Surface-Enhanced Raman Substrate Optimization for Label-Free Bacterial Detection

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Abstract

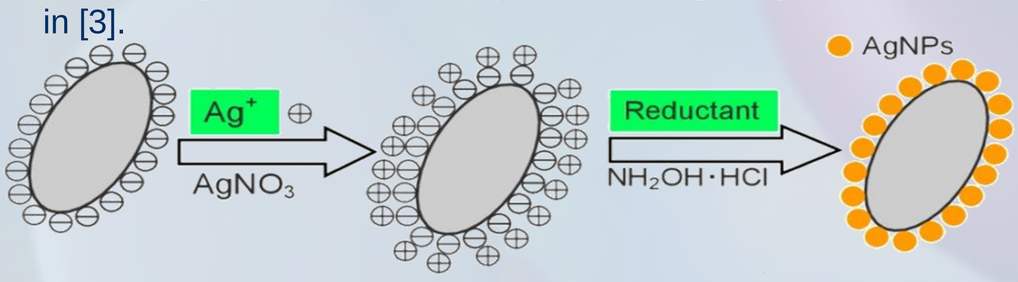
The successful use of surface-enhanced Raman scattering (SERS) based assays for bacteria sensing rely mainly on the type of the SERS active substrate used, its biological compatibility, and the consistent binding between the bacteria and the substrate. In this work, the possibility of detecting pathogenic bacteria by using polyethylene glycol (PEG) functionalized gold nanoparticles (GNPs) was investigated. The four potential SERS substrates (PEGylated AuNPs, PEGylated AgNPs, concentrated PEGylated AuNPs and Bacteria@AgNPs) were obtained by using a very effective, simple, one step synthesis method [1]. They were thoroughly characterized by means of UV-spectroscopy and Transmission Electron Microscopy (TEM). The NPs were predominantly spherical or polygonal, depending on the mixing rate of the two reagent solutions.

The SERS results obtained for *E. coli* using as prepared and concentrated by centrifugation PEG-coated GNPs were compared with the *in situ* detection method based on AgNPs coated bacteria (Bacteria@AgNPs) [1, 2]. Our efforts were focused on selecting the best SERS substrate for bacteria detection in terms of the time required, reproducibility and enhancement factor. The intensity of marker band found at 732 cm^{-1} in the SERS spectra was about 5-fold higher when using the Bacteria@AgNPs approach.

The highly reproducible and reliable vibrational profile of the Bacteria@AgNPs system endorses the successful use of SERS-based detection platform in healthcare applications.

Experimental details

- **Bacteria cultivation:** 2 ml of LB grown *E. coli* was centrifuged at 6000× (cati g?) and washed 3 times in saline buffer; the remained pellet was suspended in water or PBS environment.
- **AuPEG NPs synthesis:** as described in [1, 2] and used as prepared (AuPEG8000) or 38× concentrated by centrifugation (AuPEG200 centrifuged).
- **AgPEG NPs synthesis:** 500 µl NaOH 1%, 1.2 ml PEG200, 98 ml Millipore water and 2 ml $AgNO_3$ (10^{-3}M) were used. $AgNO_3$ solution was added to the water and heated at 200°C on a heating plate in order to boil. At the boiling point, the PEG200 solution was added rapidly and afterwards, in two steps, the NaOH solution for stabilizing the wine-colored colloidal solution. Time required: 20 min.
- ***in situ* Ag NPs synthesis (Bacteria@AgNPs):** as described in [3].

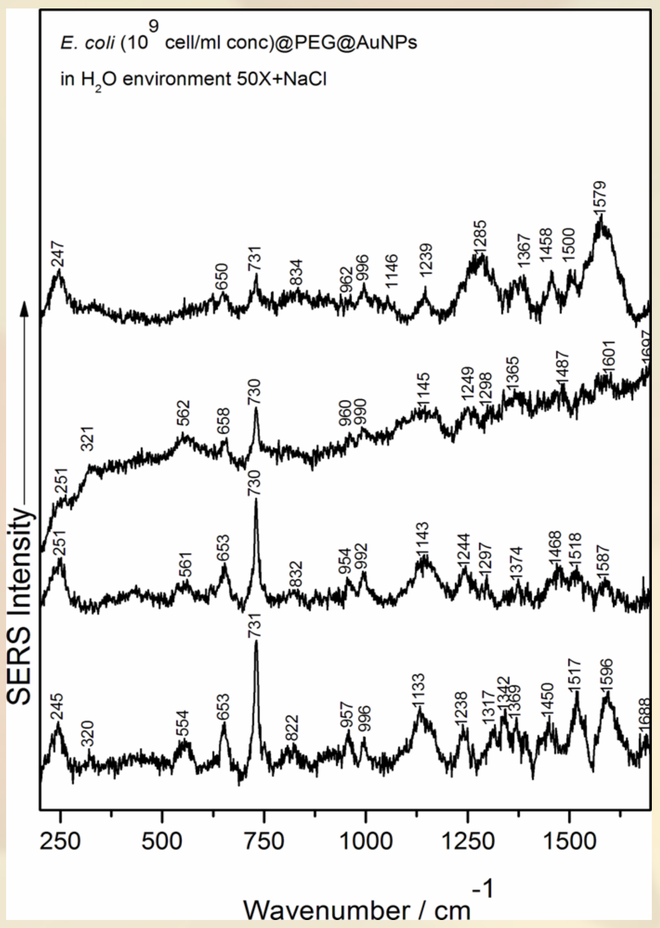


- **Preparation of samples:** 5 µl of each colloid and *E. coli* 10^9 cells/ml in PBS mixture/H_2O mixture (1:2) were dried on glass substrate prior to the measurement.

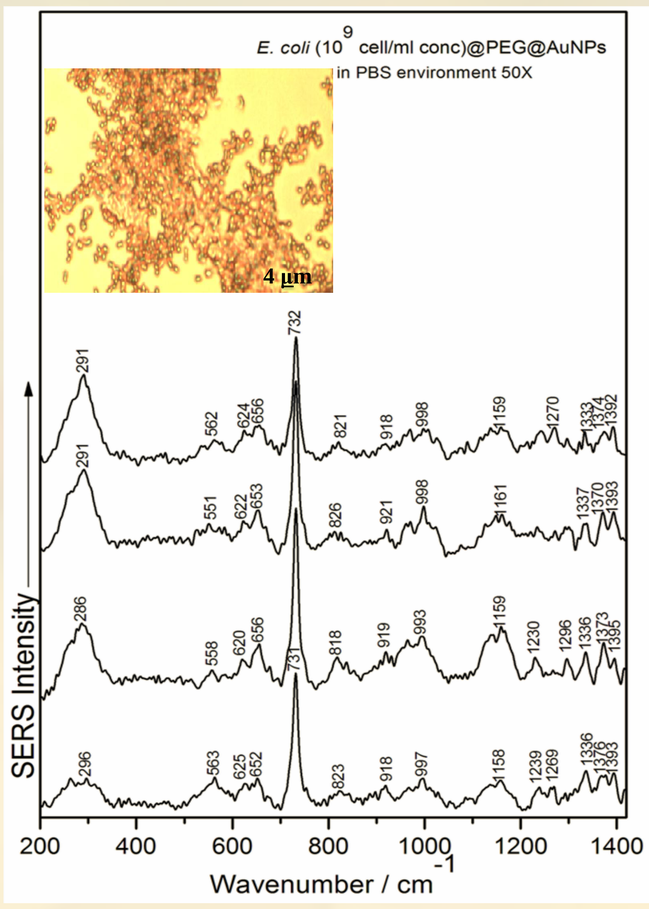
  5 µl of **Bacteria@AgNPs** were dried on glass substrate prior to measurement.

- **Measurement parameters:** 633 nm, 50× objective, 1 % laser power (0.14 mW on the source).

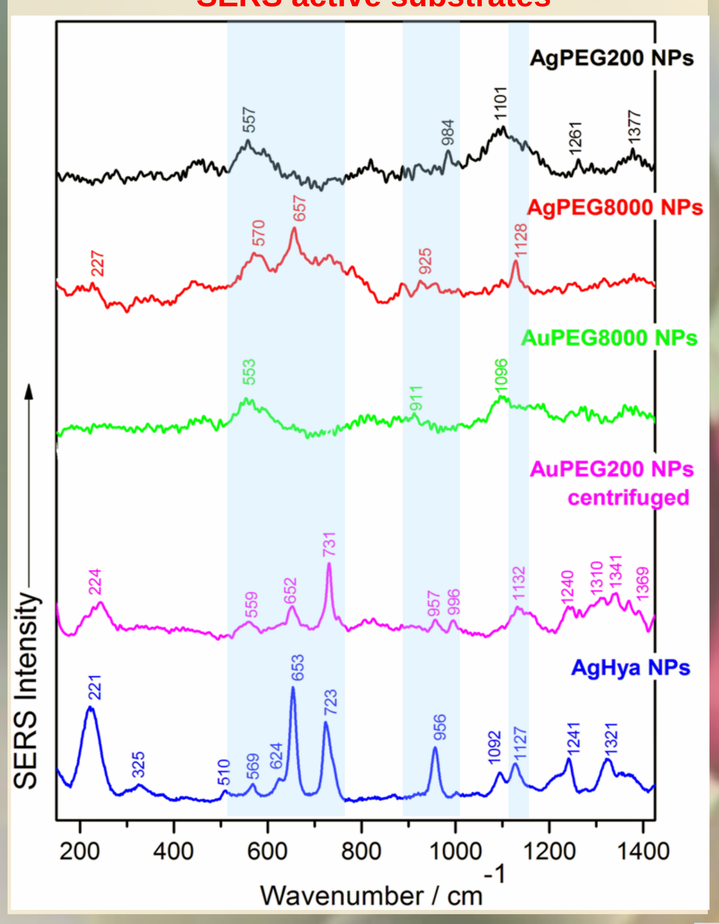
Reproducibility of the SERS spectra of *E. coli* in H_2O environment using PEG GNPs as SERS active substrates



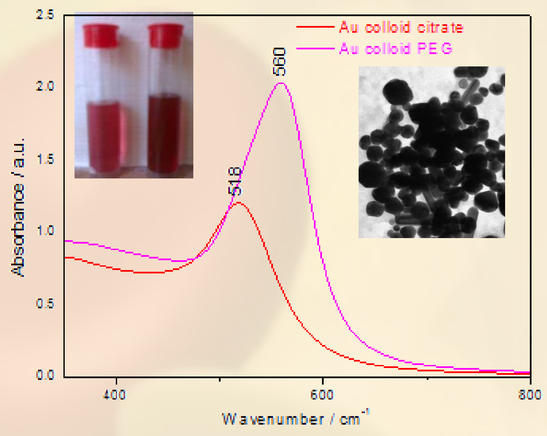
Reproducibility of the SERS spectra of *E. coli* in PBS environment using PEG GNPs as SERS active substrates



SERS spectra of *E. coli* using different SERS active substrates



UV-Vis spectrum of PEG Au colloid, in comparison with the Au colloid obtained using citrate. *Inset:* TEM image of the PEG GNPs [1]



Conclusions

- The marker regions of the bacteria are present in all the SERS spectra, regardless of the type of the active SERS substrate.
- The viability of the bacteria is revealed by the presence in the SERS spectrum of the ~730 cm^{-1} band.
- The reported results endorse the successful use of Bacteria@AgNPs as SERS-based detection platform.

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References:

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[3] H. Zhou et al., Anal. Chem. (2014) 86(3), 1525-1533.