

Ag Nanoislands Functionalized Hollow Carbon Nano Fibers as Non-Invasive Label-Free SERS Salivary Biosensor Platform for Salivary Nitrite Detection for Pre- Diagnosis of Oral Cancer

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SUPPORTING INFORMATION

TEM Analysis:

The TEM Analysis at different concentrations of AgNO₃ is given in the Figure S1. Due to the large surface area to volume ratio of HCNFs, the Ag NPs distribution is lowest on the surface of the hollow CNFs for Ag@HCNFs with a molar concentration of 10mM. When the AgNO₃ molar concentration is 20 mM, the particle distribution has been increased but, it is not sufficient to be used as the SERS substrate. The TEM image of the Ag@HCNFs at a concentration of AgNO₃ of 30 mM is depicted in the Figure S1(c). As depicted in the figure, the Ag NPs were well dispersed all over the surface of HCNFs with good uniformity and particle distribution. Hence, from the TEM images, it can be concluded that the Ag@CNFs with AgNO₃ concentration of 30mM can increase the Ag NPs distribution, generating a large number of hotspots thereby contributing to the SERS enhancement.

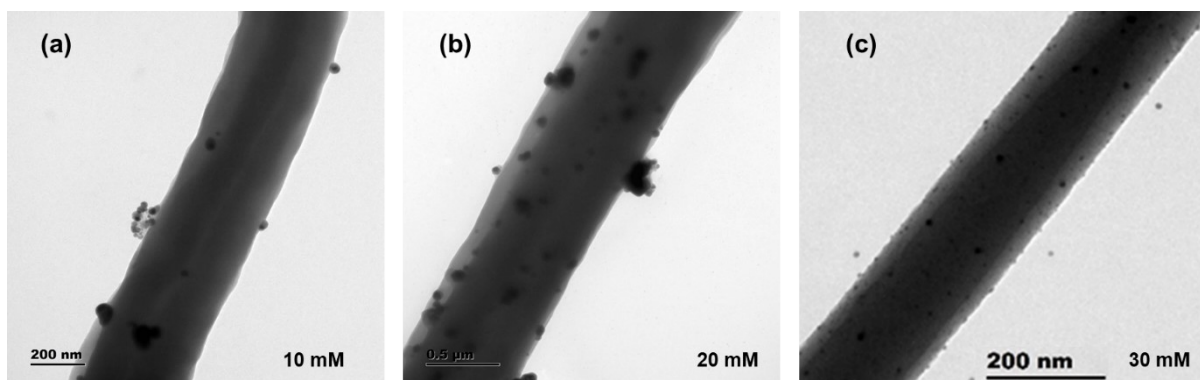


Figure S1: TEM analysis of Ag@CNFs at different AgNO_3 concentrations of (a) 10mM, (b) 20mM and (c) 30mM

UV-Visible spectra of Ag NPs

The UV-Vis absorption spectra of Ag nanoparticles was depicted in the Figure S2. The resonant stimulation of surface plasmons (SPR) in pure Ag nanoparticles resulted in a broad hump in the absorption spectrum centered at 408 nm as expected. Due to the simultaneous vibration of the free electrons in the silver nanoparticles and the resonance of the light wave, the surface Plasmon resonance (SPR) absorption band is produced. At 408 nm, a wavelength that is characteristic of Ag, a wide absorption peak was observed. The spectral analysis revealed no additional peaks, indicating that the synthesised materials comprise only Ag NPs.

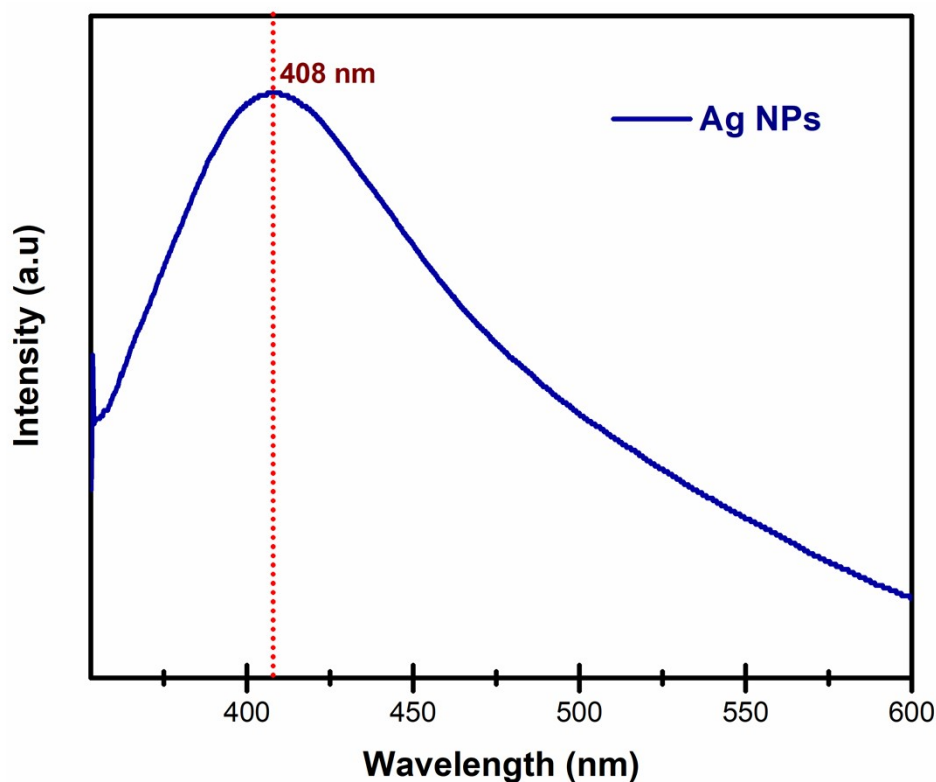


Figure S2: UV-Visible absorption spectra of Ag NPs

TEM images of Ag@HCNFs:

TEM images demonstrating the uniformity and high particle distribution of the silver nanoparticles anchored on HCNFs were included in Supporting Information Figure S3. These images confirm the uniform anchoring of silver nanoparticles, which exhibit close packing with interparticle distances ranging from 2 to 5 nm, thereby supporting the observed Surface-Enhanced Raman Scattering (SERS) properties. The uniformity in the Raman mapping images (Figure 4) is not good as the TEM images. The difference between TEM and Raman mapping images can be explained by their respective detection mechanisms and resolutions. While TEM directly images nanoparticles at high resolution (nano level), Raman mapping provides chemical information about the sample surface (micro level), which may not necessarily correlate with the spatial distribution of individual nanoparticles observed in TEM images.

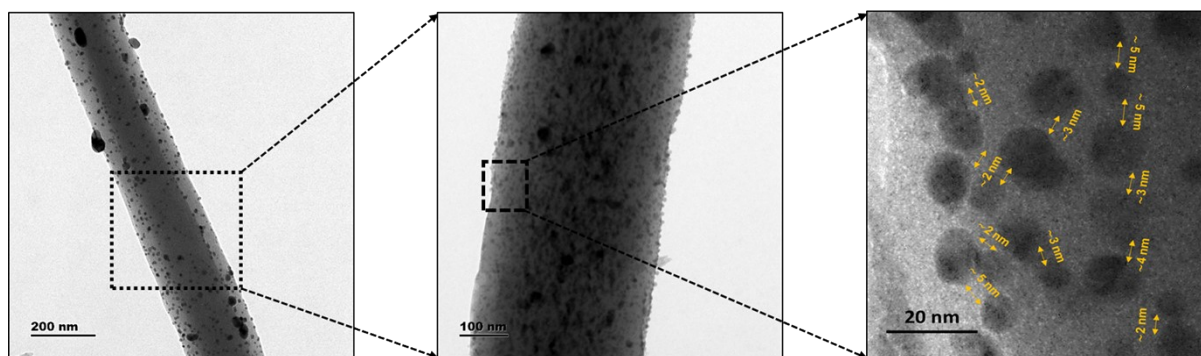


Figure S3: TEM images showing the uniform anchoring and high particle distribution of silver nanoparticles on the surface of HCNFs

As illustrated in Figure S3 TEM images, the silver nanoparticles exhibit uniform decoration throughout the surface of HCNFs, with interparticle distances falling within the range of 2-5 nm. To overcome the discrepancies in particle spacing and their impact on SERS signals, measures were taken to enhance reproducibility. Specifically, SERS analysis was conducted at three distinct locations on the substrate, ensuring repeatability and minimizing the influence of localized variations.

Evaluation of SERS Performance:

The comparison of the performance of the SERS substrate using Ag NPs anchored on normal carbon nanofibres and Ag NPs anchored on hollow carbon nanofibres was also carried out using R6G, as depicted in the Figure S4 (a). The results showed a higher enhancement in Ag@HCNFs as compared to the Ag@CNFs. This is attributed to the large surface area to volume ratio of the hollow carbon nanofibres as compared to the normal CNFs. HCNFs represent a new generation of CNFs structures, having twice the surface area of CNFs, thereby offer a promising solid base substrate for SERS applications. Due to the large surface area to volume ratio, the number of Ag NPs anchoring on the surface of HCNFs also increases leading to the formation of large number of hotspots. This leads to the enormous enhancement in the

order of 20,000 counts for Ag@HCNFs. Due to the low surface area to volume ratio of normal Ag@CNFs compared to Ag@HCNFs, the SERS intensity was found to be in the range 8000 counts due to the less number of silver nanoparticles anchored to the surface of CNFs. Hence, it can be concluded that the SERS signal can be enhanced several times using HCNFs as the support material for the plasmonic sites, demonstrating it as a potential solid base substrate for SERS applications.

In order to evaluate the SERS performance with varied surface density of AgNPs anchored on the hollow carbon nanofibers, the HCNFs were treated with various concentrations of AgNPs (10 mM, 20 mM, 30 mM) and the efficiency of the developed SERS active substrates were investigated by using R6G as the Raman reporter molecule.

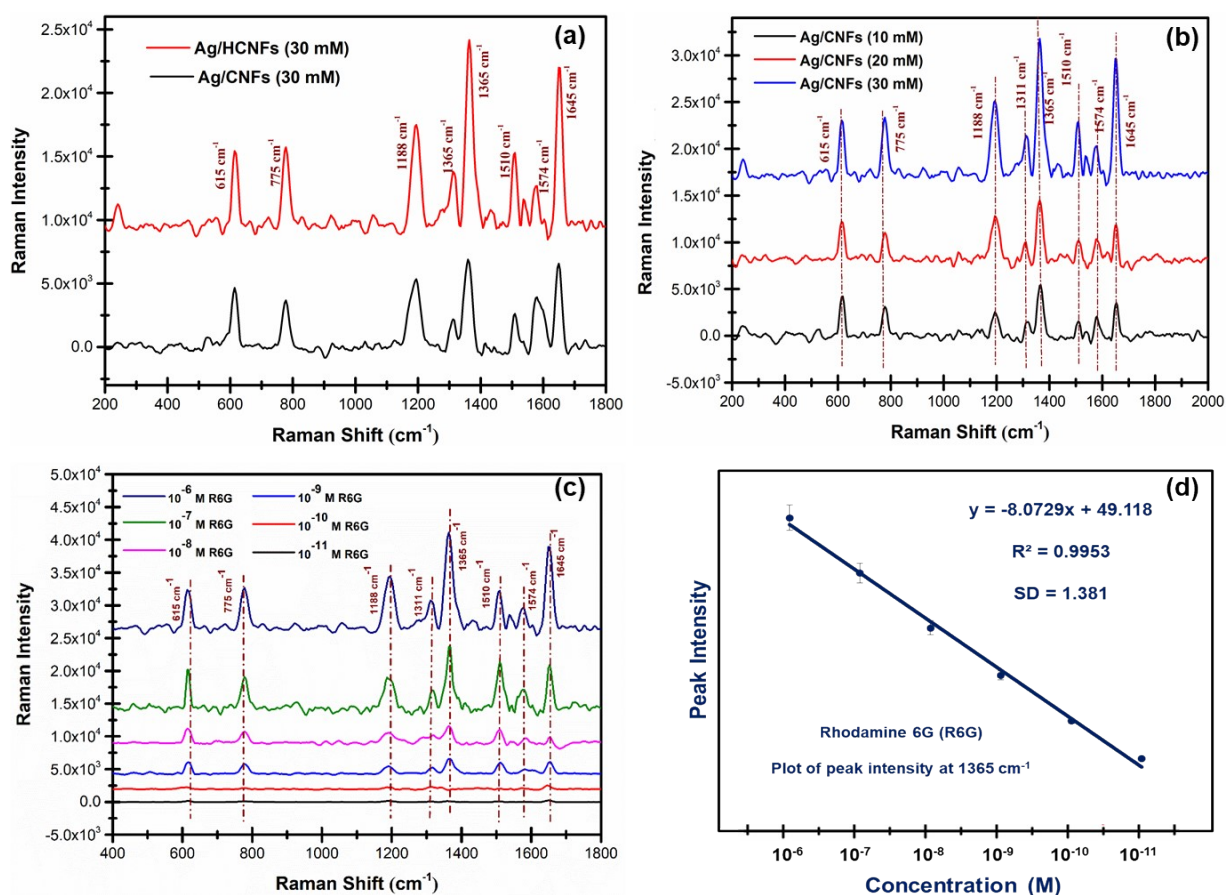


Figure S4: Comparison of the SERS spectra of R6G (a) using Ag@CNFs and Ag@HCNFs (b) different ratios of Ag@HCNFs (c) different concentrations ranging

from 10^{-6} to 10^{-11} M and (d) effect of concentration as a function of peak intensity at 1365 cm^{-1} .

Figure S4(b) shows the comparison of the SERS performance of the developed material with different concentrations of silver NPs using R6G which showed a good enhancement upto 35,000 counts using 30 mM concentration of Ag NPs. This is because hollow carbon nanofibres have a large surface area to volume ratio, providing a large area for the anchoring of silver nanoparticles. There are numerous interparticle gaps which may behave as Raman hotspots with enhanced local electromagnetic fields, producing extremely high SERS activity and sensitivity. Lower enhancement was observed for the Ag@HCNFs with precursor molar ratios of 10 mM and 20 mM, suggesting that there were insufficient silver nanoparticles to be deposited on the surface of the HCNFs. Since the deposited Ag NPs on the surface of the HCNFs are small and have a low particle distribution, they cannot produce enough hotspots for SERS at concentrations of 10 mM and 20 mM leading to the lower SERS enhancement. Hence, it can be concluded that, the structure and amount of silver loading on the surface HCNFs are significantly influenced by the silver precursor molar ratio, which also contributes towards the SERS performance.

The limit of detection of the Ag@HCNFs based SERS substrate was determined by recording the SERS spectra of different concentration range of R6G ranging from 10^{-6} M to 10^{-11} M as shown in the Figure S4 (c). The results showed that the SERS peak intensities were found to be decreased with the reduction of the concentrations of probe molecules (R6G) ranging from 10^{-6} to 10^{-11} M as expected. Also, all the main characteristic peaks of the probe molecules were still visible even at the concentration down to 10^{-11} M demonstrating the efficiency of the SERS substrate to detect trace level concentration of the analyte. The relationship between concentration and peak intensity at 1365 cm^{-1} is depicted in Figure S4 (d). It was discovered

that this relationship follows a linear trend with an R^2 value of 0.9953 and a standard deviation of about 1.381.

Limit of detection of nitrite using Ag@HCNFs:

The limit of detection of the SERS substrate using the analyte- Nitrite was also studied which is added in the supporting information S5. In order to determine the detection limit with nitrite and to evaluate the general applicability of Ag@HCNFs for potential uses in nitrite detection, concentrations ranging from 10^{-6} M to 10^{-10} M nitrite were analysed, and the SERS spectra were displayed in Figure S5.

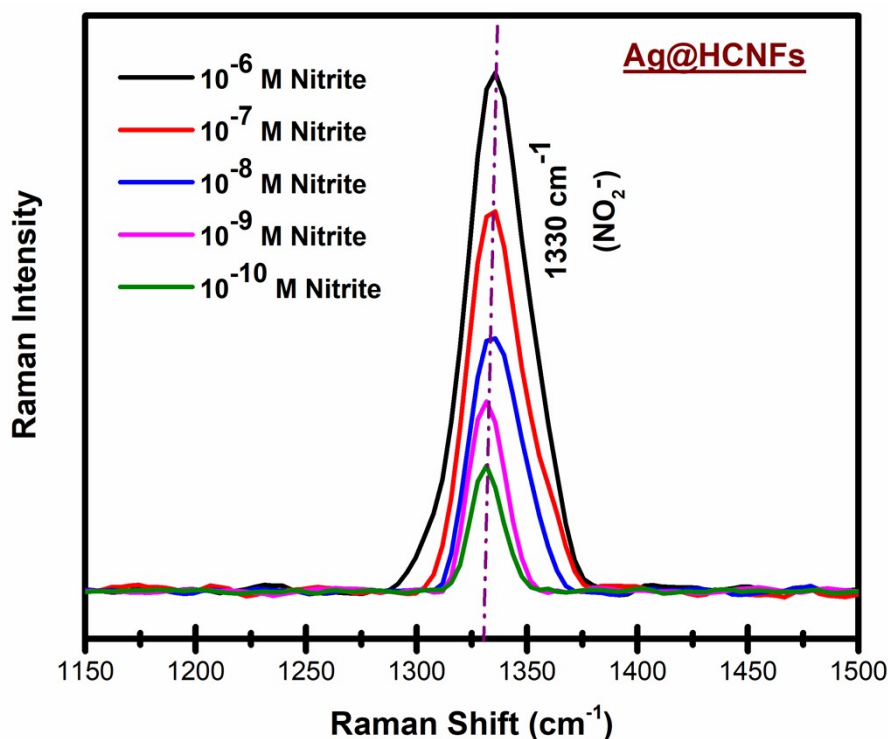


Figure S5: SERS spectra at different concentrations of nitrite ranging from 10^{-6} to 10^{-10} M

It was observed from the results that SERS peak intensities decreased as expected when nitrite concentrations were reduced from 10^{-6} to 10^{-10} M. Additionally, the distinctive peak located at 1330 cm⁻¹, which represents the symmetric N-O stretching vibrations of the nitrite, remained

discernible even at concentrations as low as 10^{-10} M, indicating the SERS substrate's effectiveness in detecting analyte traces.

This is because hollow carbon nanofibres have a large surface area to volume ratio, providing a large area for the anchoring of silver nanoparticles. The uniform anchoring and high particle distribution of the silver nanoparticles on the surface of HCNFs leads to numerous interparticle gaps (2-5 nm) which may behave as Raman hotspots with enhanced local electromagnetic fields, producing extremely high SERS activity and sensitivity as depicted in the supporting Information, Figure S3.