Bimetallic Nanostars (Ag@Au) with High Surface Enhanced Raman Scattering (SERS) Performance: Detection of β-Amyloid and Its Marker Thioflavin T

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Outline

Introduction

Fabrication and Characterization of SERS substrates

Nanostars

Synthesis

Characterization

Alzheimer Disease Markers

β-amyloid

Direct SERS Detection

Dyes (Congo Red and Thioflavin T)

SERS characterization

ThT - β-amyloid interaction

Indirect SERS Detection of β-amyloid

Tailoring the size and shape of Silver Nanostars

Ag@AuNS using AgNS as seeds

Conclusions
Surface Enhanced Raman Spectroscopy

Localized Surface Plasmon Resonance

Ag, Au, Cu

Electromagnetic mechanism
Colloids

Need external aggregation to detect low concentrations

Non reproducibility
Variation in signal/noise

SERS sensitive substrates with complex morphology

Enhanced Electromagnetic Field
Nano-Stars and nano-spheres Fabrication

AgNO$_3$ or HAuCl$_4$·3H$_2$O

Hydroxylamine

Citrate
Borohydride
Hydrochloride
Hydroxylamine

Nano-Stars
NS-Ag / NS-Ag@Au

Nanospheres

A. Garcia-Leis, J.V. Garcia-Ramos and S. Sanchez-Cortes. JPC C. (2013) DOI: 10.1021/jp401737y
Characterization of nanoparticles

UV-vis.

Extinction vs. wavelength (nm)

AgCT
AuCT
NS-Ag
NS-Ag@Au

50 nm
300 nm
Nanospheres

AgCT
(SEM)

AuCT
(SEM)
Nano-stars

(TEM and SEM characterization)
Nano-stars

(TEM and SEM characterization)

A. Garcia-Leis, J.V. Garcia-Ramos and S. Sanchez-Cortes. JPC C. (2013) DOI: 10.1021/jp401737y
**Nano-stars**

(Plasmon characterization)

\[
\begin{array}{ccc}
\text{A: 2.17/ 7.0 / 1.0} \\
\text{B: 0.21/ 7.0 / 1.0} \\
\text{C: 2.17/ 3.6 / 1.0} \\
\text{D: 2.17/ 7.0 / 0.0}
\end{array}
\]

SERS active substrates without aggregation!!

A. Garcia-Leis, J.V. Garcia-Ramos and S. Sanchez-Cortes. JPC C. (2013) DOI: 10.1021/jp401737y
Nano-stars

(Dark-Field scattering)

A. Garcia-Leis, J.V. Garcia-Ramos and S. Sanchez-Cortes. JPC C. (2013) DOI: 10.1021/jp401737y
Nano-stars

SERS activity using probenecid (sulfamid) as probe molecule

Raman in solid state ($\lambda_{\text{exc}} = 1064$ nm)

SERS ($\lambda_{\text{exc}} : 532$ nm)

A. Garcia-Leis, J.V. Garcia-Ramos and S. Sanchez-Cortes. JPC C. (2013) DOI: 10.1021/jp401737y
Tailoring the size and shape of Silver Nanostars
Preparation of a colloidal suspension of Silver Nano Stars

Chemical reduction of Ag⁺ in two steps:
Step 1: Reduction agent is a neutral Hydroxilamine solution
Step 2: After a time T₁, a 1% citrate solution is added.

The great novelty of this method is the use of neutral HA and the no use of strong surfactant agents.

Experimental Conditions
24 different samples of AgNS

[HA]
✓ 30.1 mM
✓ 60.2 mM
✓ 130 mM

[Ag⁺]
✓ 1.11 mM

V(CIT)
✓ 100 µL

T₁
✓ 5 min.
✓ 60 m0.11 mM
✓ 10 µL
✓ 30 min.
✓ in.

[HA] = concentration of hydroxylamine solution
[Ag⁺] = concentration of silver nitrate solution
V(CIT) = added volume of 1% citrate solution
T₁ = the waiting time before adding the CIT solution
PCA graphic of scores and loadings of data obtained from morphological features of 24 AgNS samples.

PCA data can be classified in seven groups formed by samples with similar features depending on: size (bigger or smaller), type of arm (longer or shorter) and type of tip (spiky or rounded).
Extinction Spectra

Scheme of possible mechanism of growing nanoparticles based on TEM images.
SERS spectra of thiophenol at 1µM on different colloids samples of AgNS: a) 105, b) 205, c) 305, d) 405, e) 505, f) 605, g) 705, h) 805 and i) Raman spectra of pure thiophenol. Excitation 532 nm.
The reported fabrication method gives rise to silver star-shaped nanoparticles with good plasmonic properties to afford a large SERS intensification. The PCA study allowed the lump together of NPs by different groups, taking account the morphological parameters. The best Ag NS according to SERS EF are those bearing an intermediate size (200 nm) displaying a moderate number of arms.
Final morphology of Ag@AuNS using AgNS as seeds

532 nm (ThT resonant wavelength)

785 nm (ThT non-resonant wavelength)
Concentrations of reagents employed for the preparation of samples A-E and final pH of colloids solutions.

<table>
<thead>
<tr>
<th>[Reagent]/mM</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>[AgNO₃]</td>
<td>0.073</td>
<td>0.37</td>
<td>0.73</td>
<td>0</td>
<td>0</td>
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<tr>
<td>[HAuCl₄·3H₂O]</td>
<td>0.27</td>
<td>0.27</td>
<td>0.27</td>
<td>0.4</td>
<td>0.2</td>
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<tr>
<td>[HA]</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>0</td>
</tr>
<tr>
<td>[CIT]</td>
<td>0.26</td>
<td>0.26</td>
<td>0.26</td>
<td>0.26</td>
<td>0.77</td>
</tr>
<tr>
<td>pH</td>
<td>5.0</td>
<td>5.0</td>
<td>4.7</td>
<td>4.4</td>
<td>5.5</td>
</tr>
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</table>
Micrograph of Ag@Au NS obtained by TEM of A (A), B (B), C (C) and (D) is a general view of sample C. (E) SEM micrograph of Sample C. (F) EDX spectra of Sample C.
Extinction spectra (left) and TEM images (right) corresponding to the samples: A (a), B (b), C (c), D (d) and E (e).

*Inset:* Pictures of the colloids obtained by methods A, B, C, D and E.
Dark-field scattering spectra observed in samples: A, C and D.

Dark-field (DF) hyperspectral image

Spectral angle mapper (SAM) image
SERS enhancement dependence on the excitation wavelength and the substrate: 532 nm (a) and 785 nm (b). Chemical formula of ThT (c). Adsorption isotherm of ThT on sample B (d). Raman spectrum of ThT 10 mM (e). SERS spectrum of ThT 1 µM on sample B (g) and sample E (g). Excitation 785 nm.
SERS spectra of ThT on Samples A and C exciting at 532nm (b and d, respectively) and 785nm on Sample A (a). The extinction spectra of samples A (c) and C (e) and the absorption spectrum of ThT (0.1 M) in water (f) is also shown for comparison.
Alzheimer disease

Neurodegenerative disease

β-amyloid

Peptide with 36-43 aa

Histological dyes used to demonstrate the presence of amyloidal deposits in tissue

Congo Red

Thioflavin T
β-amyloid (1-42)

SERS of β–amyloid(1-42) on Silver Nano-Stars

- β-Amyloid (1-42) Raman solid
- NSAg + β-Amyloid (1-42) 2.22 µM pH 9
- NSAg + β-Amyloid (1-42) 0.22 nM pH 9
- NSAg + β-Amyloid (1-42) 0.22 nM pH 7

λ exc. = 633 nm
<table>
<thead>
<tr>
<th>Wavenumber / cm⁻¹</th>
<th>Solid Raman (λ&lt;sub&gt;exc.&lt;/sub&gt; = 633 nm)</th>
<th>SERS NSAg pH 9 (633nm)</th>
<th>SERS AgCT pH 9 (785nm)</th>
<th>Assignments</th>
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<tr>
<td>1669 vs</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Am I</td>
</tr>
<tr>
<td>-</td>
<td>1623</td>
<td>1623</td>
<td>-</td>
<td>v(C=O)</td>
</tr>
<tr>
<td>1606 m</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Phe, v(C=O)</td>
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<tr>
<td>1585 w</td>
<td>1595</td>
<td>1581</td>
<td>-</td>
<td>Phe, Tyr</td>
</tr>
<tr>
<td>1565 w</td>
<td>1558</td>
<td>1567</td>
<td>-</td>
<td>Am II</td>
</tr>
<tr>
<td>-</td>
<td>1483</td>
<td>1494</td>
<td>-</td>
<td>His</td>
</tr>
<tr>
<td>1469 sh</td>
<td>1463</td>
<td>-</td>
<td>-</td>
<td>His (deprotonated)</td>
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<tr>
<td>1444 s</td>
<td>1433</td>
<td>1441</td>
<td>-</td>
<td>δ(CH&lt;sub&gt;2&lt;/sub&gt;) , δ(CH&lt;sub&gt;3&lt;/sub&gt;)</td>
</tr>
<tr>
<td>1408 sh</td>
<td>1413</td>
<td>1401</td>
<td>-</td>
<td>δ(C&lt;sub&gt;α&lt;/sub&gt;-H)</td>
</tr>
<tr>
<td>-</td>
<td>1344</td>
<td>1347</td>
<td>-</td>
<td>t&lt;sub&gt;α&lt;/sub&gt;(CH&lt;sub&gt;2&lt;/sub&gt;) or p(CH&lt;sub&gt;2&lt;/sub&gt;)</td>
</tr>
<tr>
<td>1264 w</td>
<td>1261</td>
<td>1268</td>
<td>-</td>
<td>Am III (β-sheet)</td>
</tr>
<tr>
<td>1236 w</td>
<td>1237</td>
<td>-</td>
<td>-</td>
<td>Phe, Tyr</td>
</tr>
<tr>
<td>1185 vw</td>
<td>1191</td>
<td>-</td>
<td>-</td>
<td>ω(C=O)</td>
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<tr>
<td>1179 w</td>
<td>1172</td>
<td>1171</td>
<td>-</td>
<td>v(C=O)</td>
</tr>
<tr>
<td>1123 w</td>
<td>1127</td>
<td>1128</td>
<td>-</td>
<td>His, Lys, Arg</td>
</tr>
<tr>
<td>1091 vw</td>
<td>1091</td>
<td>-</td>
<td>-</td>
<td>v(C=O) aliphatic side chains</td>
</tr>
<tr>
<td>-</td>
<td>1068</td>
<td>1064</td>
<td>-</td>
<td>Phe</td>
</tr>
<tr>
<td>1031 m</td>
<td>-</td>
<td>1034</td>
<td>-</td>
<td>Phe</td>
</tr>
<tr>
<td>1003 vs</td>
<td>-</td>
<td>1001</td>
<td>-</td>
<td>δ&lt;sub&gt;op&lt;/sub&gt;(=C-H)</td>
</tr>
<tr>
<td>969 vw</td>
<td>968</td>
<td>961</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>
Poor SERS spectra to be used for β-amyloid direct detection!!!
Use of Dyes to detect amyloid fibril formation

Nano-stars

Congo Red

Thioflavin T
Detection of Congo Red by SERS

Intensity

Wavenumber / cm⁻¹
Detection of Thioflavin T by SERS

Thioflavin T (ThT) is a fluorescent probe widely used to detect amyloid fibrils, which are a hallmark of neurodegenerative diseases such as Alzheimer's disease. In this study, the detection of ThT using Surface-Enhanced Raman Scattering (SERS) with NS-Ag@Au nanoparticles is explored. The SERS spectra show distinct peaks at 1543, 1475, 1394, 1128, 795, and 677 cm\(^{-1}\), which correspond to the vibrational modes of ThT. These peaks are not as clearly visible in the FT-Raman spectra, indicating the superior sensitivity of SERS for detection at lower concentrations. The NS-Ag@Au nanoparticles enhance the Raman signal, allowing for detection at concentrations as low as 10\(^{-6}\) M of ThT.
Scheme of the adsorption of the ThT$_1$ and ThT$_2$ species on the Ag surface which implies a rotation of the $\phi$ angle to 90°.
Detection by SERS

Langmuir Adsorption Isotherm

\[ I_s = \frac{K_{ad} I_{sm} [\text{Analyte}]}{1 + K_{ad} [\text{Analyte}]} \]

- \( I_s \) - SERS Intensity
- \( K_{ad} \) - Adsorption constant
- \( I_{sm} \) - Maximum concentration of adsorbed analyte
- \([\text{Analyte}]\) - Molecule concentration

- Concentration (10\(^{-4}\) M - 10\(^{-9}\) M)
- \( \lambda \) excitation (532 nm, 785 and 633 nm)
Detection by SERS

Langmuir adsorption isotherm

**CR**

\[ I_s = I_{sm} \times K_{ad} \times [CR]/(1 + K_{ad} \times [CR]) \]

\[ R^2 = 0.98 \]

\( I_{sm} = 6.42 \pm 0.33 \)

\( K_{ad} = 7.3E5 \pm 1.2E5 \)

**ThT**

\[ I_s = I_{sm} \times K_{ad} \times [ThT]/(1 + K_{ad} \times [ThT]) \]

\[ R^2 = 0.97 \]

\( I_{sm} = 1.43 \pm 0.03 \)

\( K_{ad} = 5.98E5 \pm 0.87E5 \)
Detection by SERS

Linear Region

\[ I_s = K_{ad} I_{sm}[\text{Analyte}] \]

Congo Red

\[ I_s = K_{ad} I_{sm}[\text{CR}] \]
\[ R^2 = 0.98 \]
\[ K_{ad} I_{sm} = 5.51 \times 10^6 \pm 0.05 \times 10^6 \]

Thioflavin T

\[ I_s = K_{ad} I_{sm}[\text{ThT}] \]
\[ R^2 = 0.95 \]
\[ K_{ad} I_{sm} = 1.2 \times 10^5 \pm 0.1 \times 10^5 \]
Absorption spectra of the studied systems
SERS on colloids

- Blue line: NS-Ag + ThT/β-Am (50µM:0.22µM) pH 9 (a)
- Green line: NS-Ag + ThT (50µM) pH 9 (b)
- Pink line: (a) - (b) difference

λexc. = 532nm

Wavenumber (cm$^{-1}$)

Intensity (a. u.)
ThT- β-amyloid complex

The effect of [β-amyloid] on the SERS spectra on Ag nanostars
(a) NSAg + [ThT:β-Am] (25µM:0.22µM) pH 6
(b) NSAg + [ThT] (25µM)
(c) [a-b] Difference

$\lambda_{exc.} = 442$ nm
Conclusions

✓ A simple method of nanostructure fabrication with high sensitivity in SERS technique has been developed.

✓ This method allows higher reproducibility in SERS measurements without aggregation.

✓ Congo Red and Thioflavine T dyes were detected at low concentrations.

✓ The adsorption isotherm of ThT over nano-stars follows a Langmuir model.

✓ SERS of β-amyloid peptide has been obtained through its interaction with ThT.
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