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

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ABSTRACT

We have developed surface-enhanced Raman spectroscopysubstrate composed of antibody-conjugated silver nanoparticles as a functional nanoprobe. In this study, we synthesized silver nanoparticles with the pMBA linker for binding to antibody Herceptin and 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 µm has been performed. Furthermore, the obtained SERS probe apparently indicated that the intensity of antibody-conjugated silver nanoparticles as a SERS sensitive probe is increased by an enhancement factor of 10^5.

Keywords: Silver nanoparticles, antibody conjugation, Raman scattering, spectroscopy, surface enhanced, SERS.

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الخلاصة

تصنيع وتوليف جسيمات مضادة نانوية للفضة باقتران هيرسبتين لاستطارة رامان ذو السطح المحسن .

في هذا البحث تم تطوير ركيزة لاستطارة رامان ذو السطح المحسن مكونة من جسيمات الفضة النانوية المضادة كمجس نانوي وظيفي،في هذه الدراسة تم تصنيع جسيمات الفضة النانوية باستخدام (pMBA) كرابط للجسيمات المضادة وتم تحليل الربط لجسيمات الفظة النانوية بواسطة جهاز(FTIR). النتائج بينت بشكل واضح الجسيمات النانوية للفضة باقطار (50 µm) علاوة على ذلك مجس سطح رامان المحسن بين بان جسيمات الفضة النانوية المضادة المقترنة قد حسنت حساسية المجس بمقدار 10^5.

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INTRODUCTION

Raman spectroscopy as a rapid, and non-destructive photonscattering 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 as a powerful analytical tool capable of chemical identification and biological species recognition. It can elucidate molecular structure and examine the surface properties. Unfortunately, the broader utility of Raman spectroscopy is restricted by the poor efficiency of Raman scattering. A 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 active investigation for its ability for biomedical diagnostics due to its high sensitivity, increased levels of multiplexing, robustness, and capability to perform detection in blood and other biological matrices (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 developedto large numbers such as 10^{11} times [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 this issue making SERS-active silver nanoparticles with great potential for intracellular bioanalysis and extracellular labeling (8-11). SERS-active plasmonic nanostructures have also been reported for photothermal treatment of shallow tumors (12-14).

The human epidermal growth factor receptor 2 (HER2) protein and the HER2/neu oncogene have been shown to be involved in cell proliferation and survival. Amplification of HER2/neu gene is present occurs in around 20% to 25% of human breast cancers and is contributed to a more aggressive disease course and poor prognosis.

HER2 amplification status has been reported to be responsible for resistance to definite endocrine and chemotherapeutic agents. Patients with HER2+ breast cancer need specific treatments that can affect HER2 activity such as using monoclonal antibodies [e.g., trastuzumab (Herceptin)] and small molecule tyrosine kinase inhibitors (e.g., lapatinib). Therefore, assessment of HER2 status is pivotal in therapeutic decision.

In the present study, we have optimized and synthesized silver nanoparticles with the linker pMBA for conjugation to a humanized anti her2/neu monoclonal antibody (Herceptin) as a SERS probe. It is the main goal of this paper to demonstrate the feasibility of antibody conjugated Ag for the highly sensitive SERS.

Materials and Methods

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. Water used in the experiments was ultrapure deionized water.

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 (15). 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

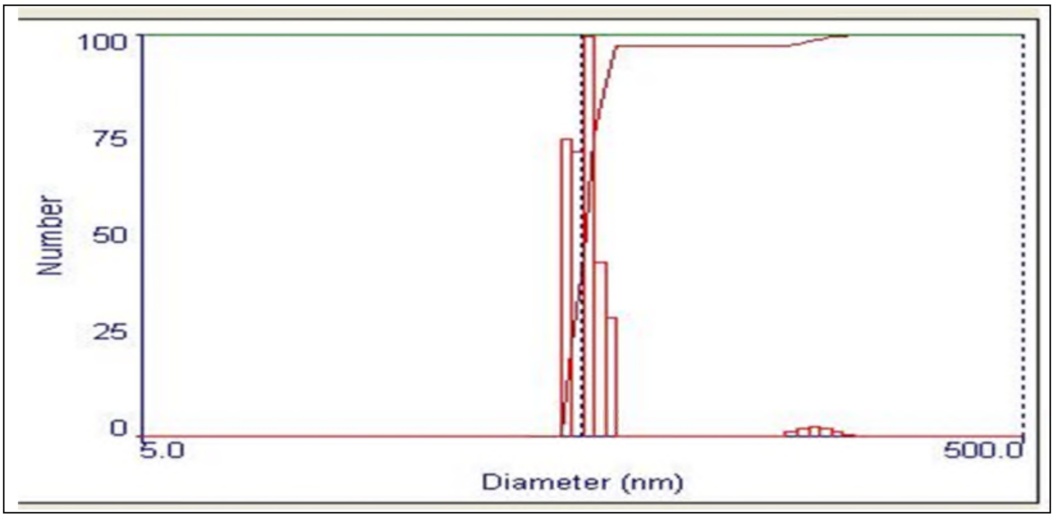
A 10-mL solution of Ag nanoparticles was mixed with pMBA solution (100 μL, 1 mM in ethanol) and then stirred for 3 h. The color of Ag reaction with pMBA changes from greenish yellow to brown. Afterwards, the solutions of EDC (10 mL, 10 mM) and NHS (1 mL, 100 mM) were added into the pMBA coated silver 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 and discussion

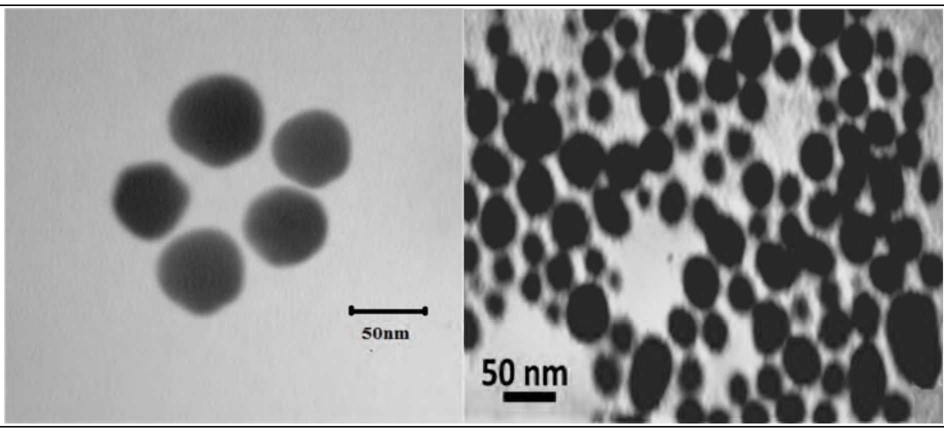
To prepare SERS probe, silver nanoparticles with an average diameter of about 50 nm (measured by DLS Fig. 1) were synthesized as the Raman enhancement substrate, as shown in the TEM image (Fig.2).



Figure(1). Average diameters of the silver nanoparticles.

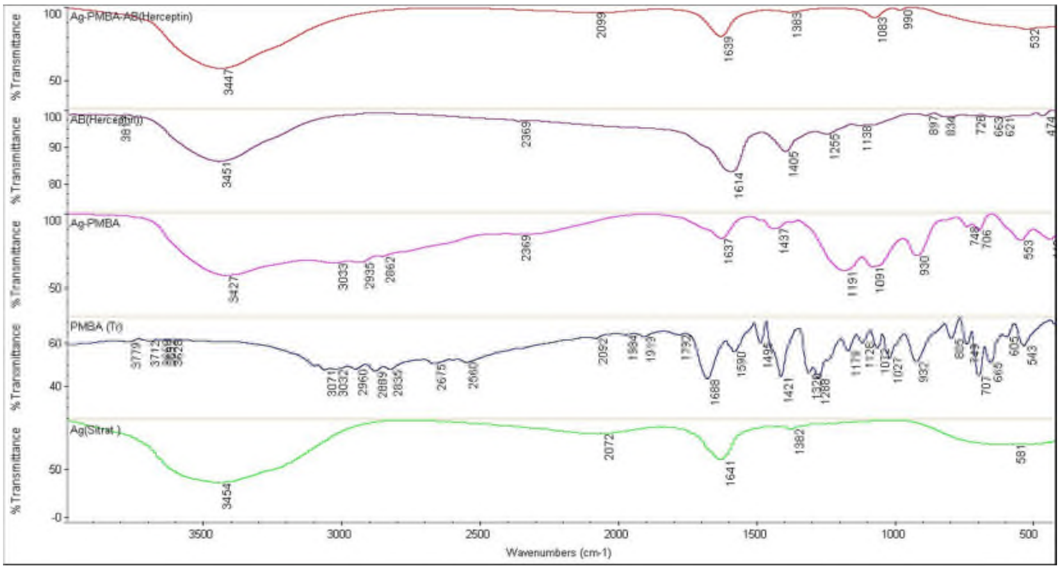
As shown in Fig.2, the shapes of nanoparticles are very smooth. This is because NABH4 was keept cooled during nanoparticles synthesis.The silver nanoparticles were functionalized with pMBA molecules to introduce carboxyl groups in order to be conjugated with antibodies through amid reactions.

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 wavenumber spectrum range of 400-4000 cm^{-1}.



Figure(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.



Figure(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 located in 1421 and 1590 cm^{-1}, C-H stretch is observed 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}. 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 assigned 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 leads to formation of new amide bands which finally cause a shift in amide 1 and amide 2 characteristic peaks. This new peaks are located in 1383 and 1639 cm^{-1}. One of the disadvantages of using FT-IR is that it gives a broad peak at high wavenumbers (~3500 cm^{-1}) caused by O-H from water (16).

The Zeta potential of nanoparticles without antibody (Table 1) and with antibody (Table 2) is measured 4 times. As seen in tables the binding of antibody to nanoparticles causes the zeta potential to rise. 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 of Jian et al. (17).

Table(1). Zeta potential of nanoparticles without antibody.

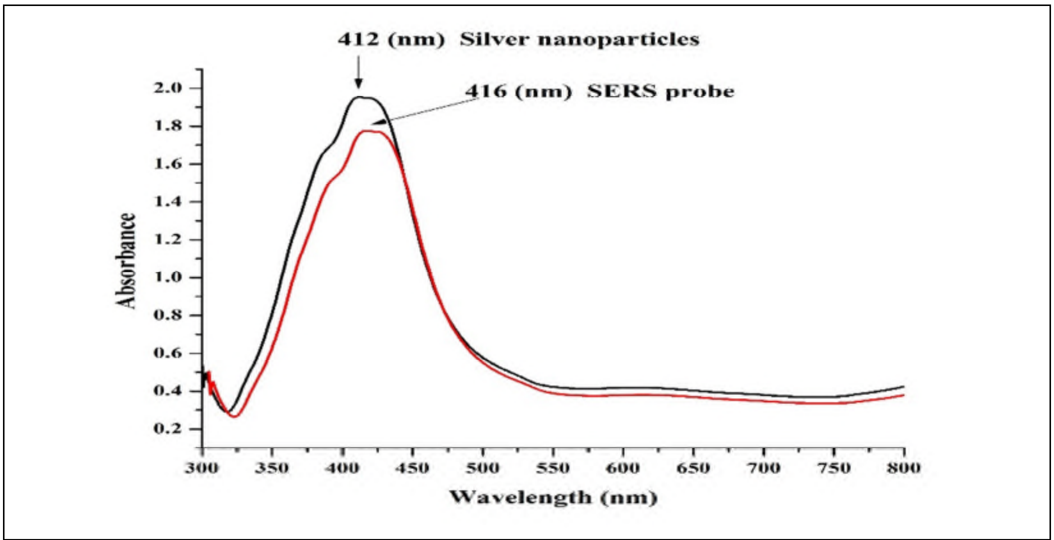
Run	Mobility	Zeta Potential (mV)
1	-1.41	-18.02
2	-1.52	-19.45
3	-1.31	-16.78
4	-1.28	-16.41
Average	-1.38	-17.66
SD	0.05	0.69

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.



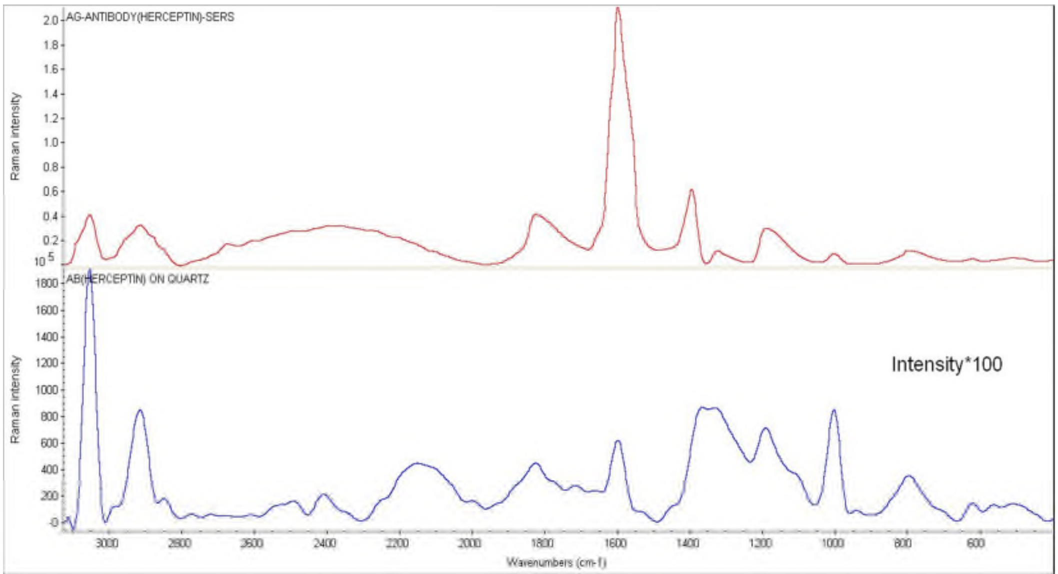
Figure(4). The extinction spectra of silver nanoparticles (black line) and SERS probe (red line).

Our SERS probe used pMBA molecules as a bridge to link silver nanoparticles and antibody molecules, resulting in a much simplified structure, as has been described before. 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.

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

Conclusion

We have used pMBA as linker between silver nanoparticles and humanized monoclonal antibody Herceptin. We have optimized the antibody-conjugated SERS probe. We showed that the SERS spectrum of the antibody conjugated SERS probe intensity tremendously increases compare to antibody alone. We suggest using our setup to investigate how the antibody-conjugated SERS probe can be internalized within HER2-overexpressing cells. By investigating the recorded spontaneous and the SERS spectra, we believe an enhancement factor of 10^5 has been achieved in this study.



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

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