diameter. By comparing SEM images of two types of SERS substrates, i.e. Ag:MTEOS film and Ag,DMCX:MTEOS film, it is shown that Ag,DMCX:MTEOS SERS substrate has a high aggregation degree of silver nanoparticles due to the addition of DMCX into sol solution.
Summary and Outlook

To demonstrate the influence of surface plasmon resonance (SPR) wavelength on SERS activity and limit of detection (LOD) for pyrene in distilled water, the naturally grown silver nanoparticle ensembles were prepared by depositing neutral silver atoms on quartz substrate under the ultrahigh vacuum (UHV) condition in cooperation with CINSaT, Kassel Universität. AFM images and optical extinction spectra of the SERS substrates revealed that as the silver atom coverage rises silver nanoparticles become more and more oblate and the surface plasmon resonance mode is shifted to long wavelength. After the silver atom deposition of $45.5 \times 10^{15}$ atoms/cm$^2$ corresponding to 491 nm of SPR wavelength, the naturally grown silver nanoparticles have a mean equivalent radius of $15 \pm 4$ nm and mean axial ratio of 0.21.

The experimental Raman set-ups which were used to characterize the fabricated SERS substrates were presented in Chapter 5. Experimental Raman set-up containing a microsystem laser diode with an emission wavelength of 671 nm was used to characterize a Ag,DMCX:MTEOS SERS substrate under the continuous flow of artificial seawater samples containing pyrene or naphthalene at different concentration. To improve the signal to fluorescence based background noise ratio in SERS measurement, a microsystem laser diode with one emission line was replaced into a microsystem laser diode module with two slightly different emission lines of 670.8 nm and 671.3 nm for SERDS. A further experimental Raman set-up containing a microsystem light source with two emission lines of 487.61 nm and 487.91 nm was also used to investigate the influence of SPR wavelength on SERS activity and LOD for pyrene in distilled water. A flow-through cell was positioned at the sample position for in-situ monitoring of PAHs in water body.

The influence of DMCX on SERS activity for some chemicals in water body as well as concentration dependent calibration curve of two PAHs e.g. pyrene and naphthalene but also Raman signal response of this substrate were presented in Chapter 6 using only SERS. In addition, SERS/SERDS investigations of three selected PAHs in seawater body were also shown in this chapter. SERS spectra of phenylacetylene, pyrene and naphthalene using Ag:MTEOS substrate and Ag,DMCX:MTEOS substrate reveal that the silver nanoparticle aggregation and the preconcentration of PAH on the silver surface due to the addition of DMCX in sol solution are two main factors for high SERS activity in Ag colloid based sol-gel substrate. The adsorption constant and LOD for pyrene in artificial seawater were determined to be $0.353 \times 10^9$ l/mol and 0.3 nmol/l (60 ppt), respectively. In that way, the adsorption constant and LOD for naphthalene in artificial seawater were also determined to be $8.2 \times 10^6$ l/mol and 13
Summary and Outlook

nmol/l (1600 ppt). Raman signal response of the in-situ chemical sensor revealed that all pyrene Raman bands in SERS spectrum disappeared 15 minutes after the 400 nmol/l pyrene in artificial seawater was changed to pure artificial seawater applying a flow rate of 30 µl/s. SERDS was introduced to remove the fluorescence based background from SERS spectrum; therefore, Raman bands of pyrene can be identified even at low concentration down to pmol/l range. Concentration dependent calibration procedure for 3 selected PAHs e.g. pyrene, anthracene and fluoranthene in artificial seawater was performed to determine LODs. These amount to 100 pmol/l (20 ppt) for pyrene, 310 pmol/l (55 ppt) for anthracene and 670 pmol/l (135 ppt) for fluoranthene, respectively. LODs for selected PAHs in water body (e.g. 1600 ppt for naphthalene using SERS, 55 ppt for anthracene using SERS/SERDS) are lower than the annual average concentrations of the environmental quality standards in inland surface water proposed by European Community.

The experimental results obtained in the present work firmly showed that SERS and SERS/SERDS techniques are quite adequate to detect the PAHs in water body and to quantify their concentrations with high sensitivity which is enough to satisfy the demand for in-situ monitoring of environmental contaminants in water body. Especially, the introduction of a laser diode module with two emission wavelengths to the nanoparticle based chemical sensor based on the Raman spectroscopy gives us a powerful potential to monitor in-situ the toxic pollutants in water body. The promising results obtained in the present work, however, would be more improved in the future by means of the further work in three directions: (1) to have the more sensitivities of SERS substrates in terms of signal amplification at an excitation wavelength of
Summary and Outlook

488 nm (or 671 nm) and PAHs enrichment on the nanoparticle surface by the hydrophobic functionalization, (2) to obtain SERS/SERDS spectra and the concentration dependent calibration curves of other PAHs in water body using a Ag,DMCX:MTEOS SERS substrate excited by 671 nm microsystem laser diode module or a naturally grown Ag nanoparticle ensemble by 488 nm microsystem light source, (3) to integrate the SERS substrate onto a marine test platform.

To develop a naturally grown metal nanoparticle ensemble suitable for in-situ seawater monitoring using 671 nm Raman set-up, spheroidal gold nanoparticles which are very stable in seawater should be optimized with respect to their size and shape having the SPR wavelength close to the required excitation one. Because PAHs are non-traditional SERS analytes, functionalization of gold nanoparticle with DMCX should be introduced for the significant enrichment of PAH molecules within the area of high electromagnetic field of excited nanoparticle.

Secondly, because Raman bands of PAHs are shifted after the adsorption to a Ag,DMCX:MTEOS SERS substrate surface the identification of Raman bands of every PAH and its calibration curve should be done to extend SERS/SERDS investigation into other PAHs and the mixture of several PAHs. Calibration procedures for other PAHs in water body using a naturally grown silver nanoparticles ensemble should be also performed to develop the nanoparticle based chemical sensor containing 488 nm microsystem light source.

Finally, to integrate SERS onto marine test platform, Raman optode combined with flow-through cell should be integrated to the existing prototype instrument. This requires some adaptation and the update with new laser technology to improve signal quality and reduce power consumption. Developed SERS substrates should be validated by this instrument.
References


References


References


References


References

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## APPENDIX I

### Chemicals used for SERS experiment

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<th>No</th>
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<th>Formula</th>
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Table of typical Raman/SERS bands of several PAHs (Ag,DMC:MTES substrate, 671 nm Raman set-up (Chapter 6))
Abbreviation of some organic compounds

Ant : Anthracene
Fla : Fluoranthene
MTEOS: Methyltriethoxysilane
Nap : Naphthalene
Pyr : Pyrene
Phe : Phenylacetylene

Abbreviation of some chemical parameters

Cₘ : Saturation concentration in water body
MW : Molecular weight
Acknowledgments

First of all I would like to thank Priv.-Doz. Dr. Heinz-Detlef Kronfeldt and Dr. Heinar Schmidt for proposing the subject of the Thesis and providing me the opportunity to be supported by the European Community in the framework of the SENSEnet project-‘An EU framework 7 funded Marie Curie Initial Training Network’.

I would like to thank Prof. Dr. Mario Dähne, Prof. Dr. Ulrike Woggon, and Priv.-Doz. Dr. Frank Hubenthal for the organization of my PhD examination.

I would like to thank all members of the SENSEnet project team, especially Dr. Douglas Connelly, SENSEnet Co-coordinator and Dr. Carla Sands, SENSEnet project manager (Southampton, UK) for their cooperation.

I would like to thank the Gottlieb Daimler-und Karl Benz-Stiftung, Ladenburg, Germany, supporting me for the first 6 months of my PhD work at Technical University Berlin.

Many thanks are expressed to the colleagues in the Laser Spectroscopy Group, Institute of Optics and Atomic Physics, Technical University Berlin: Kay Sowoidnich, Anna Kolomijeca, Bernd Geisler, Hossam Ahmad, Halah Al Ebrahim, Lutz Brechlin and Xiaofeng Shi for their continuous cooperation.

Thanks are expressed to Dr. Dirk Berger and Mr. Ulrich Gernert, ZELMI, Technical University Berlin for the SEM images of the Ag,DMCX:MTEOS SERS substrates, and Mr. Robert Ossig in CINSaT, Universität Kassel for their cooperation to develop the naturally grown Ag nanoparticle ensmsble as SERS sensor for the trace detection of PAHs in water body.

Finally, I sincerely thank to Prof. Dr. Chol-Ho Li, Director, Institute of Lasers, DPR Korea and Mrs. Hi-Ok Kim, my love wife for their care and encouragement of my research stay in Berlin for 3 years, owing to which my PhD thesis was successfully accomplished.