

LIMIT OF DRUG DETECTION WITH SURFACE-ENHANCED RAMAN SPECTROSCOPY FOR CARDIOVASCULAR TREATMENT

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Introduction

Clopidogrel Bisulfate (CL) commercially available as Plavix is a thienopyridine class inhibitor of P2Y₁₂ ADP platelet receptors. Chemically it is methyl (+)-(S)- α -(2-chlorophenyl)-6,7-dihydrothieno[3,2-c]pyridine-5(4H)-acetate sulfate (1:1) [1]. CL has been shown to prevent ischemic stroke, myocardial infarction and vascular disease and demonstrated clinical efficacy superior to that of aspirin [2]. Thus, CL is indicated for the reduction of atherosclerotic events in patients with atherosclerosis documented by recent stroke, myocardial infarction, or cardiovascular disease [2]. This article presents the Surface Enhanced Raman Scattering (SERS) of CL molecule-absorbed Au nanoparticles as the drug detection for cardiovascular treatment.

Experiment

Au nanoparticles with relatively big sizes were prepared by the reduction of gold by sodium citrate [3]. After a mixture solution of 1 mM of H₂AuCl₄ is refluxed with vigorous stirring for ~30 minutes in 550 ml of water, 50 ml of 12 mM sodium citrate is rapidly inserted and stirred for additional 30 minutes to obtain Au nanoparticles with radii of ~16.8 nm. After the reaction solutions were reached room temperature, the residual solutions were filtered through 0.45- μ m polymer membrane filters. The resulting solutions of colloidal particles were characterized by an UV-Vis spectrometer (Agilent 8453). Finally, the Au concentration was estimated to be $\sim 1.03 \times 10^{-10}$ M using Mie's scattering theory [4]. A stock solution of Plavix (CL) was prepared by dissolving a 75 mg pill in 100 ml of 99.9 % ACS spectrophotometer grade methanol. The pH of the stock CL solution was

1.57 and the CL final concentration in the stock solution was 2.33mM. The structural formula of the drug is shown in Figure 1. For SERS experiments 200 μ l of Au solution was mixed with 500 μ l stock CL solution and 800 μ l of nanopure water. The final concentration of CL for SERS samples was 7.67×10^{-7} M. The vibration modes of CL were characterized with a Raman spectrometer (Inspector, DeltaNu) with excitation wavelength at ~785 nm with ~100-mW pump power. The signal was integrated for 3 sec, and was averaged 10 times for each measurement.

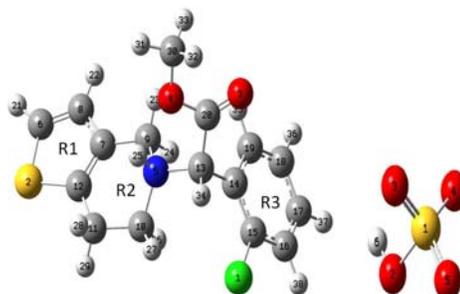


Fig. 1 Structure of CL molecule [5]. Yellow: Sulfur; green: Chloro atom; Light grey: Hydrogen; blue: Nitrogen; red: Oxygen and Grey: Carbon atoms.

Results and Discussion

The UV-Vis spectrum (Figure 2) of CL at pH~ 2 displays two peaks at ~271 and ~278 nm while the absorption spectra of the Au nanoparticles shows a localized surface plasmon resonance (LSPR) peak at ~530 nm which is modified after mixing with CL molecules. The appearance of a second LSPR peak at ~ 660 nm before and after deconvolution of CL_Au spectrum with the CL's implies that CL molecule promotes formation of dimer-like structures as seen for rhodamine molecules absorbed on silver surfaces [6]. The Raman and SERS spectra of CL

and CL_Au mixture are shown in Figure 3. The SERS enhancement factors (EF) for CL in contact with Au nanoparticles were estimated with:

$$EF = \left(\frac{I_{SERS}}{I_{Raman}} \right) \left(\frac{C_{Raman}}{C_{SERS}} \right) \left(\frac{1}{C_{Au}} \right) \quad (1)$$

where I_{SERS} and I_{Raman} are the signal intensities of SERS and Raman, C_{SERS} and C_{Raman} are the concentrations of CL for the SERS and Raman measurements, and C_{Au} is the concentration of Au nanoparticles.

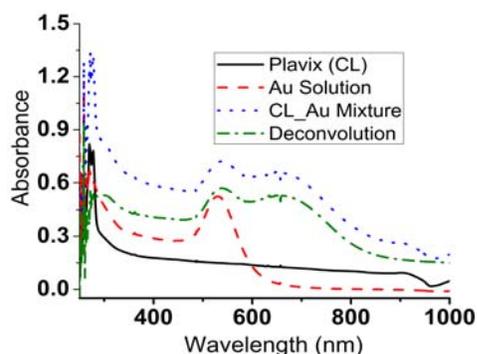


Fig. 2 Absorption spectra of CL solution (solid line); Au nanospheres in aqueous solution (---); CL mixed with Au solution (...) and its deconvolution with CL spectrum (-.-).

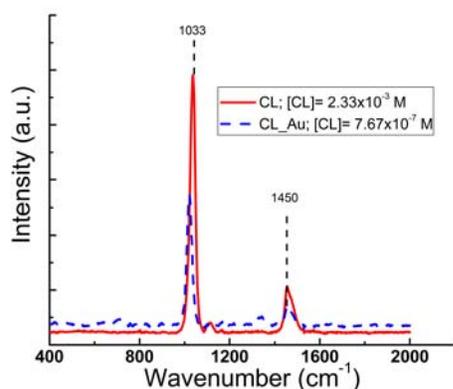


Fig. 3 Raman scattering spectrum of CL (solid line) and SERS spectrum for CL_Au mixture (dash line).

The EF were computed using two characteristic vibration modes of CL: the first one at $\sim 1033 \text{ cm}^{-1}$ which is assigned to the C–C and N–C stretching in the ring 2 of CL (in R2, Fig 1.) and the second vibration at $\sim 1450 \text{ cm}^{-1}$

which is assigned to the symmetric and asymmetric deformation of CH_3 (C30, Fig. 1) from O– CH_3 group. Using Eq (1), the EF was $\sim 3.14 \times 10^{13}$ at the vibration mode of $\sim 1033 \text{ cm}^{-1}$, and $\sim 2.5 \times 10^{13}$ at the vibration mode $\sim 1450 \text{ cm}^{-1}$.

Conclusion

SERS enhancement factor of the vibrational modes of CL at the proximity of Au nanoparticles was ~ 13 orders, which demonstrated as an excellent spectroscopic analysis technique for identifying the clopidogrel bisulphate in polymorphic mixtures for cardiovascular problems

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