Exploring metallic nanoparticles for enhanced multiplexed SERS for diagnostics.

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Exploring metallic nanoparticles for enhanced multiplexed SERS for diagnostics.

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Abstract. When it comes to diagnostics and disease management, while physicians focus on the prognosis and mortality caused by viral diseases, it is necessary to be thorough about metabolic chronic illnesses that could cause complications. Owing to its remarkable sensitivity and capability for multiplexing, Surface-Enhanced Raman Spectroscopy (SERS) emerges as a potent analytical approach with substantial promise in the realms of bioanalysis and diagnostics. This work focuses on exploring metallic nanoparticles that can be used for SERS-based pathogen and metabolic disorder-biomarkers detection for rapid viral infection and chronic disease diagnosis using SARS-CoV-2 or HIV pseudo-virus and a diabetes biomarker, glucose. Herein, metallic nanoparticles (NPs) such as gold (Au) and silver (Ag) nanoparticles, were assessed for their sensitivity in detecting both disease-biomarkers in a buffer containing HIV pseudo-virus and glucose.

1. Introduction

Infectious diseases contribute significantly to morbidity and mortality globally, with developing countries bearing a substantial burden where access to effective and affordable health care is limited [1]. According to the World Health Organization, lower respiratory infections, diarrhoeal diseases, tuberculosis (TB), human immunodeficiency virus (HIV) infection, and malaria are among the top five communicable diseases that account for most deaths globally [2]. Research indicates that several underlying health conditions such as diabetes, kidney diseases, hypertension, and cardiovascular disorders can exacerbate the severity and mortality rates in individuals with SARS-CoV-2 and HIV infections. This phenomenon may be attributed to the presence of ACE2 receptors in pancreatic β-cells, suggesting that SARS-CoV-2 could directly impact these cells, leading to glucose and metabolic dysregulation [3]. A significant study published in The Lancet Diabetes & Endocrinology, involving nearly 200,000 participants, revealed that individuals who contracted COVID-19 had an increased risk of developing diabetes within a year, even following a mild SARS-CoV-2 infection, compared to those who did not contract

the virus [4]. On the other hand, HIV infections have recently been associated with insulin resistance, hyperlipidaemia, and lipodystrophy [5-6]. While physicians focus on the prognosis and mortality of HIV and COVID-19 diseases, it is necessary to be thorough about metabolic chronic illnesses that could cause complications. Increasing plasma or urine glucose and creatinine values can indicate impaired metabolic functions. Rapid and dependable detection of multiple disease-related targets from a single biological sample is crucial for disease diagnosis and monitoring [7-8].

Disease diagnosis is often hindered by the intricate nature of biological samples, necessitating sample processing for accurate detection, and resulting in prolonged diagnostic procedures. Recent advancements in the Surface-Enhanced Raman Scattering (SERS) technique have facilitated the precise identification of biologically significant substances such as DNA and proteins. This capability holds promise for improving disease detection and management. Unlike conventional methods, SERS offers advantages like reduced sample preparation requirements and the capacity to identify target molecules directly in liquid samples. As illustrated in **Figure 1**, SERS shines in infectious disease diagnostics due to its ability to swiftly and accurately pinpoint biomarkers using their unique Raman fingerprints, derived from distinct Raman-active molecules within the analytes. [9].

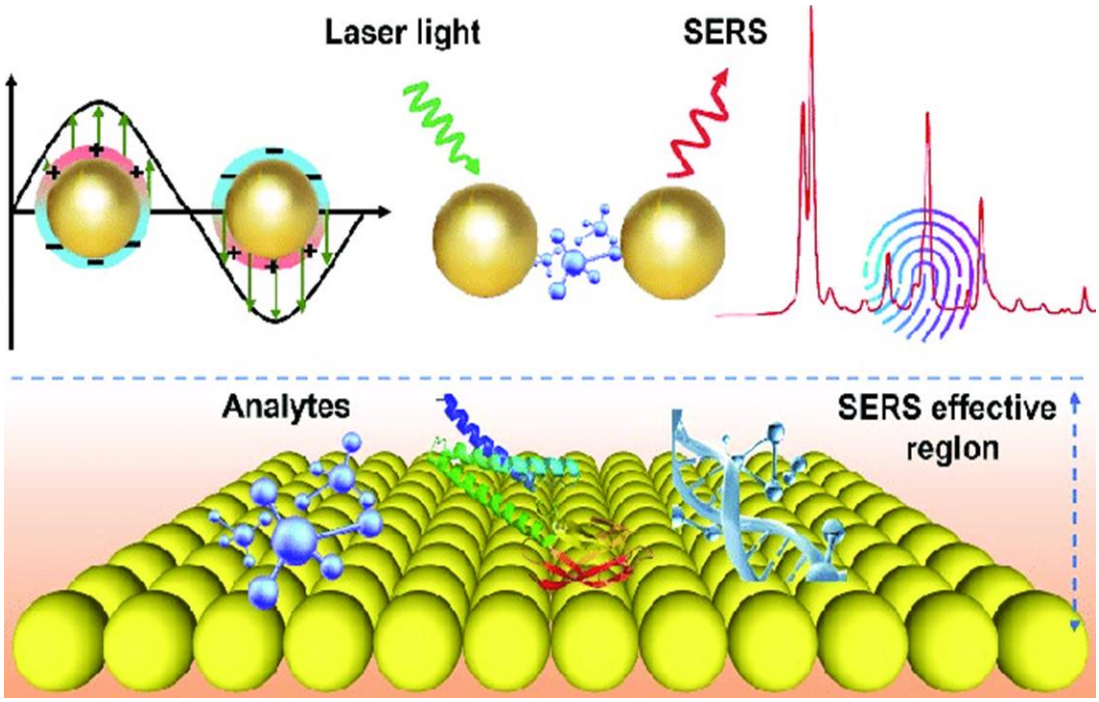

Figure 1. Schematic illustration of the mechanism of SERS for detection of analytes [8].

The primary issue with direct detection methods is the low concentrations and small Raman scattering cross-sections of biomarkers in clinical samples. This results in weak SERS signals and restricted sensitivity during disease diagnosis. To overcome this challenge, researchers have investigated various substrates for signal enhancement. In this study, electromagnetic enhancement (EM) effect using colloidal plasmonic metals like gold and silver nanoparticles was employed to boost the efficiency of Raman enhancement for detecting glucose and HIV

pseudovirus. This technique relies on enhancing light scattering within molecules attached to or combined with plasmonic metals [10].

2. Methods

2.1. Ethics Statement

This study was not performed in accordance with the Declaration of Helsinki because No human participants or tissues were used.

2.2. Preparation and characterization of nanoparticles

Citrate-stabilized AgNPs were prepared via the chemical reduction method using tri-sodium citrate [11], while AuNPs were synthesized using the sodium citrate reduction technique outlined by Igbal M., 2016 [12]. The nanoparticles were characterized using UV-visible spectroscopy (UV), Dynamic Light Scattering (DLS), and Transmission Electron Microscopy (TEM) analysis to determine their absorption wavelength, size surface charge, and shape/morphology, respectively. Samples were prepared in Phosphate Buffered Saline (PBS) and contained 2 mg/ml glucose and 10% HIV pseudovirus whilst AgNPs and AuNPs were added to samples at 20% Volume. Samples were deposited on gold-coated glass slides and analyzed using Raman as shown in **Figure 2.**

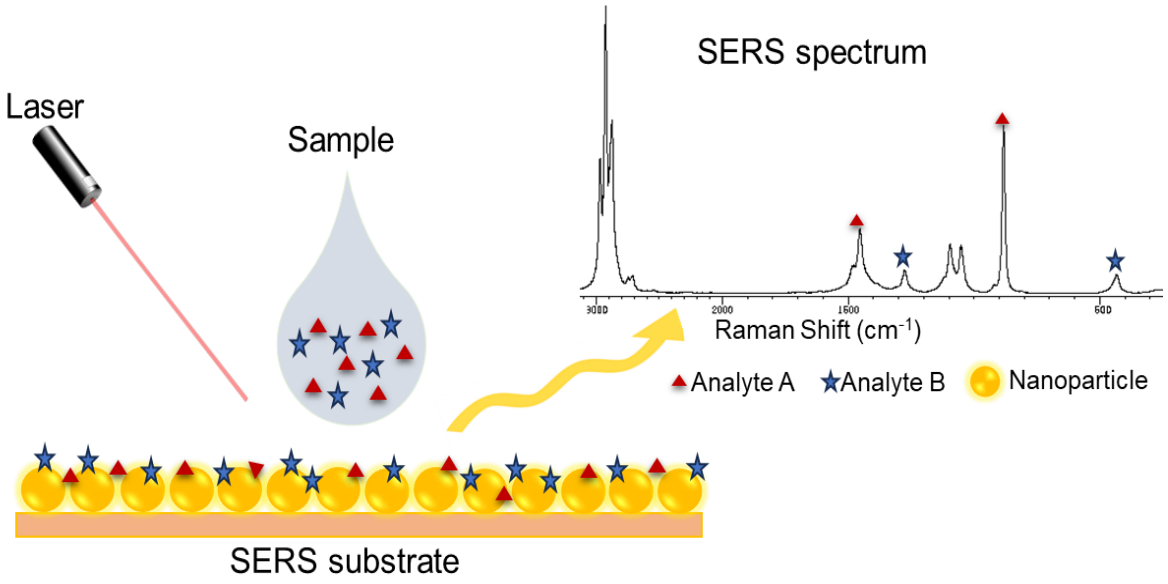

Figure 2. Schematic representation of sample preparation process and analysis.

3. Results and discussion

There is a strong need for practical and sensitive diagnostic techniques that can simultaneously detect multiple biomarkers (i.e., multiplex detection) linked to a specific disease, as the onset and progression of most diseases typically involve several biomarkers. In this study, the intrinsic surface-enhanced Raman spectroscopy (SERS)-based approach was adopted to establish the biochemical fingerprints of HIV pseudovirus, and glucose where metallic nanoparticles, gold and silver NPs, were explored as signal enhancers. Herein, nanoparticles were successfully developed by a simple chemical reduction method. The resulting AgNPs and AuNPs were characterized by UV-visible spectroscopy (UV), Dynamic Light Scattering (DLS), and

Transmission Electron Microscopy (TEM). The properties of the NPs are shown in **Figure 3** below. The UV absorbance for AgNPs was at 430 nm whilst for AuNPs at 520 nm. TEM analysis revealed that AgNPs and AuNPs are both spherical with size ranges below 100 nm and finally dynamic size and surface charge analysis by DLS shown in **Table 1**, confirmed that both NPs were below 100 nm (AuNPs at 58±3 nm and AgNps at 71±1 nm) and both NPs have negatively charged surfaces.

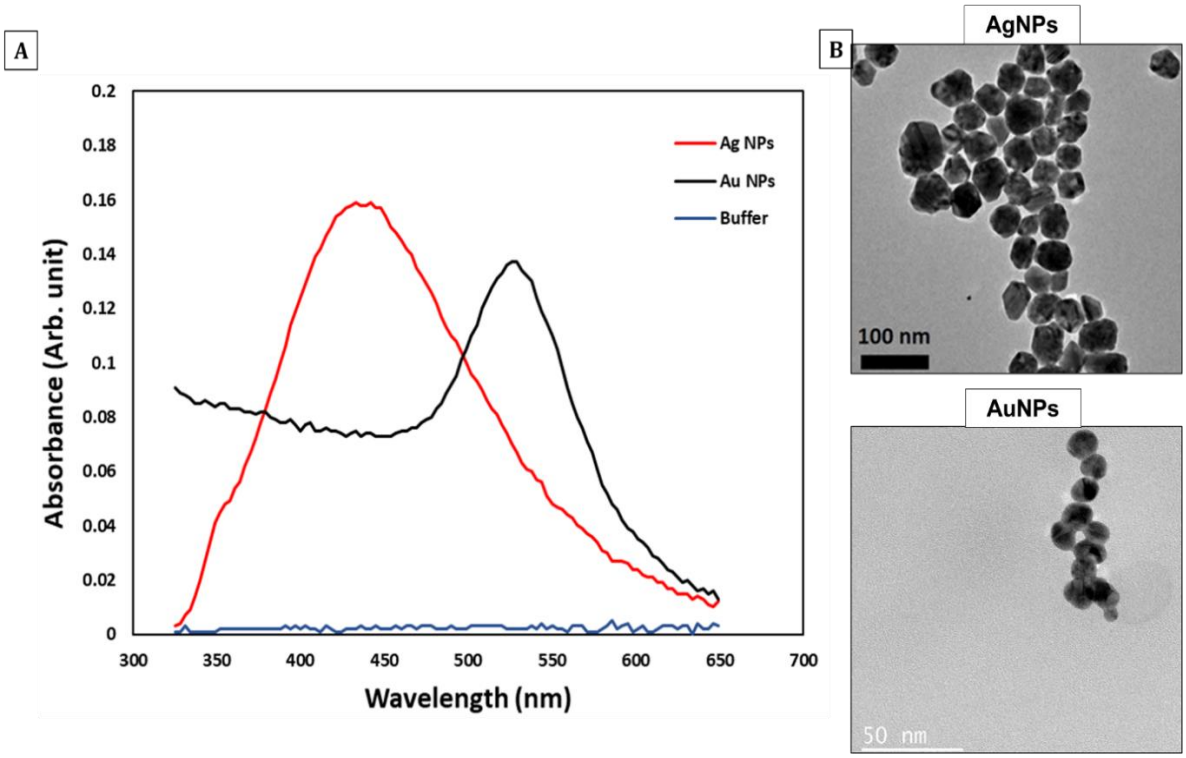

Figure 3. Characteristics of nanoparticles A) UV absorbance for AgNPs at 430 nm and for AuNPs at 520nm, B) TEM images showing AgNPs and AuNPs are both spherical with size ranges below 100nm.

Table 1. Size and surface charge analysis by DLS for both AgNPs and AuNPs showed that the NPs are smaller than 100 nm and are negatively charged.

Nanoparticle type	Size (nm)	Surface charge (ζm)
AgNPs	71+1	-12.7+0.3
AuNPs	58+3	-21.3+3.7

A comparison of Raman peaks for glucose only, HIV pseudovirus only, a combination of glucose and HIV pseudovirus only, and in the presence of Ag and Au NPs are depicted in **Figure 4A**. Raman shifts of key functional groups for the analytes; glucose and HIV pseudovirus were selected for peak area analysis and compared as shown in **Figure 4B**.

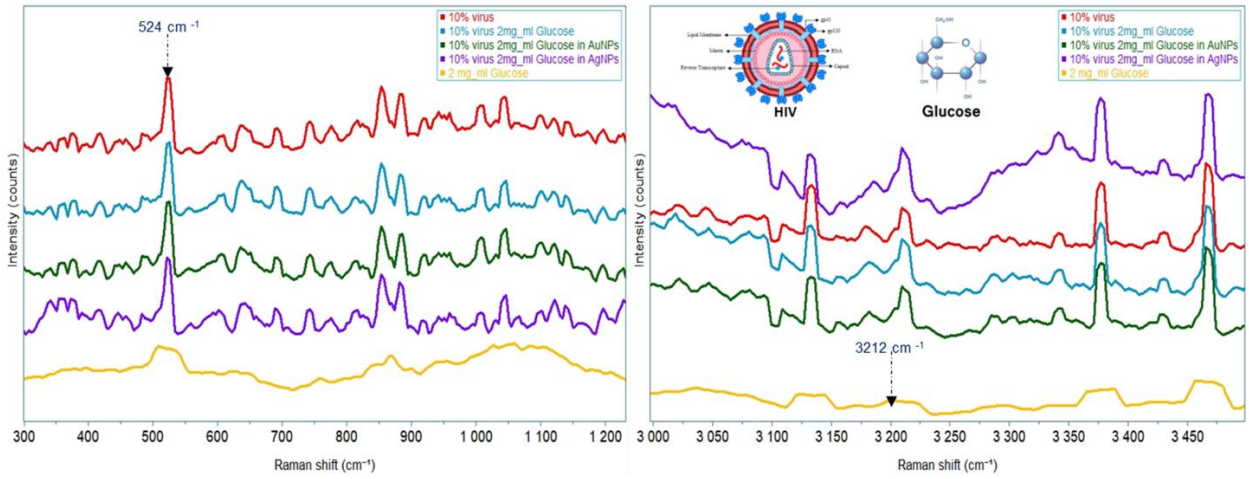

Figure 4A. Raman spectra of the analytes alone and in the presence of NPs.

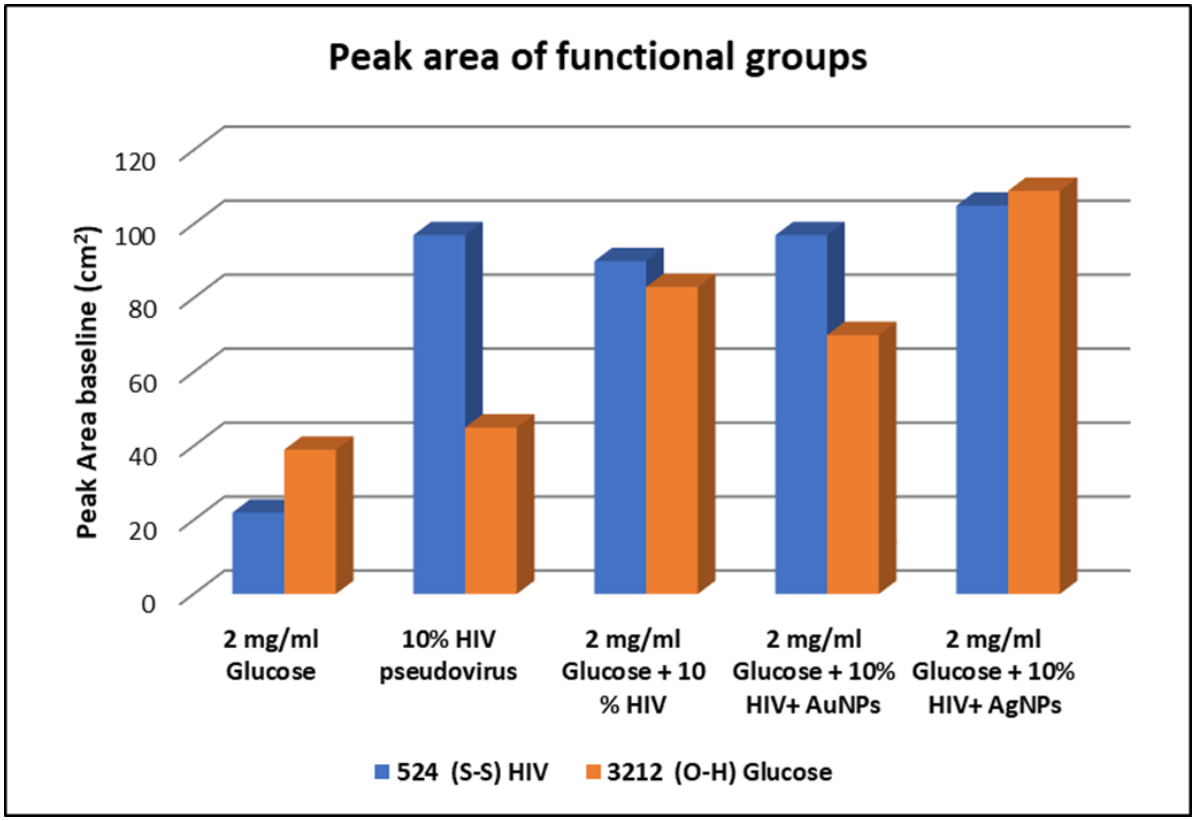

Figure 4B. Histogram of peak areas for selected key functional groups for the analytes.

The figures 4A and 4B, show that the disulphide bond at 524 cm-1 and the hydroxyl group at 3212, which are attributed to the HIV viral proteins and glucose molecules, respectively, increased significantly when analysed in the presence of nanoparticles. The peak area values show that overall, the AuNP signal was lower than the AgNPs, which suggests that the latter was more sensitive to the analytes compared to the former. These results are in agreement with the work done by Yadav *et al,* who reported label-free detection with direct adsorption of samples over Ag nanorods fabricated substrates. Moreover, the intensity of the identified peaks (534, and 1607 cm−1) in HIV-1 positive plasma samples increased with an increase in the viral load, an aspect that could be used in viral load quantification [13]. Whilst, Ceja-Fdez *et al* confirmed that colloidal SERS is an effective tool for measuring clinical concentrations of glucose, and it is three times more sensitive when nanoparticles such as gold NPs are used in conjunction with the method [14].

4. Conclusion

Successfully detected glucose and the HIV pseudovirus in a simple and rapid multiplexed-SERS assay. To evaluate the sensitivity and specificity of this technique, further optimization and direct comparisons with other testing methods are essential. The sensitive and specific analysis of biomolecules in intricate mixtures is crucial in clinical diagnostics. Disease development typically entails various biomolecules, necessitating simultaneous detection of multiple events. This approach not only saves time and expenses but also yields extensive insights from small clinical samples. Should this technique be successfully refined, a point of care (POC) SERS test conducted on samples could yield rapid results, requiring only the application of cost-effective mass-produced nanoparticles.

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