Preparation of low cost SERS-substrates for virus characterization.

Preparación de sustratos SERS de bajo coste para caracterización de virus.

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Abstract: Raman spectroscopy is a technique that allows the characterization and detection of a wide range of molecules. The characterization of biomolecules and viruses has been a novel application in the last decades. A relevant problem for applying this technique is the low intensity of the Raman signal and the low concentration of the analyte, which makes the identification of molecules and viruses quite difficult. An alternative to overcome this problem is the use of surface- enhanced Raman spectroscopy (SERS). This involves the use of SERS-substrates that generally are very expensive and can only be used once. In this work, we report the preliminary results of virus detection by SERS using low-cost homemade substrates and commercially available substrates. Characteristic Raman peaks associated with the influenza virus were detected. The results obtained with the homemade SERS-substrates are comparable to the obtained by using the comercial ones.

Keywords: SERS, virus, influenza, Coffee ring effect, nanoparticles.

Resumen: La espectroscopia Raman es una técnica que permite la caracterización y detección de una amplia gama de moléculas. La caracterización de biomoléculas y virus ha sido una aplicación novedosa en las últimas décadas. Un problema relevante para aplicar esta técnica es la baja intensidad de la señal de Raman y la baja concentración de

analito, lo que dificulta bastante la identificación de moléculas y virus. Una alternativa para superar este problema es el uso de SERS (surface-enhanced Raman spectroscopy). Esto implica el uso de sustratos SERS que generalmente cuestan una cantidad considerable de dinero y solo se pueden usar una vez. En este trabajo, reportamos resultados preliminares de la detección de virus por SERS utilizando sustratos caseros de bajo costo y sustratos disponibles comercialmente. Se detectaron picos característicos Raman asociados a virus de la influenza. Los resultados obtenidos con los sustratos SERS caseros son comparables a los obtenidos utilizando los comerciales.

Palabras clave: SERS, virus, influenza, efecto anillo de café, nanopartículas

Introduction

Viruses are the most abundant biological entities on earth (Suttle 2005). Studies covering the period of the last century have shown that globalization and industrialization played a vital role in the emergence and dissemination of viral diseases (Lipkin and SJ 2015). Current virus detection methods include Enzyme-Linked Immuno Sorbent Assay (ELISA), Polymerase Chain Reaction (PCR), virus isolation, and serology (Lu 2003; Spackman et al. 2002).

Although effective, these are generally time-consuming and their applications are limited by detection sensitivity, specificity, versatility, and portability (Tong et al. 2019). Optical spectroscopy is a good tool for the characterization of different types of cancer, pathogens and virus (Ambartsumyan et al. 2020; Fernández Ramírez et al. 2019). Due to its excellent chemical specificity, Raman spectroscopy has become one of the most promising alternatives to overcome the limitations of the current virus detection methods (Ambartsumyan et al. 2020). However, Raman scattering has not found wide application with biological samples due to its inherently weak signal (Xia 2017). Enhanced Surface Raman Spectroscopy (SERS) compensates for this deficiency through an electromagnetic or chemical enhancement of the signal (). The main characteristics of this technique include speed to identify biological samples, the ability to detect analytes at low concentrations and perform measurements without sample preparation (Kukushkin et al. 2019; Ambartsumyan et al. 2020). To date, SERS technique has been successfully implemented to detect many different types of viruses (Shanmukh et al. 2006; Luo et al. 2014; Otange et al. 2018) and, various kinds of bacteria (Galvan and Q 2018).

Although many SERS substrates with

excellent enhancement effects exist in the market, the applications of SERS are still limited. The main reasons for this are the complex manufacturing process and expensive fabrication (Wang et al. 2018). Therefore, it is very important to develop SERS substrates using a simple, low-cost, and time-saving method. In this study, we make a preliminary report of virus detection by SERS measurements. We present an inexpensive and rapid method for the manufacture of SERS substrate using commercial gold nanoparticles on cellulose paper.

Materials and Methods

Raman measurements were carried out in samples of the commercial vaccine for influenza (flu) (virus strains: A/California/7/2009 [H1N1], A/Hong Kong/4801/2014 [H3N2] and B/Brisbane/60/2008) in humans (Vaxigrip). For the homemade SERS substrate fabrication, we placed 1.5 µl of highly concentrated and previously centrifuged gold nanoparticules, (colloidal gold nanoparticles, 80 nm in diameter, Stellnet Inc) on cellulose paper. Then, we allowed it to dry at room temperature for 15 minutes. A drop of vaccine was placed on the substrate for Raman measurements. For comparison, measurements using commercial gold SERS substrates were also performed. The samples were excited with a 785 nm diode laser (75 mW power) using a microscope (40x objective lens). The scattered light were collected in a backscattering configuration by using an spectrometer (RamanHR-TEC-785, StellarNet, with an spectral resolution around 4 cm−1). A sketch of the experimental setup is shown in figure 1.

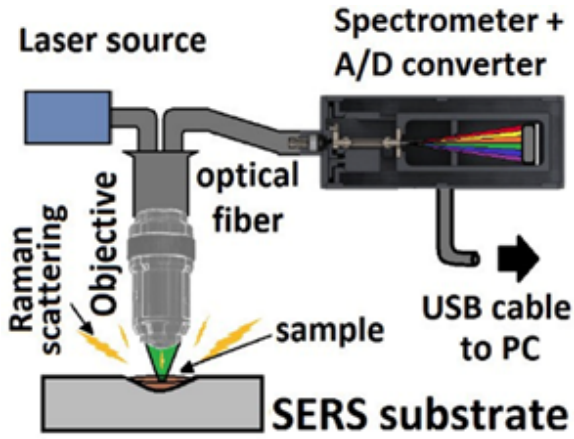

Figure 1: Diagram for the Raman setup used in the SERS measurements

For the measurements an integration time of 5 s and 10 scans were used. The Raman spectra shown in this paper are the average of 25 measurements taken at different spots on the sample. The monotonous part of the spectra associated to sample luminescence was subtracted. Then the resulting spectra were smoothed by a weighted average taken 25 points. The Raman signal that appears at 256 cm−1 associated with the dispersion of nanoparticles of the SERS, was used for the normalization of the spectra. A gaussian deconvolution of all the measured spectra was performed for Raman peak identification.

Figure 2: Photograph of an influenza vaccine sample on the homemade SERS substrate (gold nanoparticle+cellulosa paper). The coffee ring is clearly observed.

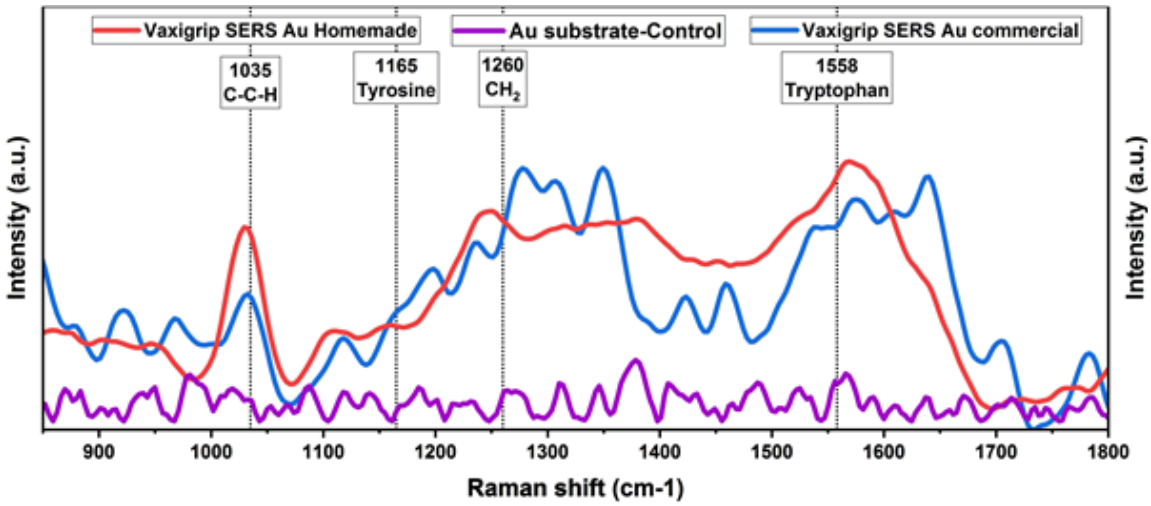

Figure 3: Raman spectra of a commercial influenza vaccine. These spectra were obtained using a commercial (blue line) and a homemade (red line) gold SERS substrates. The purple line shows the control spectrum for the measurement performed on the homemade SERS substrate.

Results and Discussion

The Raman spectra were obtained exciting on the edge of the Au nanoparticle + vaccine drop, as shown in figure 2, the so called coffee ring (Kumar et al. 2020). A high signal amplification is obtained in this region. In figure 3, the measured Raman spectrum of the vaxigrip vaccine using a homemade (red line) and a commercial

(blue line) gold SERS substrate are shown. The corresponding control for the measurement using the homemade substrate is shown in purple. An important result is that the Raman spectrum obtained using the homemade SERS substrate is comparable to the spectrum obtained by using the commercial one. In fact, the Raman spectrum of the vaxigrip vaccine obtained using the homemade SERS substrate exhibits bands with greater intensity than the spectrum obtained using the commercial one (peaks around 1035 cm -1 and, 1558 cm -1).

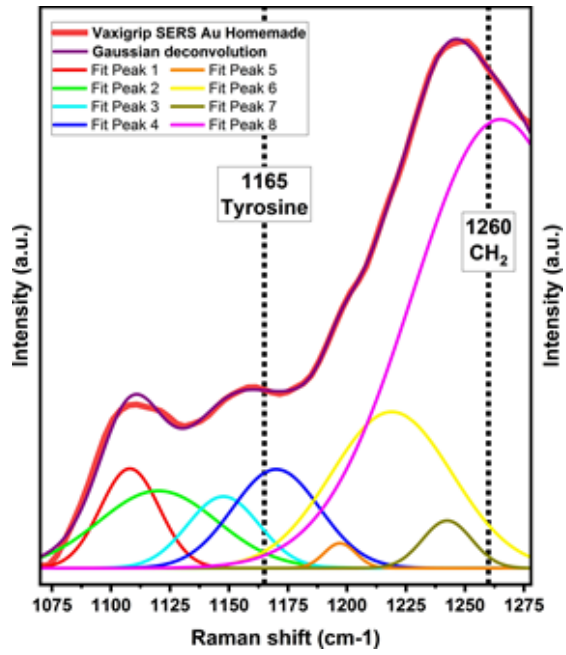

Figure 4: Gaussian deconvolution for a Raman spectrum obtained with the homemade SERS substrate.

Some bands for influenza virus at 1035 cm−1 (C-C-H bending in ribose ring), 1165 cm−1 (Tyrosine), 1260 cm−1 (CH 2 in-plane deformation) and, 1558 cm−1 (Tryptophan) have been reported (Pezzotti et al. 2019; Chang et al. 2011; Lin et al. 2011). They are shown as vertical dotted lines in figure 3, and are clearly observed using both types of SERS substrates. In particular, the peaks related to CH2 in-plane deformation, associated to the envelope of the influenza virus according to Lin and coworkers (2011), seem to be shifted from the reported value, as observed in the spectrum deconvolution shown in figure 4. A similar shift is observed for the Tyrosine peak. These shifts are probably related to the different absorption behavior of the sample on the gold SERS substrate as reported by other authors (Fan et al. 2010).

The Raman spectrum of the vaxigrip vaccine obtained using the homemade SERS substrate exhibits bands that showed greater intensity than the spectrum obtained using the commercial one (peaks at 1035 cm−1 and, 1558 cm−1).

A relevant issue is the relative low cost of the SERS substrates developed in this work, which are around a factor of 100 less expensive than the commercial substrates. These facts open the possibility of manufacturing these substrates for detecting different molecules by a simple, low cost, and time saving method.

Conclusions

We prepared SERS substrates based on cellulose paper and gold nanoparticles with an straightforward and low cost method. Raman spectra obtained using these substrates show similar results to the obtained with commercial ones. These preliminary results open the way to improve the overall performance of the fabricated SERS substrate prototypes, but also for further work and exploration with other types of SERS substrate materials.

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