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Investigation of Magnetic Field enriched Surface Enhanced Resonance Raman Scattering Performance Using Fe$_3$O$_4$@Ag Nanoparticles for Malaria Diagnosis

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**ABSTRACT**

Recently, we have demonstrated the magnetic field-enriched surface-enhanced resonance Raman spectroscopy (SERRS) of $\beta$-hematin by using nanoparticles with iron oxide core and silver shell (Fe$_3$O$_4$@Ag) for the potential application in the early malaria diagnosis. In this study, we investigate the dependence of the magnetic field-enriched SERRS performance of $\beta$-hematin on the different core and shell sizes of the Fe$_3$O$_4$@Ag nanoparticles. We note that the core and shell parameters are critical in the realization of the optimal magnetic field-enriched SERRS $\beta$-hematin signal. These results are consistent with our simulations that will guide the optimization of the magnetic SERRS performance for the potential early diagnosis in the malaria disease.

**Keywords:** Raman, surface enhanced Raman scattering (SERS), hemozoin, resonance Raman, hematin, malaria, discrete dipole approximation (DDA)

1. **INTRODUCTION**

We have previously demonstrated the development of magnetic field-enriched surface enhanced resonance Raman spectroscopy (SERRS) for early malaria diagnosis.$^1$ This magnetic field-enriched SERRS strategy dramatically enhanced the Raman spectra of $\beta$-hematin, which is equivalent to hemozoin (a malaria biomarker generated by malaria parasites) in Raman features, by using the nanoparticles with iron oxide core and silver shell (Fe$_3$O$_4$@Ag) enable SERRS. Although the plasmonic properties related to the different core and shell sizes of other types of nanoparticles have been examined in the literature,$^2$ the modified SERRS performance of these nanoparticles under an external magnetic field has never been evaluated before. Moreover, the paramagnetic $\beta$-hematin crystal under investigation in this strategy is much larger than the commonly used test molecules in surface-enhanced Raman spectroscopy such as Rhodamine 6G that are non-magnetic, which further complicate the optimization of nanoparticles for SERRS.

In this work, we investigate the dependence of magnetic field enriched SERRS performance of $\beta$-hematin on the core and shell sizes of Fe$_3$O$_4$@Ag nanoparticles. We note that Raman enhancement is improved in Fe$_3$O$_4$@Ag with a larger Fe$_3$O$_4$ core and a thicker Ag shell in the absence of a magnetic field. On the contrary, in the presence of an external magnetic field, Fe$_3$O$_4$@Ag with a smaller Fe$_3$O$_4$ core and a thinner Ag shell lead to the formation of more aggregates and hot spots that render a stronger Raman enhancement. These results are consistent with our simulations that will guide the optimization of magnetic SERRS for early malaria diagnosis.

2. **MATERIALS AND METHODS**

2.1 Core-shell Fe$_3$O$_4$@Ag nanoparticles synthesis

A coprecipitation method was used to fabricate the Fe$_3$O$_4$ nanoparticles.$^3$ NaOH at concentration of 4M was added drop-wise to the dissolved mixture of FeCl$_2$·4H$_2$O and FeCl$_3$·6H$_2$O at concentrations of 0.168 M and 0.333 M, respectively, at 60°C under vigorous mixing. The introduction of NaOH was ceased at pH values of 11, 13, and 14 to create different sizes of Fe$_3$O$_4$, followed with another 1-hour heating at 70°C under stirring.

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After cooled to room temperature, the nanoparticles were washed with deionized water after segregated from the mixture by using magnet. Next, 0.15 g of polyacrylic acid in 80 ml of ethanol was used to graft the aforesaid Fe$_3$O$_4$ nanoparticles in 20 ml ethanol at a concentration of 16.2 mM. This mixture was sonicated for 15 min and then the Fe$_3$O$_4$ nanoparticles were isolated from the mixture by using a magnet. The isolated Fe$_3$O$_4$ nanoparticles were washed and re-dispersed in ethanol and deionized water at a concentration with volume percent ratio of 80.6:19.4%. A seed-growth reduction method was used to fabricate Ag shell. In order to synthesize Ag shells of different thicknesses, we performed the followings in an ultrasonic bath to the aforesaid Fe$_3$O$_4$ nanoparticles suspension:

Set 1. Drop-wise AgNO$_3$ at concentration of 2.8 mM was added and mixed for 30 min, followed with the addition of 4.1 mM hydroxylamine hydrochloride, 8.1 mM NaOH, in triton X-100, ethanol, and deionized water at a concentration with volume percent ratio of 0.9:70.8:28.3 %;
Set 2. Additional 9.7 mM of AgNO$_3$ was added to set 1, in triton X-100, ethanol, and deionized water at a concentration with volume percent ratio 2:65.3:32.7%;
Set 3. 19.4 mM instead of 9.7 mM of AgNO$_3$ was used during the second-time AgNO$_3$ addition in the procedures as mentioned set 2;
Set 4. Same set 3 procedures were carried, except that an additional 9.7 mM was added into the mixture, prior to the introduction of triton X-100, ethanol, and deionized water.

Lastly, the Fe$_3$O$_4$@Ag nanoparticles were isolated by using a magnet and resuspended in 15 ml of methanol.

2.2 Raman measurements

A 785-nm laser was used to analyze all β – hematin samples at an excitation power of 0.1 mW by using the above Fe$_3$O$_4$@Ag nanoparticles with a magnetic field of 0.198T and a magnetic field gradient of 26.6 Tm$^{-1}$ as stated in our other publication. An integration time of 10 s and a spectral resolution of 2 cm$^{-1}$ was employed for each acquired spectrum.

2.3 Discrete dipole approximation (DDA)

We calculated the electromagnetic field ($|E|^2$) distributions and the extinction efficiency ($Q_{ext}$) values of the Fe$_3$O$_4$@Ag nanoparticles with different core sizes and shell thickness using discrete dipole approximation (DDA) model.$^4$ We employed the DDA model since the technique is more efficient and flexible (comparing to the multiple multipole method and the finite difference time domain method).$^5$ The Amsterdam DDA code$^6$ was used for all the DDA calculations in this paper. All volumes under investigation were assumed to be comprised of only Fe$_3$O$_4$, Ag and β – hematin only with electric constants$^7, 8, 9$ obtained from the literature. $Q_{ext}$ and $|E|^2$ of the Fe$_3$O$_4$@Ag nanoparticles with different core and shell parameters were calculated to evaluate against the experimental SERS performance, based on the fact that $Q_{ext}$ and $|E|^2$ are closely correlated to the SERS signals.

3. RESULTS
We characterized the SERRS performance of the fabricated Fe₃O₄@Ag nanoparticles with different dimension C₄₀S₆₀, C₄₀S₅₀, C₄₀S₄₀, C₄₀S₁₀, C₂₅S₆₀, C₂₅S₄₅, C₂₅S₂₅, C₂₅S₁₀, C₁₀S₅₀, C₁₀S₄₀, and C₁₀S₃₀, in which the first number indicates the Fe₃O₄ core radius and the second number gives the shell thickness. Figure 1 shows the SERRS spectra of β-hematin (a) without and (b) with the influence of an external magnetic field by using different Fe₃O₄@Ag nanoparticles. Prominent Raman peaks were observed at 754, 1120, 1570, and 1628 cm⁻¹, which correspond to ν₁₅ (D₄h notation system for resonance Raman peaks studies on myoglobin), ν₂₂, ν₂, and ν₁₀, respectively. We note that the SERRS performance trend [Figure 1(a)], which has a greater SERRS signal with an increase in the core size and shell thickness in the Fe₃O₄@Ag nanoparticles, is dissimilar from that with the magnetic field enrichment [Figure 1(b)].

Figure 1. Experimental SERRS spectra of β-hematin at (a) concentration 0.5 mM without magnetic-field enrichment and (b) concentration of 0.5 μM with magnetic-field enrichment by using Fe₃O₄@Ag nanoparticles of different core/shell parameters.

Figure 2. Calculated extinction efficiencies of Fe₃O₄@Ag nanoparticles with Fe₃O₄ core radius ranging from 5 nm to 50 nm and Ag shell thickness ranging from 10 nm to 70 nm using the DDA model at unpolarized 633-nm wavelength excitation.

Figure 2. Calculated extinction efficiencies of Fe₃O₄@Ag nanoparticles with Fe₃O₄ core radius ranging from 5 nm to 50 nm and Ag shell thickness ranging from 10 nm to 70 nm using the DDA model at unpolarized 633-nm wavelength excitation.
We first employ DDA to calculate the extinction efficiency ($Q_{ext}$) of Fe$_3$O$_4$@Ag nanoparticles with Fe$_3$O$_4$ core radius ranging from 5 nm to 50 nm and Ag shell thickness ranging from 10 nm to 70 nm (Figure 2) since SERS intensities and the extinction efficiencies are related to the induced electromagnetic field, which is also applicable to other plasmonic structures. The overall trend of the graph shows that $Q_{ext}$ value increases with the increase in the core radius and the shell thickness. This observation is consistent with $\beta$–hematin SERRS in the case of without magnetic enrichment [Figure 1(a)]. The extinction efficiencies can predict the enhancement trends in the case of without an external magnetic field. In contrast, $\beta$–hematin SERRS in the case of magnetic-field enrichment [Figure 1(b)] gives a different trend.

One of the possible factors that modify the SERRS trends in the magnetic field-enriched SERRS performance could be attributed to aggregation of Fe$_3$O$_4$@Ag nanoparticles and $\beta$–hematin. As for example, the extinction efficiency of the Fe$_3$O$_4$@Ag dimer (Figure 3) is different from a single Fe$_3$O$_4$@Ag nanoparticle (Figure 2). This further augmentation effect is noted even if the Fe$_3$O$_4$@Ag nanoparticles are separated from each other by a $\beta$–hematin crystal of 40 nm (Figure 4). More details of this study can be found in our paper.3

![Graph showing extinction efficiencies of Fe$_3$O$_4$@Ag nanoparticles in dimer configuration with Fe$_3$O$_4$ core radius ranging from 5 nm to 50 nm and Ag shell thickness ranging from 10 nm to 70 nm at unpolarized 633-nm wavelength excitation.](image)

Figure 3. Extinction efficiencies of Fe$_3$O$_4$@Ag nanoparticles in dimer configuration with Fe$_3$O$_4$ core radius ranging from 5 nm to 50 nm and Ag shell thickness ranging from 10 nm to 70 nm at unpolarized 633-nm wavelength excitation.

![Diagram showing $|E|^2$ field distribution for two Fe$_3$O$_4$@Ag (C25S25) with a gap excited by vertically polarized 633-nm excitation, ranging from 5 nm to 40 nm, using the refractive index of $\beta$–hematin for the surrounding medium. White circles demarcates locations of nanoparticles and number inside indicates the maximum $|E|^2$ intensity.](image)

Figure 4. $|E|^2$ field distribution for two Fe$_3$O$_4$@Ag (C25S25) with a gap excited by vertically polarized 633-nm excitation, ranging from 5 nm to 40 nm, using the refractive index of $\beta$–hematin for the surrounding medium. White circles demarcates locations of nanoparticles and number inside indicates the maximum $|E|^2$ intensity.
4. CONCLUSIONS

In conclusion, we have investigated theoretically and experimentally the magnetic field enriched SERRS phenomenon of $\beta-$hematin by using Fe$_3$O$_4$@Ag with different core sizes and shell thicknesses. These results show that the magnetic field enriched SERRS by using Fe$_3$O$_4$@Ag shows great potential for early malaria diagnosis.

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