**Advances in Bioresearch**
Adv. Biores., Vol 7 (6) November 2016: 76-81
©2016 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
ICV Value 8.21 [2014]

Advances in Bioresearch

## ORIGINAL ARTICLE

# Synthesis and optimization of silver nanoparticles-antibody Herceptin conjugation for surface-enhanced Raman scattering (SERS)

**Naser Jafarzadeh[1*], Mohammad Javad Rasaee[2] , Kambiz Gilany[3] and Rasoul Malekfar [4]**
[1]Department of Medical Physics, Tarbiat Modares University, Tehran, I.R. Iran,
[2]Department of Medical Biotechnology , Tarbiat Modares University, Tehran, I.R. Iran,
[3]Reproductive Biotechnology Research Center, Avicenna Research Institute, ACECR, Tehran, I.R. Iran.
[4] Atomic & Molecular group, Department of Physics, Tarbiat Modares University, Tehran, I.R. Iran.

**ABSTRACT**
*Here, we have a developed surface-enhanced Raman spectroscopy substrate composed of antibody-conjugated silver nanoparticles as a functional nanoprobe. In this study, we synthesized silver nanoparticles with the para-mercaptobenzoic acid (pMBA) linker that binds to antibody Herceptin Here, we analyzed the binding of Herceptin to silver nanoparticles by Fourier Tranform Infrared (FT-IR). Our results clearly showed that a spherical shape of silver nanoparticles with a diameter of 50 nm. Furthermore, our SERS probe apparently indicated that the intensity of antibody-conjugated silver nanoparticles as a SERS sensitive probe is increased by an enhancement factor of 105.*

*Key words: Silver nanoparticles, antibody conjugation, Raman scattering, spectroscopy, surface enhanced, SERS.*

Received 19/07/2016 Accepted 29/10/2016 

**How to cite this article:**
Naser Jafarzadeh, Mohammad Javad Rasaee, Kambiz Gilany and Rasoul Malekfar. Synthesis and optimization of silver nanoparticles-antibody Herceptin conjugation for surface-enhanced Raman scattering (SERS), Adv. Biores., Vol7 [6] November 2016: 76-81. DOI: 10.15515/abr.0976-4585.7.6.7681

## INTRODUCTION

Raman spectroscopy as a rapid, and non-destructive photon scattering method, presents fine spectral aspects with specific information based on vibrational energy stages of analyte molecules. The ability for multiplexed detection and molecular specificity can make this technique a powerful analytical tool capable of chemical identification and biological species recognition. It can elucidate molecular structure and examine the surface properties. This technique has been reported to greatly improve the efficiency of Raman scattering which is the surface-enhanced Raman scattering (SERS), in which analytes are localized near plasmonically active surfaces or substrates [1-4].

Surface-enhanced Raman scattering (SERS) is under rigorous investigation in biomedical application because of its strength , sensitivity and enhanced levels of multiplexing as well as its capability to perform recognition in blood and other biological sources [5]. The surface-enhanced Raman scattering (SERS) effect has been shown to be responsible for the enhancement of Raman signal of molecules adsorbed on metallic nanostructures. The enhancing factor can be developed to large numbers such as $10^{11}$ times more [1–4], allowing to get SERS spectrum from very diluted solutions [6-7].

Although unlabeled silver nanoparticles show limited use in SERS-based biochemical analysis due to their lack of molecular specificity. Addition of biochemically responsive labels or ligands can solve it, making SERS-active silver nanoparticles with great potential for intracellular bioanalysis and extracellular labeling [8-11]. The human epidermal growth factor receptor 2 (HER2) proteins and the HER2/neu oncogene have been shown to be involved in cell proliferation and survival. Amplification of HER2/neu gene occurs in around 20% to 25% of human breast cancers and is apparently associated with a more aggressive disease course and poor prognosis. HER2 amplification status has been reported to be responsible for resistance Patients with HER2+ breast cancer need specific treatments that can affect

HER2 activity such as using monoclonal antibodies and small molecule tyrosine kinase inhibitors. Therefore, assessment of HER2 status is pivotal in therapeutic decision.

In the present study, we synthesized and then optimized the silver nanoparticles for conjugation to a humanized anti her2/neu monoclonal antibody. (Herceptin) with the linker pMBA. The main goal of this study is to demonstrate antibody conjugated silver nanoparticles potential application in SERS.

## RESEARCH METHOD

### Materials

A humanized monoclonal antibody Herceptin was purchased from Genentech. Silver nitrate ($AgNO_3$), sodium borohydride (NaBH(4)), pMBA, 1-Ethyl-3-[3-dimethy-laminopropyl] carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) were obtained from Sigma-Aldrich. Trisodium citrate dehydrate purchased from Merck.

### Silver nanoparticles preparation

The Ag nanoparticles were prepared as described by Hashemifard et al. Briefly, 15 mL solution of 160 mM $AgNO_3$ was added dropwise into 50 mL of the freshly prepared 20 mM solution of $NaBH_4$ while in ice bath under vigorous stirring until the solution turned into greenish yellow in color [12]. The solution in ice bath causes the synthesis reaction to be faster and the morphology of the silver nanoparticles to be smoother. The morphology of the prepared AgNPs was characterized by transmission electron microscopy (TEM).

### Antibody-conjugated silver nanoprobe

A 10-mL solution of Ag nanoparticles was mixed with pMBA solution (100 µL, 1 mM in ethanol) and then stirred for 3 h. The color of Ag reaction with pMBA changes from greenish yellow to brown. Afterwards, the solutions of EDC (10 mL, 10 mM) and NHS (1 mL, 100 mM) were added into the pMBA coated silver nanoparticles. The carboxylic groups on the particles surfaces were activated to form reactive NHS ester intermediates. After 30 min of stirring, 10 µL of anti-HER2 monoclonal antibody (Herceptin) was added into the carboxylic group activated silver nanoparticles and then stirred for another 3 h on ice bath. The amine groups on the antibody molecules reacted with the active ester groups on the silver nanoparticles surfaces to form stable amide bonds. The antibody conjugated silver nanoparticles were further purified by centrifugation at 6,000 rpm for 8 min. The supernatant solution was removed and the precipitated particles redispersed in 10 mL deionized water.

### Instrument and measurement

Extinction spectra were collected using a spectrophotometer T80+UV/VIS PG Instruments, Leicester, UK. Transmission electron microscopy (TEM) images characterizing the morphology of the SERS probe were obtained using a transmission electron microscope (Zeiss-EM10C Company). For analysis of the silver nanoparticles conjugated antibody stability FT-IR model Nexus IR 670, Thermo Scientific, was used. The Raman scattering light was recorded with a X100 microscope objective and in the spectral range of 600-3000 $cm^{-1}$ and by a Almega Thermo Nicolet Dispersive Raman Spectrometer with a second harmonic @532 nm of a Nd:YLF laser with apower of 100 mW.

## RESULTS

To prepare SERS probe silver nanoparticles , as Raman enhancement substrates, spherical shapes with an average diameter of about 50 nm (as measured by DLS Fig. 1) were synthesized as determined by TEM image (Fig. 2).

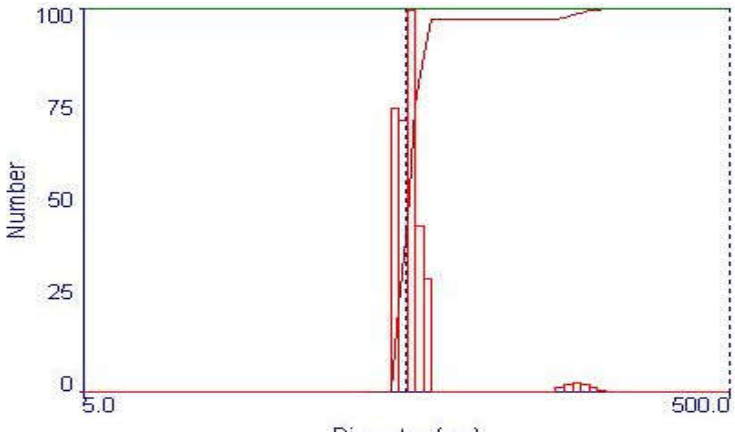


Fig. 1. Average diameters of the silver nanoparticles.

As shown in Fig. 2, the shapes of nanoparticles are very smooth. This is because, NABH4 was kept cooled during nanoparticles synthesis. The silver nanoparticles were functionalized with pMBA molecules due to introduction of carboxyl groups in order to be conjugated with antibodies through amid reactions.

The successful conjugation of antibody molecules to the surfaces of silver nanoparticles was confirmed by FT-IR (Fig. 3). The scanning process was conducted on the wave number spectrum range of 400-4000 $cm^{-1}$.

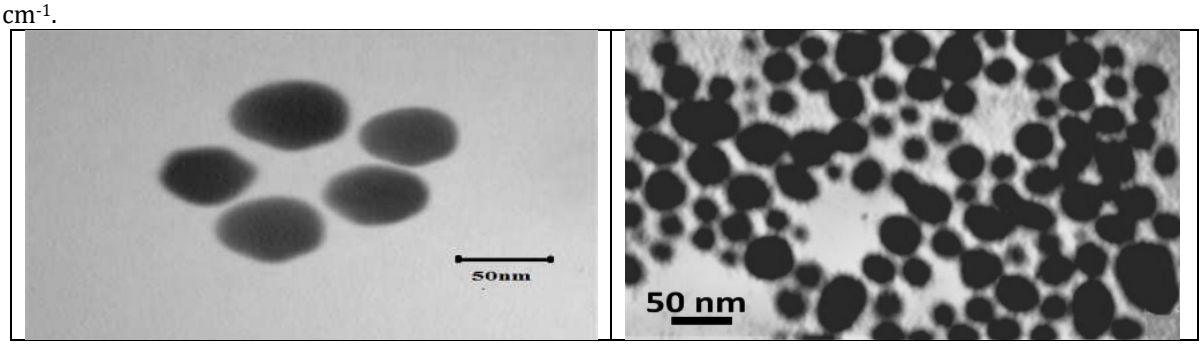


Fig. 2. Transmission electron microscope images of synthesized silver nanoparticles with different magnifications.

The FTIR spectra recorded for Ag nanoparticles, pMBA, pMBA coated Ag nanoparticles, Herceptin anti-body and also anti-body conjugated nanoparticles are shown in Fig.3, from bottom to top, respectively. In Ag nanoparticles the band observed at 1641 $cm^{-1}$ is related to metal-carbonyl vibration and confirms the presence of citrate groups on the surface of nanoparticles.

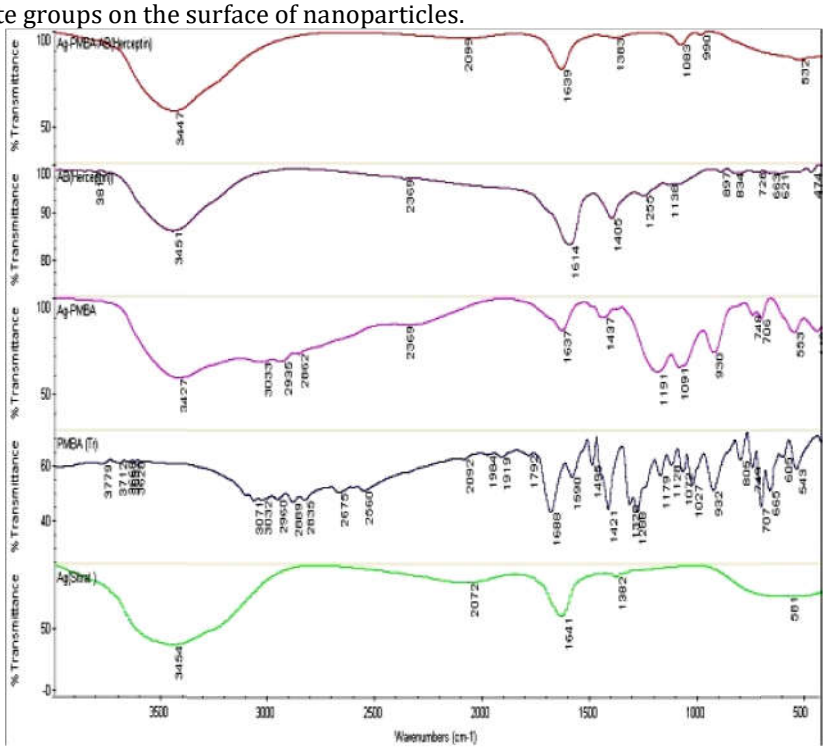


Fig. 3. FT-IR spectra (from bottom to top) of: A) Ag nanoparticles B) pMBA C) Ag-pMBA D) Herceptin antibody E) Antibody conjugated silver nanoparticles.

pMBA spectrum has three sets of peaks associated with aromatic ring, carboxyl group and finally thiol group. Characteristic peaks related to aromatic ring (C-C stretches) are observed in 1421 and 1590 $cm^{-1}$, C-H stretch is detected in 3071 $cm^{-1}$ and finally peaks on 805 and 1919 $cm^{-1}$ are the clear evidences for para substitution in this compound. The stretching vibration of the carbonyl group in carboxyl group is located in 1688 $cm^{-1}$, conjugation with a double bond or benzene ring lowers this stretching frequency in comparison with normal condition, C-O stretch is observed in 1320 $cm^{-1}$. In addition, a weak stretch band located in 2560 $cm^{-1}$ is correlated to thiol group. In the anti-body spectrum the peaks located at 1405 and 1614 $cm^{-1}$ are correlated to amide 1 and amide 2, respectively. The absence of S-H stretch band in the pMBA coated Ag nanoparticles spectrum confirms the bonding of pMBA to the surface of Ag

nanoparticles, Characteristic peaks related to aromatic ring remain intact in this spectrum. As we expected in the anti-body conjugated nanoparticles spectrum the prominent peaks belong to anti-body due to their high molecular weight. Anti-body conjugation via EDC-NHS protocol could result in formation of new amide bands which finally cause a shift in amide 1 and amide 2 characteristic peaks. These new peaks are located in 1383 and 1639 $cm^{-1}$. One of the disadvantages of using FT-IR is a broad peak at high wavenumbers (~3500 $cm^{-1}$) caused by O-H from water [13].

The Zeta potential of nanoparticles without antibody (Table 1) and with antibody (Table 2) are measured 4 times. The binding of antibody to nanoparticles causes an increase the zeta potential. It must be mentioned that the increase of zeta potential is because of positive charge of antibody of amino acids and is more than negative charge of amino acids. Binding of antibody to nanoparticles changes the average zeta potential from -17.66 to -12.93. This shift of 4.73 is very close to the reported value [14].

Table 1. Zeta potential of nanoparticles without antibody.

| Run | Mobility | Zeta Potential (mV) |
|---|---|---|
| 1 | -1.41 | -18.02 |
| 2 | -1.52 | -19.45 |
| 3 | -1.31 | -16.78 |
| 4 | -1.28 | -16.41 |
| Average | -1.38 | -17.66 |
| SD | 0.05 | 0.69 |

Table 2. Zeta potential nanoparticles with antibody.

| Run | Mobility | Zeta Potential (mV) |
|---|---|---|
| 1 | -0.82 | -10.46 |
| 2 | -1.16 | -14.86 |
| 3 | -0.85 | -10.82 |
| 4 | -1.22 | -15.57 |
| Average | -1.01 | -12.93 |
| SD | 0.01 | 1.33 |

The UV/Visible extinction spectra of the pure silver nanoparticles and the antibody-conjugated SERS probe are shown in Fig. 4.

The spectrum of the pure silver nanoparticles showed a maximum absorption at 412 nm due to plasmon resonance. The antibody-conjugated SERS probe showed a slight increase of the maximum absorption peak and a red shift of ~4 nm.

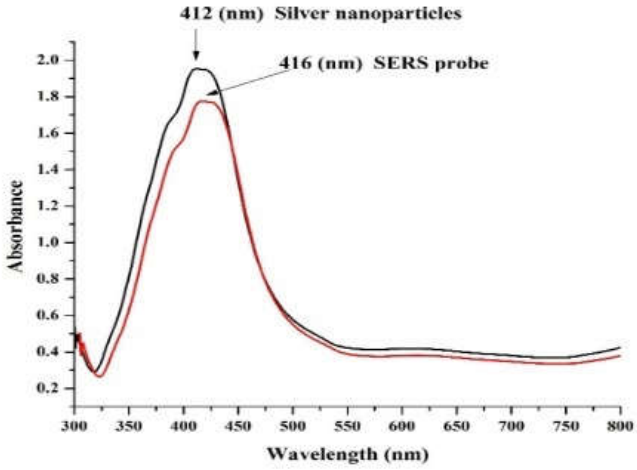


Fig. 4. The extinction spectra of silver nanoparticles (black line) and SERS probe (red line).

In this study, we used pMBA molecules as a bridge to link silver nanoparticles and antibody molecules. In order to find an optimum aspect ratio for a maximum SERS effect, SERS spectra of 7µg/µl anti-HER2 monoclonal antibody (Herceptin) was measured. Furthermore, the SERS spectra of the antibody conjugated were measured. The concentration of the pure silver nanoparticles and the antibody-conjugated SERS probe was 7 µg/mL.

the intensity of the SERS signal of the antibody conjugated rises tremendously in compared with antibody in normal conditions. Spontaneous Raman spectrum (middle spectrum) has expanded by 100 times (Fig.5).

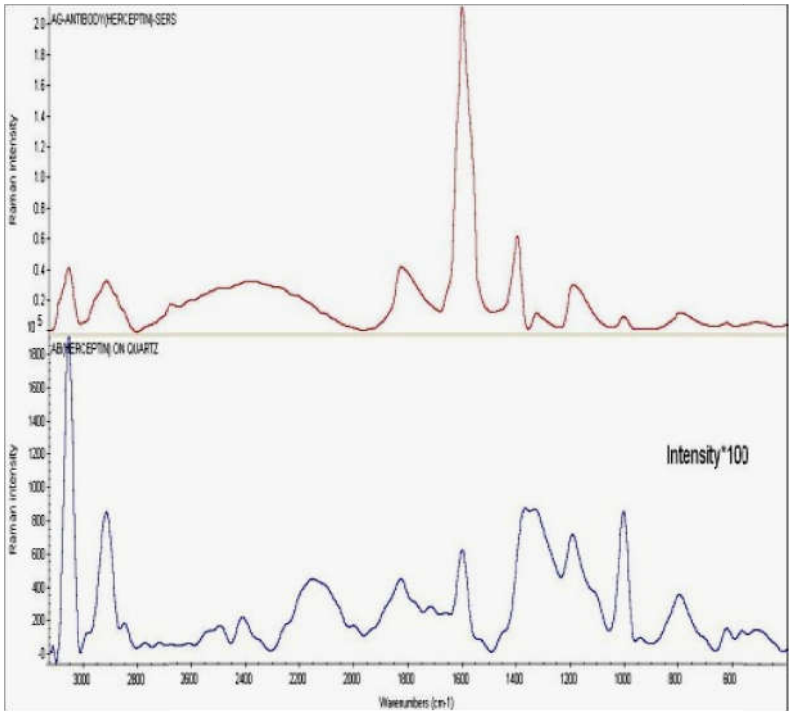


Fig. 5. Spontaneous Raman scattering of Herceptin (on quartz) (bottom spectrum) and SERS spectrum of silver nanoparticles and the antibody-conjugated SERS probe of Herceptin (top spectrum).

We calculated the enhancement factor (EF) by using the expressions (15):

$$EF = \frac{I_{Surf}/N_{Surf}}{I_{Raman}/N_{Raman}} = \frac{I_{Surf}}{N_{Surf}} \times \frac{N_{Raman}}{I_{Raman}}$$

The value of EF is about $\mathbf{1 \times 10^5}$

## CONCLUSION

We have used pMBA as a linker between silver nanoparticles and humanized monoclonal antibody Herceptin and optimized the antibody-conjugated SERS probe. We showed that the SERS spectrum of the antibody conjugated SERS probe intensity tremendously increases compared to antibody alone. It suggests the using of our setup to investigate how the antibody-conjugated SERS probe can be internalized within HER2-overexpressing cells. In this study, we demonstrated an enhancement factor of $10^5$ in intensity by investigating the recorded spontaneous and the SERS spectra.

## REFERENCES

1. Jeanmaire DL, Van Duyne RP. [1997]. Surface Raman spectroelectrochemistry: Part I. Heterocyclic, aromatic, and aliphatic amines adsorbed on the anodized silver electrode. Journal of Electroanalytical Chemistry and Interfacial Electrochemistry, 84(1):1-20.
2. Vo-Dinh T, Hiromoto M, Begun G, Moody R. [1984].Surface-enhanced Raman spectrometry for trace organic analysis. Analytical Chemistry, 56(9):1667-70.
3. Schatz GC. [1984]. Theoretical studies of surface enhanced Raman scattering. Accounts of Chemical Research, 17(10):370-6.
4. Kerker M. [1984].Electromagnetic model for surface-enhanced Raman scattering (SERS) on metal colloids. Accounts of Chemical Research, 17(8):271-7.
5. Sha MY, Xu H, Natan MJ, Cromer R. [2008].Surface-enhanced Raman scattering tags for rapid and homogeneous detection of circulating tumor cells in the presence of human whole blood. Journal of the American Chemical Society, 130(51):17214-5.
6. Le Ru E, Blackie E, Meyer M, Etchegoin PG. [2007].Surface enhanced Raman scattering enhancement factors: a comprehensive study. The Journal of Physical Chemistry C, 111(37):13794-803.
7. Haynes CL, McFarland AD, Duyne RPV. [2005]. Surface-enhanced Raman spectroscopy. Analytical Chemistry, 77(17):338 A-46 A.

8. Isola NR, Stokes DL, Vo-Dinh T. [1998].Surface-enhanced Raman gene probe for HIV detection. Anal Chem., 70(7):1352-1356.
9. Wabuyele MB, Vo-Dinh T. [2005]. Detection of human immunodeficiency virus type 1 DNA sequence using plasmonics nanoprobes. Anal Chem, 77(23):7810-7815.
10. Vo-Dinh T, Allain LR, Stokes DL. [2002]. Cancer gene detection using surface-enhanced Raman scattering (SERS). Journal of Raman Spectroscopy, 33(7):511-516.
11. Michota A, Bukowska J. [2003]. Surface-enhanced Raman scattering (SERS) of 4-mercaptobenzoic acid on silver and gold substrates. Journal of Raman Spectroscopy, 34(1):21-25.
12. Hashemifard N, Mohsenifar A, Ranjbar B, Allameh A, Lotfi AS, Etemadikia B. [2010]. Fabrication and kinetic studies of a novel silver nanoparticles-glucose oxidase bioconjugate. Analytica chimica acta, 675(2):181-184.
13. Gilany K, Pouracil RS, Sadeghi MR. [2014]. Fourier transform infrared spectroscopy: a potential technique for noninvasive detection of spermatogenesis. Avicenna journal of medical biotechnology, 6(1):47-52.
14. Jiang W, Kim BY, Rutka JT, Chan WC. [2008]. Nanoparticle-mediated cellular response is size-dependent. Nature nanotechnology, 3(3):145-150.
15. Liu Y, Xu S, Li H, Jian X, Xu W. [2011]. Localized and Propagating Surface Plasmon Co-Enhanced Raman Spectroscopy Based on Evanescent Field Excitation. Chem Commun (Camb), 47(13):3784-3786.