

Organometallic nanoprobe to enhance optical response on Polycyclic Aromatic Hydrocarbons (PAHs) immunoassay using SERS technology.

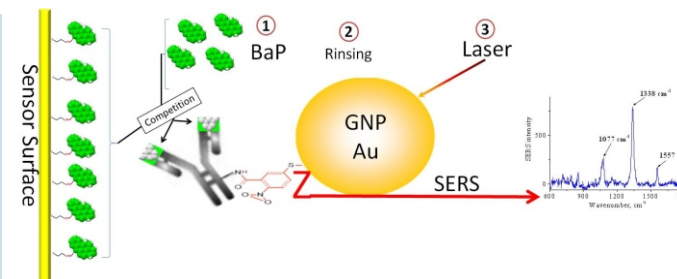
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Aim:

Coupling of electromagnetic phenomenon related to the transduction by SERS effect with a biochemical phenomenon of specific biosensing for the development of an innovative method able to detect and quantify benzo[a]pyrene (BaP) at low concentrations in seawater.

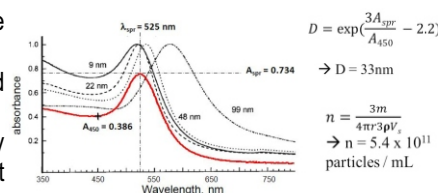
Principle of the sensing method :

Our method is based on the competition between benzo[a]pyrene to be determined and benzo [a] pyrene grafted on the surface of the sensor towards the antibodies grafted on organometallic probes. Thus, as more molecules of BaP are present, the lower the SERS signal is. Inversely, if the initial concentration of antigen is low, the signal is strong.



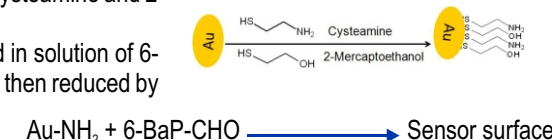
- 1 - Preparation of Raman Reporter-Labeled Immunogold Colloids

- Gold NanoParticles (GNPs) were prepared according to the Turkevich-Frens method; reduction of HAuCl₄ by sodium tricitrate.
- Size and concentration of gold nanoparticles were determined from UV-Vis Spectra.
- 5,5'-Dithiobis(succinimidyl-2-nitrobenzoate) (DSNB) is obtained by conversion of corresponding dithiobis(benzoic acid) using a reagent mixture carbodiimide / succinimide.
- GNP coating of the thiolate of DSNB, which can couple to the primary amines of a anti-BaP antibody by formation of an amide linkage.



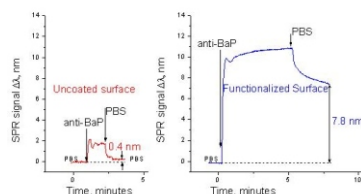
- 2 - Functionalization of the sensor surface with a SAM presenting BaP head

- Direct synthesis of 6-Benzo[a]pyrenecarboxaldehyde from BaP was accomplished via Vilsmeier-Haack reaction using N-methylformamide and POCl₃.
- Attachment of BaP to a gold surface of the sensor begins with a self-assembled mixed monolayer formation of cysteamine on this surface. This step was assured by means of gold-alkanethiolate self-assembly process using cysteamine and 2 mercaptoethanol.
- Afterwards, the substrate was firstly treated in solution of 6-BaP CHO. This forms a schiff base with were then reduced by cyanoborohydride.



- 3 - Activity control of sensing components by SPR

In order to control the surface grafting of benzo [a] pyrene, we have used Surface Plasmon Resonance (SPR) spectroscopy. Thus, we have studied two types of surface, functionalized and non-functionalized (uncoated) surfaces. The analysis begins with the injection of phosphate buffer at pH 7. The BaP13 antibody at 50 ppm in PBS was then injected. The analysis is finished by a rinsing step with PBS to remove non-specific antibodies adsorption.

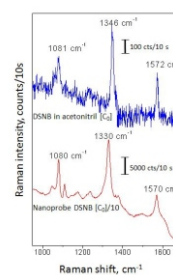


With the two surfaces, we observe an increase in the SPR signal after antibodies injection.

However, we note that the increase obtained with the functionalized surface is considerably higher.

- 4 - SERS assay of nanoprobe

Spectrum of free DSNB is dominated by the symmetric nitro stretching at 1342 cm⁻¹. The band at 1572 cm⁻¹ is assigned to an aromatic ring mode, and the band at 1081 cm⁻¹ is a succinimidyl N-C-O stretch overlapping with aromatic ring modes. The same bands in the solution spectrum are present in the spectrum of DSNB nanoprobe.

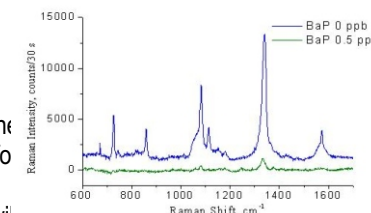


The ratio of areas under these Raman bands reported to the quantities of DSNB in the analyzed samples allows as to calculate an enhancement factor of 36000.

- 5 - BaP detection

Raman analysis steps:

- Dilute the probe suspension with 6 volumes of PBS.
- Mix one volume of the suspension thus obtained with one volume of seawater containing benzo[a]pyrene and incubate for 30 minutes.
- Place a microdrop (60 μl) of this mixture on the surface of a substrate previously covered with benzo[a]pyrene for 30 minutes.
- Rinse the substrate with water and analyze with the Raman spectrometer (excitation at 785 nm, water immersion objective x 100).



Thus, the molecules of BaP present in the sample to analyze will block the antigenic sites of antibodies in the organometallic probes.

Based on these preliminary results, the analysis of samples of seawater polluted by benzo[a]pyrene using the SERS phenomenon and Raman probe will allow detection of this analyte at concentrations around 0.5 ppb.

Conclusion:

We have demonstrated the possible use of the new organometallic nanoprobe for competitive surface-enhanced Raman scattering (SERS) immunoassay for a PAH such as BaP in seawater. Before use in SERS detection, the nanoprobe activity was controlled by our own Surface Plasmon Resonance sensor. The present method allows us to detect benzo[a]pyrene at trace concentration (sub-ppb).