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Sol–Gel and Electrospinning Synthesis of Silica–Hydroxyapatite–Silver Nanofibers for SEIRAS and SERS

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Received: 6 July 2020; Accepted: 8 September 2020; Published: 23 September 2020

Abstract: Surface-enhanced Raman spectroscopy (SERS) and Surface-enhanced infrared absorption spectroscopy (SEIRAS) are both novel techniques favored by the excitation of surface plasmons onto metal nanostructures. The light emitted from the metal surface couples with the vibrational transitions of molecules in proximity, enhancing its spectral response and leading to more sensitive and effective spectroscopic analysis. The absence of inexpensive and reproducible substrates is among the major impediments to the accurate implementation and optimal performance of the technique. The development of a low-cost active substrate based on silica–hydroxyapatite through sol–gel synthesis and electrospinning is addressed in the present study. Fibers of 512 ± 199 nm diameter were produced after sintering at 1150 °C on the electrospun mats. The fibers are fixed to an indium tin oxide (ITO) glass base for electrodeposition with 10 and 20 mM AgNO₃ at 1.5 and 3.3 V at different time periods. Electrodeposition produced silver nanorods and nanocubes on the fibers. The SERS and SEIRAS activity of each one of the nine supports was tested using pyridine 1 nM, comparing it with the spectrum of pyridine 1 mM. An enhancement factor of 2.01×10^6 for the band at 3335 cm^{-1} was obtained during a SEIRAS essay for the support doped for 2 min at 3.3 V with 10 mM silver nitrate solution. The highest SERS enhancement factor was 3.46×10^8 , for the band at 1567 cm^{-1} in the substrate doped for 5 min at 1.5 V with silver nitrate solution at 10 mM. After testing both samples with 10^{-4} M violet crystal solution, no SERS enhancement factor was found, but higher band resolution in the spectra was observed.

Keywords: electrospinning; sol–gel; silica; hydroxyapatite; silver; SEIRAS; SERS; nanofibers

1. Introduction

The infrared and Raman spectroscopy techniques are classic chemical characterization tools that offer high sensitivity combined with the capability of single molecular fingerprint differentiation for a wide range of compounds, including relatively simple sample handling procedures [1]. Low signal intensity from analyte traces and the manifestation of relaxation phenomena such as fluorescence limit their application. Surface-enhanced Raman spectroscopy and surface-enhanced infrared absorption spectroscopy make possible the detection of species at extremely low concentrations. The use of nanometric designed metal substrates has proven to be useful for the detection of a vast variety of substances, featuring molecules of environmental and industrial relevance. Such supports possess multiple applications in the areas of biotechnology [2], biosensors design [3,4] and nanocatalysis [5], among others. The detection and amplification in surface-enhanced Raman spectroscopy (SERS) and Surface-enhanced infrared absorption spectroscopy (SEIRAS) depend synergistically on the

metal nanostructure and surface morphology. The type of analyte–metal molecular bond is of great importance, and in nearby metallic island gaps, the signal shows improved amplification [1].

Typically, silver nanoparticles have been employed to carry out Raman enhancement essays, with results that reach augmentations in the order of 10^{15} [6] and infrared signal amplification essays which report enhancement factors up to 150 [7]. In each case, the care to the surface morphology in the support is key to obtaining large enhancement factors. Classical colloidal techniques provide control over the size of the particle and a quick route for the synthesis of a substantial quantity of particles, thus supporting the plasmon resonance effect, yet those solutions tend to be rather heterogeneous [8]. Homogeneous nanoparticles can be yielded through electrolytic deposition procedures, offering a regulation of shape and extent of sharp features on the surface of the substrate, leading to larger enhancements [9]. Nonetheless, the obtention routes of these substrates are commonly complex and appeal to the usage of expensive reagents. Many of the prototypes are neither chemically stable nor reproducible [1,10–12]. Composites based on semiconductors of TiO_2 or SiO_2 , which include noble metals such as silver and gold, have been shown to increase the activity in SERS, for which reason the investigations look for SERS substrates with higher sensitivity, uniformity, and reproducibility. Recent works have shown the synergistic effect of electromagnetic enhancement and charge transfer as the main reason for the increase in the SERS effect in titanium dioxide silver films [13].

The hydroxyapatite structure has many advantages such as high biocompatibility, high surface area and porosity, characteristics that are necessary in the administration of active substances and adsorbent material. In SERS and SEIRAS, porous structures functionalized with noble metal nanoparticles are a new concept of sensors that can help detection and adsorption in a simple way. However, complicated or expensive pretreatment steps are required for the coating or preparing of their surfaces to aid in signal amplification. The adaptation of sol–gel and electrospinning techniques allow the creation of fibers with a homogeneous and rough surface for easy functionalization with metal nanoparticles, thus allowing the formation of hot spots for a good amplification of spectroscopic signals [1]. The sol–gel and electrospinning techniques allow the control and modification of morphology of new materials at nanoscale, in a simple way and normal temperature and pressure conditions, hence reducing the cost and production time for the obtention of substrates [1,10–12]. In the present study, a synthesis of stable, reusable, and low-cost active substrates for SEIRAS and SERS is proposed. The substrate based on silica–hydroxyapatite–silver provides a support for Raman and infrared signal enhancement through the coupling of the sol–gel, electrospinning, and electrodeposition methods.

2. Materials and Methods

2.1. Silica and Hydroxyapatite Sol–Gel Obtention

The silica sol–gel was prepared starting with the organometallic precursor tetraethylorthosilicate (98%, Fluka®, Buchs, Switzerland) dissolved in absolute ethanol (99.5%, Sigma Aldrich®, St. Louis, MO, USA). Molar proportions for TEOS, ethanol, water and HCl (Sigma Aldrich®, 99%) were 1:2:2:0.1, respectively, according to the methodology proposed by Garibay-Alvarado [10]. The mixture was put under constant stirring for 30 min at 25 °C. The hydroxyapatite sol–gel was elaborated from the combination of a calcium nitrate tetrahydrate solution ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$) (Sigma Aldrich®, 99%) in one of ethanol and another of triethyl phosphite ($\text{C}_2\text{H}_5\text{O}$)₃P (Sigma Aldrich®, 99%). The mixture was put under constant magnetic stirring for 1 h at 25 °C. Then, for 24 h at 40 °C and finally it was aged for 6 h at 60 °C using magnetic stirring.

2.2. Electrospun Composite Obtention

A 7% *w/v* solution of polyvinylpyrrolidone (PVP) (P.M. 1,300,000; 99%, Sigma Aldrich®) dissolved in ethanol was prepared using a water bath and constant magnetic stirring. Later it was combined with each one of the gels obtained before to produce solutions with silica at 10% *v/v* and with hydroxyapatite at 20% *p/v*. Both solutions were loaded into 35 mL syringes mounted in a Nanobond model NEU-Pro

device for electrospinning in a coaxial setup. A modified method proposed by Garibay-Alvarado was employed [10]. The electrospun fibers were dried at 50 °C for 24 h, once dried, the fibers were placed in an electric muffle to be progressively treated and sintered at 200, 400, 600, 800, 1000 and 1150 °C. The heating ramp applied were 0.5 °C/min for 200 and 400 °C, 1 °C/min for 600 and 800 °C, 5 °C/min for 1000 and 1150 °C. Subsequently, the fibers were doped with silver particles by electrodeposition.

2.3. Electrodeposition of Silver Nanoparticles

The electrodeposition of the sintered composite was carried out using an electrolytic cell, employing silver as the anode. The ceramic fibers were fixed upon a 1 × 1 cm² indium tin oxide (ITO) glass with a carbon tape to form the SERS/SEIRAS support. Afterwards, both electrodes were immersed in a silver nitrate solution at 60 °C. Initially, a voltage of 1.5 V was used, with 10 mM AgNO₃. Then, the voltage used was 3.3 V for 10 and 20 mM AgNO₃ solutions. The electrodeposition intervals were 2, 3 and 5 min for each iteration according to Table 1.

Table 1. Electrodeposition parameters for the SiO₂–hydroxyapatite (HA) supports.

Sample	Voltage (V)	AgNO ₃ (mmol/L)	Time (min)
F1	1.5	10	2
F2			3
F3			5
F4	3.3	10	2
F5			3
F6			5
F7	3.3	20	2
F8			3
F9			5

2.4. Characterization

IR characterization was performed using a Bruker Platinum FTIR device set to the Attenuated Total Reflectance mode, making 48 scans per sample with a resolution of 4 cm^{−1} in a spectral range from 4000 to 400 cm^{−1}. The evolution of the electrospun material throughout the thermal treatment was evaluated, taking samples before and after the processing at every heating cycle. Using a Raman Confocal alpha 300 WiTec spectrometer (Agilent Technologies, Inc., Santa Clara, CA, USA), an integration time of 0.5 s, 10 accumulations and a 532 nm excitation laser source, the characterization of the electrospun composites was performed. The composites were placed over carbon tape on a slide for its microstructural analysis, which was made in a JEOL[®] (Tokyo, Japan) JSM-6400 equipment with an energy of 20 keV, an energy dispersive X-ray spectroscopy (EDS) essay was carried out to determine the elemental composition at the surface of the fibers. X-ray diffraction is a method widely employed for the identification of crystalline compounds [14]. The as-spun and thermal-treated fibers at 400 °C, 800 °C and 1150 °C were analyzed by the powder diffraction technique, with a Kα Cu of 1.5406, performing a scan from 10° to 80°.

2.5. SERS and SEIRAS Effects Evaluation

The SERS and SEIRAS activities from the doped composite were evaluated in a Raman Confocal alpha3000 spectrometer with a laser source of 532 nm and a Bruker Platinum FTIR infrared spectrometer, respectively. Utilizing 1 mM and 1 nM pyridine solutions in deionized water the area deconvoluted under the curve of the main bands was obtained. The supports that showed higher SERS/SEIRAS activity were tested again using a 10^{−4} M violet crystal solution.

3. Results and Discussions

A viscous silica gel with translucent aspect was obtained. After the addition of PVP, the gel acquired greater opacity and viscosity. A low viscosity was preferred for electrospun use. After aging, the appearance of the hydroxyapatite sol–gel resembled a white paste that contained fine crystalline lumps. Once the PVP was added, the mixture turned transparent and viscous. The features of the synthesized gel are in conformity with those described by Garibay-Alvarado [10]. The as-spun silica–hydroxyapatite fibers, stored at 50 °C, are white and fragile. The texture of the mat is soft, and the distribution of fibers is homogeneous. In some sites the fiber mat lost continuity due to the accumulation of material at the tip of the dispenser and drop sprinkling during electrospinning. High moisture levels cause the dissolution of the polymer, damaging the mat's integrity. After the sintering treatment, the fibers remained white and adopted a shiny look, additionally, their flexibility decreased, and they turned fragile.

3.1. FTIR Characterization

The infrared absorption spectra of the SiO₂–hydroxyapatite (HA)–PVP fibers are represented in Figure 1a. There can be several multiple bands corresponding to vibrations from the PVP used for electrospinning. The bands located at 2951 and 1420 cm^{−1} correlate to asymmetrical stretching vibrations of methylene (CH₂) and out of plane bending vibrations of the C–H bond, respectively. An intense and narrow band is observed at 1643 cm^{−1}, characteristic for the C=O bond stretching. According to [15], the location of this PVP band varies from 1633 to 1644 cm^{−1} depending on the molecular weight of the polymer. Likewise, it is reported that, for a weight of 1,300,000 just as the polymer used, the absorption peak should be found at 1633 cm^{−1}, while there is a possibility in which this maximum is blue-shifted because of the formation of coordination bonds between the oxygen in the carbonyl group and some metal ion such as Ca²⁺ in the hydroxyapatite. The band at 1312 cm^{−1} belongs to the stretching vibration of C–N in the PVP. In Figure 1a, it is primarily observed that bands around 3400 and 2950 cm^{−1} drastically decrease as the temperature rises due to the progressive decomposition of the polymer and the residual organic matter calcination. The same occurs for the polymer bands at 1643, 1420, 1313, 1167 and 738 cm^{−1}: they all remain visible at 400 °C. From 200 °C on, the band of Si–O–Si bending turns evident and at 400 °C a band emerges at 821 cm^{−1} analogous to the early formation of silanol groups (Si–OH), indicative of chemical instability in the silica, which at current sintering temperature has not yet lost its –OH groups [1]. At 579 cm^{−1} there is a band caused by the phosphate group stretching, more noticeable at 200 and 400 °C. The fibers sintered at 600 °C in Figure 1b present a well-defined and intense band near 1445 cm^{−1} related to the carbonate group. It is appreciated only until the calcium carbonate's decomposition temperature at 800 °C. The Si–OH band gradually reduces from 600 to 1150 °C, proving the loss of –OH groups from silica. A protruding band at 1046 cm^{−1} associated to the ν_3 mode of the phosphate group is found from 600 to 1150 °C. A shoulder at 1083 cm^{−1} becomes prominent from 1000 °C on and can be attributed to a contribution from both the phosphate ν_3 mode and the stretching of the O–Si–O bond. Other vibrations corresponding to the ν_2 and ν_4 modes of phosphate are appreciated at 473 and 579 cm^{−1}, respectively, along all different sintering temperatures, and more clearly at 1150 °C just as Figure 1b displays.

Du et al. [16] report three major bands located at 460, 808 and 1090 cm^{−1} as part of the characterization of large-scale produced silica fibers by a vapor transport process. The band at 1090 cm^{−1} is attributed to the asymmetric stretching mode of the Si–O–Si bond, while bands at 460 and 808 cm^{−1} are assigned to the absorption of the symmetric stretching and bending of Si–O–Si. The same bands were also observed in the IR spectrum of pulverized silica. Furthermore, according to the reports from [17], this confirms the subsistence of bands at 963, 1028 and 1110 cm^{−1} for the phosphate group in samples of hydroxyapatite obtained by hydrothermal and growth on silica gels methods, and from [18], who ascribes the bands at 472, 574, 962, 1046 and 1087 cm^{−1} to the modes ν_2 , ν_4 , ν_1 , ν_3 and ν_3 , respectively, from a phosphate group in silicon-substituted hydroxyapatite samples, it is inferred that the bands at 943, 1036 and 1083 cm^{−1} identified in the IR spectra of the silica–hydroxyapatite

coaxial fibers developed in the present investigation belong exclusively to hydroxyapatite. However, the red-shifting of the bands related to the ν_1 and ν_3 modes (1087 cm^{-1}) and the blue-shifting of the mode ν_3 (1046 cm^{-1}) can be explained in terms of a substitution of the phosphate groups by silicate within the hydroxyapatite cell. In Figure 1b it is observed that the IR spectrum for the SiO_2 -HA fibers sintered at $1150\text{ }^\circ\text{C}$. A prominent band is present at 1036 cm^{-1} , and another less intense band appears at 579 cm^{-1} : both referring to the stretching of the P-O bond from phosphate groups. Those bands found at 870 and 473 cm^{-1} correspond to the asymmetric stretching and bending of Si-O-Si, respectively.

Figure 1. (a) Infrared spectra of the SiO_2 -HA-polyvinylpyrrolidone (PVP) fibers at 50, 200 and $400\text{ }^\circ\text{C}$, and (b) the infrared spectra of the SiO_2 -HA at 600, 800, 1000 and $1150\text{ }^\circ\text{C}$.

3.2. Raman Spectroscopy Characterization

The comparison of the Raman spectra for the fibers treated at 50, 200 and $400\text{ }^\circ\text{C}$ in Figure 2a reveals the presence of PVP characteristic bands, around 1400 cm^{-1} due to the C-H bending and near 1650 cm^{-1} due to carbonyl stretching. The existence of the band at 945 cm^{-1} reveals, as reported by [19], the persistence of amorphous calcium phosphate that decreases but does not disappear after sintering. At 200 and $400\text{ }^\circ\text{C}$, the elevated amount of fluorescence in the spectra prevents the appreciation of more bands. At $1150\text{ }^\circ\text{C}$, the band at 945 cm^{-1} remains noticeable, and it is assigned to the presence of acid phosphate coming from the tricalcium phosphate. Tricalcium phosphate coexists in two phases, α and β [20], and they are less stable phases than pure hydroxyapatite. Its existence under $1300\text{ }^\circ\text{C}$ implies an accelerated disintegration process of hydroxyapatite caused by the exchange of tetrahedral phosphate for silicate. It is known that silica can disrupt the symmetry of the hydroxyapatite's hexagonal grid and speed up its decomposition into tricalcium phosphate in the normal range of 1300 – $1400\text{ }^\circ\text{C}$ to a minor interval between 750 and $1150\text{ }^\circ\text{C}$ (Figure 2b). We also observed the rise of the triply degenerated asymmetric stretching (ν_3) vibrational mode of the phosphate group as well, commonly observed at 1090 cm^{-1} , and a band for the triply degenerated bending mode (ν_4) of phosphate, normally found at 578 cm^{-1} for pure hydroxyapatite [21]. The analysis executed reveals analogous bands at 1074 and 589 cm^{-1} , respectively. The shift in the bands for the ν_3 and ν_4 modes evidences the diffusion of silica towards the crystalline network of the hydroxyapatite coating the coaxial fibers. The polyvinylpyrrolidone seems to have completely disappeared by $800\text{ }^\circ\text{C}$. Figure 2c details a higher amplification for the Raman spectrum of the fibers sintered at $1150\text{ }^\circ\text{C}$.

Figure 2. (a) Raman spectra of the SiO₂–HA–PVP fibers from 50 to 400 °C, (b) Raman spectra of the SiO₂–HA fibers from 600 to 1150 °C and (c) the Raman spectrum of the SiO₂–HA fibers sintered at 1150 °C.

3.3. Scanning Electron Microscopy Characterization

Four samples of the composite originated from distinct stages of the thermal treatment and sintering process were observed via scanning electron microscopy (SEM) to study the fibrillar arrangement and the surface features of the fibers. As seen in Figure 3a, the as-spun fibers presented an aleatory orientation and a smooth surface, as well as intersection zones between fibers that contain nodules. Defects can be attributed to the accumulation of collected material and the interruption of the electrospinning flow. The as-spun fibers' diameter was 685 ± 166 nm. The elemental analysis by EDS (Figure 3c) revealed a Ca/P ratio of 3.3, and the presence of silicon (3.19%), nitrogen (6.28%) and oxygen (29.07%) as well. Fibers at 400 °C were morphologically comparable to the as-spun fibers. The amount of organic material and cluster-like defects diminished considerably. The arrangement of the fibers remained aleatory and their surfaces soft and flawless (Figure 3d). Their average diameter was 304 ± 57 nm, exhibiting a reduction of approximately 56% with respect to the as-spun fibers' diameter. Elemental analysis (Figure 3f) shows a light decrease in the Ca/P ratio in the analyzed zone (3.0) and in the silicon and oxygen atomic proportions of 4.72 and 30%, respectively. No nitrogen was detected in the sample, confirming the removal of PVP. The silica–hydroxyapatite fibers sintered at 800 °C denoted an average diameter of 256 ± 49 nm, 16% narrower than fibers treated at 400 °C.

It was noted that the fibers possessed multiple fractures that interrupted their arrangement (Figure 3g). Ca/P ratio stands constant, and so do the silicon (4.92%) and oxygen (30.91%) percentages (Figure 3i).

Figure 3. (a) Micrograph at 10,000×, (b) diameter distribution plot and (c) EDS spectrum of the as-spun SiO_2 -HA-PVP fibers; (d) micrograph at 10,000×, (e) diameter distribution plot and (f) EDS spectrum of the as-spun SiO_2 -HA-PVP fibers at 400 °C; (g) micrograph at 10,000×, (h) diameter distribution plot and (i) EDS spectrum of the as-spun SiO_2 -HA-PVP fibers at 800 °C; (j) micrograph at 10,000×, (k) diameter distribution plot and (l) EDS spectrum of the as-spun SiO_2 -HA-PVP fibers at 1150 °C.

In Figure 3j, the morphology of the silica-hydroxyapatite fibrillar composite sintered at 1150 °C is appreciable. It is characterized by an apparent fusion of individual fibers into bigger aggregates. The fibers' shape resulted consequently affected by the fusion of hydroxyapatite, but their defined arrangement was preserved. The average diameter of these fibers was 512 ± 199 nm, containing nodules between fibers and porous surface, which are ideal sites for silver depositing. The EDS analysis (Figure 3l) revealed a reduction in the Ca/P ratio, from 3:1 as a previous sample evidenced, to 6:1. In its place, the Ca/Si ratio was 2.98. This change is explained as a consequence of the substitution of phosphate groups by silicates within the hydroxyapatite cell after sintering at 1150 °C and it is in agreement with the increment in the intensity of Si-O bands in the infrared and Raman spectra.

3.4. X-ray Diffraction Characterization

The diffractograms from the as-spun and 400 °C-sintered silica–hydroxyapatite fibers on Figure 4 display marked shoulders for silica amorphous regions with a low degree of crystallinity. The amorphous phases belong to the formation of glass with the loss of polymer and precursor salts. A more defined pattern emerges at 800 °C because of the formation of stable hydroxyapatite. The diffraction pattern of the composite after sintering at 1150 °C discloses peaks in 2θ at 18.3°, 23.1°, 26°, 29.2°, 32.2°, 34.3°, 40.1°, 47°, 49.5°, 54.4° and 64.2° due to the presence of the crystallographic planes (101), (111), (200), (120), (211), (202), (310), (222), (213), (400) and (304), attributed to the hexagonal structure of hydroxyapatite, according to the diffraction pattern (R050512), obtained from the Ruff database [21]. At 1150 °C there was no formation of other phases and there is a decrease in the amorphous phase of silica.

Figure 4. X-ray diffraction pattern of SiO₂–HA fibers at 50, 400, 800 and 1150 °C.

3.5. Characterization of the Doped Composite

The silica–hydroxyapatite composite was doped with silver through electrolysis. Table 1 shows the details of the voltage employed, silver nitrate concentration and the electrodeposition time for each one of the nine samples. Initially, a voltage of 1.5 V and time intervals of 2, 3 and 5 min were used in agreement with the favorable results on silver dendrite obtention reported by Cabello [22]. As the doped supports did not exhibit the presence of dendrites, a greater voltage and higher silver nitrate concentration were adopted.

As seen in Figure 5, at a voltage of 1.5 V and a deposition time of 2 min, silver is not homogeneously distributed onto the fibers surface. Those regions that contain abundant silver exhibit the barely perceptible growth of irregular cubic crystals, especially in the center and upper right zone of Figure 5a. However, these structures are fragmented and surrounded by smaller semispherical particles; the ovoid and elongated form of particles favored a nucleation process (Figure 5b). The silver nanocubes are more easily recognizable as the electrodeposition time. Stacked cubic crystals were detected, as shown in Figure 5c. Figure 5d presents the existence of smaller spherical structures growing upon the cubic

phases, morphologically alike those particles observed on the 2 min doped support. An increment in the deposition time produced bigger and more defined crystalline arrangements. After 5 min of doping, silver deposits on the fibers acquired more elongated shapes than those in previous supports. There is no trace of nanocubes, but thin needles and rods are distributed all along the fibers' surface and into deeper regions of the mat (Figure 5e). It is well known that the morphology of the deposits can be manipulated in function of the applied potential. Liu et al. [23] registered the achievement of aleatory silver architectures because of a quick nucleation in low reduction potentials, down to -0.9 V vs. Ag/AgCl, producing dendrites since the reduction rate of silver is elevated when the current density is high. By increasing the reduction potential, the later manifestation of nanorods happened. Therefore, congruently to [24], it is assumed that at 1.5 V nucleation occurs slowly, for the presence of a few cores, their continuous nucleation and the advent of crystals with various sizes are characteristic elements of low-rate nucleation increases.

Figure 5. Micrographs of the SiO₂–HA–Ag composite from supports F1 to F3: (a) F1 at 20,000× and (b) 40,000×; (c) F2 at 20,000× and (d) 40,000×, and (e) F3 at 20,000× and (f) 40,000×.

Figure 6a belongs to the support doped for 2 min at 3.3 V using AgNO₃ 10 mM. The manifestation of silver cores along numerous individual fibers, with an enlarged concentration at the cross-region between fused fibers, is noticeable. The settlements present variable shapes, most of them resembling tiny spheres arranged into disperse clusters, like the results obtained after the 1.5 V doping. Some others show planar growth like hexagonal laminated flakes (Figure 6b). The pores on the fibers are preferential sites for the deposition and accumulation of silver. The microscopy results do not present evidence of significant difference with respect to the growth of silver nanoparticles in samples doped at a lower voltage. Tsai et al. [25] registered the electrochemical deposition of silver nanoparticles with diameters between 100 and 200 nm onto carbon nanotubes using 10 mM AgNO₃ and a voltage of −0.3 V vs. Ag/AgCl. Analogous studies report the appearance of hexagonal crystals and dendrites isolated on silica–titania-based fibers after two minutes of electrodeposition at 1 V, utilizing a 10 mM AgNO₃ solution [22]. Hydroxyapatite's low conductivity, near $5.07 \times 10^{-10} \text{ S cm}^{-1}$ according to Suresh et al. [26], is a determinant factor that could justify the mitigated nucleation of silver in contrast to the silica–titania samples. For a 3 min doping, silver was handsomely deposited on the fibers' surface in the form of little particles adopting distinct morphologies, predominantly sphere and ovoid shapes, without displaying crystal growth (Figure 6c). As the magnifications grew, it was possible to observe how those particles agglutinate and become part of a series of disoriented systems. With a doping time of 5 min, the silver deposits in sample F6 exhibited unique morphologically features among all the analyzed cases: as seen in Figure 6e, multiple cubic crystals with a side length of $303 \pm 75 \text{ nm}$ were produced and they accumulated on the fiber membrane. Figure 6f grants a more explicit look to the corner sharpening of some nanocubes, conceding them a nanostar-like appearance, these being preferential sites for the nucleation of new silver cores. It denotes the proliferation of smaller particles on the cubes. Figure 6e also presents the elongation of one of the silver cores into a 1.1 μm -long needle. At higher magnifications, the subsistence of spherical silver particles is noteworthy, yet the nanocubes remain recognizable.

Micrographs of support F7 in Figure 7a,b for 3.3 V, 20 mM of AgNO₃ and doped for 2 min do not evidence significative silver accumulations. Figure 7a, at its central left portion contains signs of growth of some undefined cuboid crystal and many spherical bodies. Figure 7b shows silver clusters with no evident crystalline growth, composed of a wide variety of spherical nanoparticles. At 3 min of deposition, sample F8 (Figure 7c) presents increasing cuboid crystals smaller than nanocubes on support F6. At this doping time, crystal growth is clouded by the manifestation of the so-observed spheroid nanoparticles. At higher magnifications, the morphology of the clusters resembles more a group of disordered spheres rather than uniform cubic crystals. At last, 5 min after electrodeposition, the fibers were found remarkably coated by silver. In the first instance, it appeared that the dominant morphology among silver clusters is that of assembled spheroids, but Figure 7e enables the recognition of a few cuboids growing, far more defined than in samples F7 and F8.

The concentration of silver nitrate does not seem to influence the morphology of the deposited silver nanoparticles as much as the doping time does, but the rise in the concentration does generate more prominent clusters in less time, understanding that conductivity in the electrolyte depends on the concentration of ionic species [27]. By increasing the concentration of nitrate, the migration of silver ions towards the support is favored, leading to the saturation of certain sites on the fiber mat. Regarding the effect of enlarging the voltage, the cell potential is intrinsically related to a free energy variation, and hence, to the reaction constant for Ag⁺:

as represented in Nernst equation. In this case, by increasing both the voltage and the silver nitrate concentration, the reduction rate of silver and the accumulation of nanoparticles on the fibers were favored. The prolongation of the electrodeposition time lapse led to a more ordered crystallization.

Figure 6. Micrographs of the SiO_2 -HA-Ag composite from supports F4 to F6: (a) F4 at 20,000 $\times$ and (b) 40,000 $\times$; (c) F5 at 20,000 $\times$ and (d) 40,000 $\times$, and (e) F6 at 10,000 $\times$ and (f) 20,000 $\times$.

From the SEM results, it was concluded that the supports doped for 5 min at 1.5 and 3.3 V employing a 10 mM silver nitrate solution hold the best characteristics for their employment as SERS-active substrates, since the surface plasmon can be tuned between 415 and 623 nm [28–30], depending on the architecture of the nanoparticle and especially on the dimensions of the cube or rod. If the plasmon is located nearby the excitation wavelength (532 nm), then it is possible to create a resonance condition between both and make the electromagnetic enhancement prevail for the molecules adsorbed at the metal surface. Likewise, cornered ends and sharp edges become hot spots due to charge gathering. The contribution from electromagnetic enhancement to the final enhancement factor is crucial and depends on the near field properties [31].

Figure 7. Micrographs of the SiO_2 -HA-Ag composite from supports F7 to F9: (a) F7 at 20,000 $\times$ and (b) 40,000 $\times$; (c) F8 at 20,000 $\times$ and (d) 40,000 $\times$, and (e) F9 at 20,000 $\times$ and (f) 40,000 $\times$.

3.6. X-ray Diffraction Characterization

The powder diffraction analysis of a silica-hydroxyapatite sample doped for 5 min with 10 mM silver nitrate at 1 V in Figure 8 manifested marked shoulders for amorphous silica regions with a low degree of crystallinity. The silver characteristic planes (111), (200), (220) and (311), placed at 37°, 45.2°, 66.2° and 76.9° of 2 θ are visible. They belong to the face-centered cubic structure of silver [21]. Larger concentrations of deposited silver would lead to more intense peaks. The most outstanding peak corresponds to the (111) plane, suggesting the preferential elongation of silver nanorods [32].

Figure 8. X-ray diffraction pattern of the SiO₂–HA composite doped with 10 mM AgNO₃ at 1 V for 5 min.

3.7. SEIRAS Activity Evaluation

The SEIRAS analysis generated IR spectra for 1 nM pyridine adsorbed on every support and in each case, it was compared to the spectrum of a 1 mM pyridine solution measured in conditions foreign to the substrate. All samples evidenced an intense band around 3350 cm^{−1} corresponding to the C–H bond stretching of pyridine in aqueous systems. The spectrum of 1 mM pyridine also indicates a low band at 1638 cm^{−1} along with another, notoriously broader, at 560 cm^{−1} attributed to the 8a mode (C–N stretching) and to the ring deformation, respectively. Numerous bands exhibit a shift with respect to the fundamental position of some vibrational modes of pure pyridine reported by Johnson et al. [33] and Partal-Ureña et al. [34]. The band shift can be associated to the interaction of the analyte with silver. Figure 9 shows the spectra obtained during the essays with supports F1–F3. The characteristic pyridine bands observed in the spectrum of the 1 mM pyridine solution are clearly appreciable on the spectra of 1 nM pyridine placed on the silica–hydroxyapatite–silver supports. On the 2 min doped support there is an important band at 1024 cm^{−1} due to ν_3 vibrational mode of the phosphate group in the hydroxyapatite of the substrate. Bands at 983, 874 and 559 cm^{−1} (see Table 2), which are assigned to the ν_3 mode of silicate, the Si–O–Si bond’s asymmetrical stretching and the ν_4 mode of phosphate, become attenuated as the electrodeposition time rises, indicating that those modes could be affected by electromagnetic enhancement because of the proximity of the fibers to the silver nanoparticles. It is imperative to point that several bands between 1080 and 1003 cm^{−1} that belong to the pyridine could possibly be overshadowed by the phosphate modes from the support, particularly the band at 1020 cm^{−1} attributed to transition 12. The bands at 992 and 943 cm^{−1} may owe their magnitude to a combined contribution from the phosphate modes and pyridine (see Table 2).

The enhancement was defined depending on the area beneath the curve of the main bands, through the expression in Equation (1):

$$EF = \frac{I_{SEIRAS}C_{FTIR}}{C_{SEIRAS}I_{FTIR}} \quad (1)$$

Equation (1): enhancement factor (FA) determination, I_{SEIRAS} being the intensity of a band, expressed in terms of its area, obtained the analyte of concentration C_{SEIRAS} on the substrate; I_{FTIR} corresponds to the intensity of the same band for the analyte in conditions foreign to the substrate at a concentration C_{FTIR} .

Figure 9. Infrared spectra of 1 mM pyridine on the supports doped for 2, 3 and 5 min in a 10 mM AgNO_3 solution at 1.5 V, and of 1 mM pyridine (black).

Table 2. Main infrared bands and the vibrational modes they belong to from a 1 mM pyridine solution placed on the SiO_2 –HA doped at different time intervals (2, 3 and 5 min) using 1.5 and 3.3 V at 10 mM of AgNO_3 ; and 3.3 V at 20 mM of AgNO_3 .

Condition: 1.5 V at 10 mM		Condition: 3.3 V at 10 mM		Condition: 3.3 V at 20 mM	
Wavenumber (cm ⁻¹)	Vibrational Mode	Wavenumber (cm ⁻¹)	Vibrational Mode	Wavenumber (cm ⁻¹)	Vibrational Mode
3348	C–H ν	3337	C–H ν	3362	C–H ν
1639	8a	1638	8a	1638	8a
1527	8b	1466	8b	1541	8b
1406	19b	1450	19b	1456	19b
1024	$\text{PO}_4^{3-} \nu_3$	1046	$\text{PO}_4^{3-} \nu_3$	1019	$\text{PO}_4^{3-} \nu_3$
984	$\text{SiO}_4^{4-} \nu_3$	986	$\text{SiO}_4^{4-} \nu_3$	983	$\text{SiO}_4^{4-} \nu_3$
874	Si–O–Si ν_{as}	876	Si–O–Si ν_{as}	860	Si–O–Si ν_{as}
–	–	–	–	788	$\text{SiO}_4^{4-} \nu_1$
711	11	713	11	708	11
559	$\text{PO}_4^{3-} \nu_4$	561	$\text{PO}_4^{3-} \nu_4$	558	$\text{PO}_4^{3-} \nu_4$

By SEIRAS, enhancement factors up to 1.93×10^6 for a band at 711 cm^{-1} , related to the C–H stretching, and 1.16×10^6 for the band at 1639 cm^{-1} , concerning the C–N stretching in pyridine, were both originated on the 3 min doped support. In that same device appears a low intensity shoulder at 1468 cm^{-1} because of the enhancement of pyridine's mode 19a, which corresponds to the stretching of C–N and C–C. The latter vibration mode mitigates in the spectrum of the 5 min doped support and the band at 1406 cm^{-1} for the C–C stretching intensifies. Infrared spectra of the pyridine set on the support series doped at 3.3 V with 10 mM silver nitrate (F4–F7) are shown in Figure 10. A more pronounced tendency for the bands at the 1100 – 500 cm^{-1} region in comparison to the series doped at 1.5 V is observed, peculiarly in the band at 1046 cm^{-1} , manifesting the presence of silica and hydroxyapatite vibrational modes. Only the 2 min doped support presented an enhancement for the 8a and 11 modes localized at 1638 and 713 cm^{-1} , with factors of 1.32×10^6 and 1.49×10^6 , respectively. In addition, all three substrates exhibited a broad and low-intensity band around 1466 cm^{-1} , generated by transition 19a, and a shoulder close to 1450 cm^{-1} , due to pyridine's mode 19b (see Table 2). Li et al. [35] solely report the bands 1603 , 1445 and 1447 cm^{-1} during a SEIRAS essay with pyridine adsorbed on a silver

electrode. They also pointed a band-shifting for mode 19b from 1437 to 1447 cm^{-1} , comparable to the deviation in the spectra on Figure 10, indicating a coordination bond between silver and the nitrogen-free electron pair.

Figure 10. Infrared spectra of 1 mM pyridine on the supports doped for 2, 3 and 5 min in a 10 mM AgNO_3 solution at 3.3 V, and of 1 mM pyridine (black).

Figure 11 highlights a more uniform behavior for all the bands in every support. A probable cause is the high silver accumulation generated by doping with 20 mM silver nitrate, which was demonstrated to minimize the morphological cluster variation among distinct time intervals and thus lead to the aggregation of spherical nanoparticles, as observed in the micrographs. Table 2 summarizes the major bands found in the IR spectra of the F7–F9 series and the vibrations they belong to. A broadening for the C–H stretching band at 3362 cm^{-1} occurs as the electrodeposition time increases. The bands at 1638, 1541, 1456 and 708 cm^{-1} related to their respective pyridine transitions 8a, 8b, 19b and 11 are noticeable in all three supports. The 2 min doped support displays the highest enhancement for the bands at 1541 cm^{-1} , owning a factor of 1.09×10^6 , and 708 cm^{-1} , with a factor of 1.34×10^6 . Bands at 1019 and 558 cm^{-1} corresponding to the phosphate modes ν_3 and ν_4 , and at 983, 860 and 788 cm^{-1} , related to the ν_3 , asymmetric stretching and ν_1 silicate transitions are present on the three spectra, presenting an amplitude increment in the 3 min support graph, suggesting the enhancement of such modes or even pyridine modes that help the enlargement of these bands.

The variation in the modes enhanced amidst different supports is caused because of the fact that a determined adsorbed pyridine orientation over the metal nanoparticle favors the coupling of the localized electric fields to specific vibrational transitions, and is consistent with the presence of diverse morphologies including spheres, cubes and rods or needles observed in the supports doped for different time intervals. The amplification effect originates from the excitation of the electrons at the surface of metal nanoarrays due to their interaction with the electromagnetic field of the incident beam. The resonance between the plasmon at the metal and the excitation radiation generates localized electric fields that, thanks to their proximity to the adsorbed analyte, couple with the vibrational transitions of pyridine and magnify its spectral signal [36,37].

Figure 11. Infrared spectra of 1 mM pyridine on the supports doped for 2, 3 and 5 min in a 20 mM AgNO_3 solution at 3.3 V, and of 1 mM pyridine (black).

As displayed in Table 3, the top enhancement factor was 2.01×10^6 for the band at 3335 cm^{-1} in the F4 support, doped with 10 mM silver nitrate at 3.3 V for 2 min. The same substrate presented the enhancement of the bands at 1639 and 711 cm^{-1} . Here, the enhancement effect is assigned mainly to the hot spots on the surface of the fibers generated by silver nanospheres and flakes distributed along the fibers, as appreciated in micrographs. Support F2 also presented the enhancement of these three main bands. In this case, enhancement is linked to the presence of nanocubes gathered over the substrate, whose pointy features and sharpened edges favor charge accumulation and the advent of hot spots [38]. Despite support F6 showed to have the higher number of cubic nanostructures and defined crystals compiled, its infrared enhancement turned poor in comparison, because of circumstantial factors such as the absence of highly doped regions, and the consequence of non-uniform metal clusters' distribution. The rest of the supports demonstrated the enhancement of at least one band. Supports F5 and F9 did not show apparent amplification of any characteristic pyridine band. Table 3 indicates how all enhanced vibrational transitions can be classified into modes with A_1 or B_1 symmetry [39]. The amplification of A_1 modes by supports F2, F4 and F6 suggests molecular orientation perpendicular to the metal surface, typically referred to as “end-on” orientation, which hints the formation of a bond via the lone electronic pair of nitrogen with silver [40].

Table 3. Registered enhancement factors for the main bands of the FTIR spectrum of pyridine.

Support	3335 cm^{-1}	1639 cm^{-1}	711 cm^{-1}
Symmetry	A_1	A_1	B_1
F1	—	—	1.83×10^6
F2	1.12×10^6	1.16×10^6	1.93×10^6
F3	—	—	1.60×10^6
F4	2.01×10^6	1.32×10^6	1.49×10^6
F6	1.02×10^6	—	—
F7	—	1.09×10^6	1.34×10^6
F8	—	—	1.00×10^6

3.8. SERS Activity Evaluation

The SERS activity of all supports was evaluated by comparing the Raman spectrum of 1 mM pyridine tested in conditions foreign to the silica–hydroxyapatite–silver substrate with the spectrum of

1 nM pyridine placed upon the supports doped at different time intervals (2, 3 and 5 min). Figure 12 shows the SERS spectra of 1 nM pyridine evaluated on the series of supports doped at 1.5 V (F1–F3). Several enhanced bands can be, as described in Table 4. Bands at 586 and 1097 cm^{-1} on the 1 mM pyridine spectrum are highlighted: they belong to the 6a and 18a modes, respectively, which represent the distortion and stretching of the aromatic ring. As observed, support F3, doped for 5 min, exhibits a considerably superior enhancement of these bands with respect to the other supports, as well as for those bands located at 963, 1340 and 1593 cm^{-1} , attributed to the modes 5, 14 and 18a, related to the C–H wagging, stretching and in-plane aromatic ring bending, and C–N/C=N stretching. Only the bands for the modes 6a and 5 suffered a slight red-shifting in comparison to the normal Raman vibration modes [34], while the transitions correlated to the ring bending and stretching experienced a blue shifting. Bindhu et al. [41] says that the shift of the ring stretching modes suggests that the pyridine adopted an end-on orientation on the surface of silver. They also pointed out that a displacement of Raman normal modes occurs because of the modification of the adsorbate's structure caused by the overlapping of its molecular orbitals with the metal orbitals.

Figure 12. Surface-enhanced Raman spectroscopy (SERS) spectra of 1 nM pyridine on supports doped at 2, 3, and 5 min with 10 mM AgNO_3 at 1.5 V, and Raman spectra of 1 mM pyridine (black).

Figure 13 shows the SERS spectra obtained from 1 nM pyridine on supports F4–F6. A clear band sharpening in the region from 500 to 1600 cm^{-1} is especially visible on the spectra from the 2 and 3 min doped supports. The spectrum of support F4, deposited for 2 min, shows prominence in the bands at 595, 960, 1082, 1372 and 1595 cm^{-1} , assigned to the pyridine modes 6a, 5, 18a, 14 and 8a. In contrast, the spectrum of the 3 min doped support rises with a superior resolution degree for several bands and less spectral noise. The transitions 11, 10a, 12, 15, 3, 14 and 8b, localized at 719, 846, 1041, 1174, 1260, 1372 and 1539 cm^{-1} are acutely identified. Support F6, doped for 5 min, did not evidence the amplification of the vibrational modes 6a and 18a observed on the spectrum of 1 mM pyridine, respectively, located at 586 and 1097 cm^{-1} . Nonetheless, the bands at 1202, 1485 and 1611 cm^{-1} for vibrations 9a, 19a and 8a are sufficient. The other vibration modes presented in Figure 13 are enlisted in Table 4. Although the pyridine molecule could adopt a parallel orientation to the adsorbent surface, interacting through the electrons in the aromatic ring, it is well known that the formation of an N–Ag bond, perpendicular to the silver face, is the geometric configuration most energetically stable [42]. This is the case of pyridine adsorbed upon the supports of this series, considering that the bands enhanced by supports F4 and F6 relate to stretching transitions of C–C, C–N and C=N, and nearly all modes in support F5 are attributed to in-plane bending, stretching and aromatic ring breathing vibrations. It is worth noting that modes 3 and 10a seen in the 3 min doped substrate are equivalent

to C–C and C–N stretching, as well as C–H bending, both out of the plane. Out-of-plane vibrations indicate a planar pyridine orientation and the possible development of a multilayer [40,41].

Table 4. Main Raman bands and their corresponding vibrational modes of a 1 nM pyridine solution placed on SiO₂–HA doped with a 10 mM AgNO₃ solution during different time intervals (2, 3 and 5 min) using 1.5 and 3.3 V at 10 mM of AgNO₃; and 3.3 V at 20 mM of AgNO₃.

Condition: 1.5 V at 10 mM		Condition: 3.3 V at 10 mM		Condition: 3.3 V at 20 mM	
Wavenumber (cm ^{−1})	Vibrational Mode	Wavenumber (cm ^{−1})	Vibrational Mode	Wavenumber (cm ^{−1})	Vibrational Mode
586	6a	595	6a	572	6a
690	11	719	11	747	4
–	–	846	10a	844	10a
963	5	960	5	938	10b
–	–	982	17a	–	–
1034	12	1041	12	–	–
1097	18a	1082	18a	1092	18a
–	–	1174	15	1174	15
1203	9a	1207	9a	–	–
–	–	1260	3	–	–
1340	14	1372	14	1336	14
–	–	1487	19a	1428	19b
–	–	1539	8b	1571	8b
1593	8a	1595	8a	1607	8a

Figure 13. SERS spectra of 1 nM pyridine on supports doped at 2, 3, and 5 min with 10 mM AgNO₃ at 3.3 V, and Raman spectra of 1 mM pyridine (black).

Figure 14 is shown next, presenting the SERS spectra of pyridine put on the supports doped with 20 mM silver nitrate at 3.3 V (F7–F9). The spectrum of the 2 min support is highly active, emphasizing the bands at 747, 844, 938, 1174 and 1428 cm^{−1} referent to the modes 4, 10a, 10b, 15 and 19b, respectively. The 3 min doped support spectrum discloses the enhancement of the bands at 572, 952, 1336, 1571 and 1607 cm^{−1} for the pyridine modes 6a, 10b, 14, 8b and 8a. The spectral strength decays drastically once the electrodeposition time reaches 5 min. Substrate F9 owns defined but low intensity bands compared to its precursors at 860, 1031, 1135, 1386 and 1608 cm^{−1} for vibrational modes 10a, 12, 15, 14 and 8a (see Table 4). As a result of the preferential enhancement of modes 10b (C–H in-plane wagging) and 15 (C–C–H bending) from support F7, and of modes 8a (C–C stretching), 12 (aromatic ring breathing)

and 14 (C–C–H in-plane bending) from F9, a perpendicular orientation to the silver nanoparticle surface is suggested for adsorbed pyridine. The existence of an intense band linked to the out-of-plane stretching (mode 8b) in the spectrum of support F8 indicates the combination of various orientation types for pyridine, involving different bond angles to silver [33,43–45]. All supports presented SERS activity. When the radiation interacts with metal nanoparticles smaller than the incident wavelength, a displacement in the electronic density occurs and the particle polarizes, turning into a dipolar antenna that emits light. Surface plasmons are excited when the excitation wavelength is resonant with the plasmon absorption profile in the nanoparticle. Consequently, a strong electromagnetic field is induced and the Raman modes of a molecule nearby the metal surface are drastically intensified [46].

Figure 14. SERS spectra of 1 mM pyridine on supports doped at 2, 3, and 5 min with 20 mM AgNO_3 at 3.3 V, and Raman spectra of 1 mM pyridine (black).

Figure 15 and Table 5 show the behavior of the calculated four bands, at 1264, 1355, 1480 and 1567 cm^{-1} , as a function of silver electrodeposition time for each device. It is necessary to underline that an inverse performance is shared for all bands among the supports doped with 10 mM silver nitrate and 1.5 V, and those doped with a 20 mM silver nitrate and 3.3 V: in the first case, the area climbs with a positive slope as the doping interval increases, with a maximum at 5 min, while in the second case the calculated area reaches its maximum earlier, at 2 min of electrodeposition; from there, it drops until 5 min, where amplification is practically null. On the other hand, the area of bands at 1480 cm^{-1} (Figure 15b) and 1567 cm^{-1} (Figure 15d) for the series doped with 10 mM silver nitrate and 3.3 V finds its top value after 3 min of doping and later it descends significantly. The area of the bands at 1264 (Figure 15a) and 1355 (Figure 15c) from this same series maximizes at 2 min and after that it radically decreases after 5 min of doping. Such behavior leads to note that at minor voltages the silver reduction rate on the fibers is low, and at short time intervals, nucleation is supported and hence producing a sequential growth by silver cores stacking.

Figure 15. Calculated areas, in arbitrary unities (a.u.) for the bands: (a) at 1264 cm^{-1} , (b) at 1355 cm^{-1} , (c) at 1480 cm^{-1} and (d) at 1567 cm^{-1} of a 1 mM pyridine solution (0 min) and a 1 nM pyridine solution on each spectrum obtained from the $\text{SiO}_2\text{-HA-Ag}$ supports doped for 2, 3 and 5 min.

Table 5. Calculated areas of the SERS enhanced bands.

Support	Time (min)	Bands (cm^{-1})			
		1567	1480	1355	1264
		Area (a.u.)	Area (a.u.)	Area (a.u.)	Area (a.u.)
1.5 V 10 mM	2	6.78×10^2	2.69×10^3	3.39×10^3	3.08×10^3
	3	7.64×10^2	1.53×10^3	2.48×10^3	2.11×10^3
	5	2.17×10^5	1.86×10^5	2.61×10^5	2.07×10^5
3.3 V 10 mM	2	8.24×10^4	9.30×10^3	9.17×10^4	6.16×10^3
	3	2.84×10^4	2.48×10^4	4.90×10^4	2.17×10^4
	5	1.09×10^4	1.33×10^4	9.28×10^3	8.72×10^3
3.3 V 20 mM	2	1.12×10^5	1.03×10^5	1.15×10^5	9.70×10^4
	3	1.02×10^5	8.19×10^4	9.85×10^4	8.56×10^4
	5	2.90×10^3	2.61×10^3	2.65×10^3	2.47×10^3

Accordingly, at early doping stages the enhancement of the electromagnetic field over precise spherical nanoparticles, as the microscopy analysis points out, is subtle compared to the response generated once the silver nanostructures accumulates in the form of rods and needles. Although the voltage intensification to 3.3 V suggests an elevated silver crystallization rate, allowing the observation of nanocubes growth, the employment of an elevated nitrate concentration and the prolongation of doping time resulted in the amassing of larger deposits over the substrate, which inhibits the

generation of localized hot spots. The enhancement factor (EF) for each selected band was calculated in an analogous way to the SEIRAS analysis, using Equation (1). Table 6 shows details for the factors for the main four bands on the 1 nM pyridine spectrum resulting from the essays carried out with each one of the nine experimental supports. As appreciated, the highest enhancement factor was 3.46×10^8 , obtained from support F3, doped for 5 min with 10 mM silver nitrate at 1.5 V, for the band at 1567 cm^{-1} , however all the bands on this substrate presented a factor in the order of 10^8 . The degree of amplification in support F3 is primarily attributed to the presence of silver nanorods and needles over the fibers. The Raman amplification effect due to nanorods has been already tested [47], obtaining enhancement factors up to 1.44×10^8 in essays with $1.6 \times 10^{-7} \text{ M}$ Nile blue in the presence of nanorods synthesized by silver seeds growth aid by Cetyl Trimethyl Ammonium Bromide (CTAB). The overlaying of the transversal and longitudinal plasmon bands constitutes the basis of electromagnetic enhancement on nanorods, so that the top SERS activity expresses in perpendicular direction to the major axis [48].

Table 6. Enhancement factors obtained for four Raman bands of pyridine placed on the $\text{SiO}_2\text{-HA-Ag}$ supports.

Bands (cm^{-1})	1.5 V–10 mM			3.3 V–10 mM			3.3 V–20 mM		
	2 min	3 min	5 min	2 min	3 min	5 min	2 min	3 min	5 min
1264	1.88×10^6	1.28×10^6	1.26×10^8	3.76×10^6	1.33×10^7	5.32×10^6	5.92×10^7	5.22×10^7	1.50×10^6
1355	2.68×10^6	1.96×10^6	2.06×10^8	7.24×10^7	3.87×10^7	7.33×10^6	9.06×10^7	7.78×10^7	2.10×10^6
1480	2.85×10^6	1.62×10^6	1.98×10^8	9.89×10^6	2.63×10^7	1.41×10^7	1.09×10^8	8.70×10^7	4.15×10^6
1567	1.08×10^6	1.22×10^6	3.46×10^8	1.31×10^8	4.52×10^7	1.74×10^7	1.78×10^8	1.62×10^8	3.08×10^6

Silver nanocubes are considered as excellent scaffold for enhanced Raman spectroscopy. Zeng et al. [49] detailed a SERS test using elongated silver cubes (nanobars), made by seed growth assisted by growing control agents and 1, 4-benzenedithiol, obtaining an EF of 8.6×10^4 ; Tegegne et al. [50] performed the synthesis of silver nanocubes with a side length of 50 nm by the polyol route, applied to the detection of deoxynivalenol, where they registered a maximum EF of 1.82×10^7 , mentioning how a previous polydopamine coating increased the analyte's adsorption capacity and therefore its signal magnification, pointing to the relevance of the chemical enhancement; Kundu et al. [30] used silver nanocubes with a side length of $335 \pm 5 \text{ nm}$, obtained by the reaction of silver nitrate with the sodium salt of poly (styrene sulfonate) in the presence of gold seeds, and achieved an EF of 1.04×10^5 for a band of 1 mM blue methylene. They highlight size as a decisive factor above the nanoparticles' shape and architecture by comparing the EF obtained for the same band adsorbed on 9 nm diameter nanospheres, resulting two orders of magnitude higher than the factor exhibited by nanocubes. Bhattacharjee et al. [28] stated an EF of 7.52×10^9 for 4-mercaptobenzoic acid with silver nanocubes with a gold core and described how the close proximity between the excitation wavelength and the surface plasmon band on the nanocubes contributed to such elevated enhancement. Finally, Ben-Jaber et al. [51] informed the enhancement factors of 1.19×10^{11} for Rhodamine-6G and 9.26×10^{10} for cyclotrimethylenetrinitramine in tests with 153 nm-long silver nanocubes. The polyol technique is frequently used for the synthesis of silver nanocubes [52]. Zhang et al. [53] developed the synthesis of silver nanocubes with a side length between 30 and 700 nm by a modified polyol method, using silver trifluoroacetate as a precursor; Sun and Xia [54] synthesized $175 \pm 13 \text{ nm}$ -long nanocubes working with ethylene glycol at high temperature; Chang et al. [55] employed this polyol technique to make 70–80 nm-long nanocubes, and Huang et al. [56] produced 2 nm-long nanocubes by UV irradiation.

From the two mechanisms responsible for the Raman enhancement, charge transfer, also called chemical interaction, depends on the interaction between the analyte molecule and the metal surface, and provides only in a factor of 10–100 to the final amplification, whereas the electromagnetic enhancement contributes in a factor of 10^{10} . Electromagnetic enhancement is influenced in turn by the close field properties and is subject to the electric field distribution on the nanoparticle. The main reason of why pointy architectures and sharp-edged features, such as nanocubes, produce amplification spots is because they promote the plasmon red-shifting by its oscillation rate attenuation, which follows the

charge separation by the accumulation of electrons on those spikey structures [29,31]. In the present research, the interaction involving the pyridine and the silver nanoparticles is flagrant, as most of the observed transitions are shifted with regard to the normal Raman modes, so it is recognized that the existence of a charge transfer contribution aids the final spectral response. Concerning the factors implied in the electromagnetic enhancement, it is considered that the incisive features, for instance the pointed corners and prominent borders from the cubic morphology of silver settlements, decidedly supported the intensity raise of the pyridine Raman modes. The size of those nanocubes could have inhibited the obtention of higher enhancement factors. Moskovits [57] determines that, for coinage metals, the optimal size of the SERS responsible nanoparticle is from 10 to 100 nm, lower than the incident wavelength but larger than the conduction electron's path.

The SERS activity of the substrates with outstanding performance (F3, F4 and F7) was tested again with a 10^{-4} M crystal violet solution ($C_{24}H_{28}N_3Cl$). The Raman spectrum of the analyte of interest is shown in Figure 16. Nine of its key vibrational modes are significant. Table 7 approaches with clearness the location and nature of those vibrations. It is also noticed that the baseline of the spectrum presents an acute slope due to the high degree of fluorescence. Background fluorescence emerges as spectral noise and is generated because the emission band of crystal violet reaches its maximum at about 575 nm, very close to the excitation wavelength from the laser used for the SERS essay at 532 nm [58]. A comparison between the SERS spectra from the tested supports is outlined in Figure 17. There, no evident difference was noticed between the spectrum of the sample in support F7 and the crystal violet spectrum by itself. Both share a comparable slope, indicating that fluorescence still prevails upon the substrate doped with an elevated concentration of silver nitrate. It was deduced that the great nanoparticle clusters, mainly spheroids, do not sustain an electric field intensity suitable for the magnification of Raman signals, neither an unimpeded surface for the adsorption of crystal violet. Alternatively, fluorescence disappears in supports F3 and F4, though the intensity of the primary bands results insignificant compared to the Raman spectrum of the sample. Table 7 indicates the enhancement factors gained for the bands of crystal violet. As seen, all factors are less than 1, which is translated into a seemingly null amplification.

Figure 16. Raman spectrum of a 10^{-4} M crystal violet acquired under conditions foreign to the SiO_2 -HA-Ag substrate.

Table 7. Enhancement factors acquired for the bands of 10^{-4} crystal violet on supports F3, F4 and F7 and the main vibrational modes these bands belong to.

Bands (cm^{-1})	Vibrational Modes	F3	F4	F7
207	$\tau(\text{CH}_3)$	0.11	0.19	0.15
432	$\delta(\text{CNC})$	0.11	0.21	0.15
812	C-H w *	0.10	0.20	0.14
917	$\delta(\text{CC}_{\text{central}}\text{C})$	0.09	0.64	0.15
1183	$\nu_{\text{as}}(\text{CC}_{\text{central}}\text{C})$	0.09	0.22	0.15
1304	$\nu_{\text{as}}(\text{CC}_{\text{central}}\text{C})/\delta(\text{CCC}_{\text{ring}})/\delta(\text{CH})$	0.08	0.22	0.13
1370	$\nu_{\text{as}}(\text{CC}_{\text{central}}\text{C})/\delta(\text{CCC}_{\text{ring}})/\delta(\text{CH})$	0.07	0.21	0.13
1452	$\delta_{\text{as}}(\text{CH}_3)$	0.08	0.23	0.12
1471	$\delta_{\text{as}}(\text{CH}_3)$	0.07	0.21	0.11
1546	$\nu(\text{C}_{\text{ring}}\text{N})/\delta_{\text{as}}(\text{CH}_3)$	0.07	0.23	0.11
1590	C=N/C-C _v	0.07	0.22	0.13
1623	C=N/C-C _v	0.06	0.20	0.11

* Wagging vibration.

Figure 17. SERS spectra of the 10^{-4} M crystal violet placed on supports F3, F4 and F7, and the Raman spectrum of 10^{-4} M crystal violet (black), confined to a spectral interval between 1200 and 1700 cm^{-1} .

The SERS substrate with less sensitivity to crystal violet is marked. The phenomenon may be due to a lower interaction of the violet crystal with silver at this wavelength, Chadha et al. [59]. In 2013, they demonstrated that the SERS spectra of CV adsorbed on Ag and Au nanoparticles increase in intensity with increasing laser excitation wavelengths of 532 and 660 nm. Even when none of the supports exhibited an increase in the area on the bands studied, supports F3 and F4 indeed displayed more defined spectra for crystal violet. From the 31 vibrational transitions for crystal violet studied in detail by Cañamares et al. [60], at least 15 are clearly identifiable on the spectra of F3 and F4 (Figure 18) while only nine are visible on the spectrum of the analyte placed out of the supports. A reason that would explain these low enhancement factors is the significant contribution of fluorescence to the band's amplitude, as denoted by the elevated intensity count in the spectrum plot of Figure 16, an issue that aided the Raman bands to be superior to the SERS bands. In that sense, the Raman enhancement effect has proved to weaken fluorescence [61]. Additionally, the strength of the Raman dispersion varies according to the adsorption angle of the analyte to the substrate surface; in this case, the analyte molecules own three phenyl rings bonded to a central carbonium [62]. The sensitivity increment

provided by F3 and F4 is owed to the piercing features of the nanoarchitecture of the supports and to a favorable analyte–substrate interaction. High SERS yields have been demonstrated in studies carried out with the nanoarrangements produced by this approach, primarily for nanocubes with a side length around 100 nm. The amount and form of silver particles electrodeposited on the fibers modify the number of hot spots in the substrate, causing the substrate to have a greater amplification effect.

Figure 18. SERS spectra of 10^{-4} M crystal violet placed on supports F3 and F4.

4. Conclusions

Coaxial silica–hydroxyapatite-based fibers, with a diameter of 685 ± 166 nm were developed by the sol–gel and electrospinning methods. The as-spun fibers were sintered at 1150 °C and their final diameter was 0.51 ± 0.2 μ m. Their microscopic and spectroscopic characterization revealed the presence of silica-substituted hydroxyapatite. Nine supports were produced through the electrodeposition of silver over the fibers placed on ITO glass, using two voltages, 1.5 and 3.3 V, and two silver nitrate concentrations, of 10 and 20 mM, at different times. The deposited silver nanoparticles presented variable architectures such as spheres, rods, needles, stars and cubes, as well as clusters. All supports presented SEIRAS activity. The larger enhancement factors were 2.01×10^6 and 1.93×10^6 for the bands of 1 nM pyridine at 3335 (C–H stretching) and 711 cm^{-1} (mode ν_{11}), respectively, acquired in the 2 min doped support, using 10 mM silver nitrate and 3.3 V. The supports also evidenced SERS activity, and an enhancement factor of 3.46×10^8 was reached for the band of 1 nM pyridine at 1567 cm^{-1} , in the 5 min doped support, employing 10 mM silver nitrate and 1.5 V. The nanorods and nanocubes, the latter with a side length of 303 ± 75 nm, proved being adequate substrates for Raman and infrared enhancement essays. The SERS activities of such supports was not evident after being tested with 10^{-4} M crystal violet. No enhancement factor was higher than 1, but the spectra showed better definition, demonstrating an improvement of the technique’s sensitivity and significantly weakening the fluorescence during the analysis. Thus, silver in the hydroxyapatite silica fibers significantly contributes to the amplification mechanism, and the effective range of signal amplification in the support studied was found near concentrations of 1 nM.

Author Contributions: Conceptualization, S.Y.R.-L.; methodology, S.Y.R.-L., R.F., and F.S.-N.; formal analysis, S.Y.R.-L., R.F., and F.S.-N.; investigation, S.Y.R.-L. and F.S.-N.; resources, S.Y.R.-L.; data curation, S.Y.R.-L., R.F., and F.S.-N.; writing—original draft preparation, S.Y.R.-L., R.F., and F.S.-N.; writing—review and editing, S.Y.R.-L., R.F., and F.S.-N.; visualization, S.Y.R.-L.; supervision, S.Y.R.-L.; project administration, S.Y.R.-L.; funding acquisition, S.Y.R.-L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: Thanks to PRODEP, Universidad Autónoma de Ciudad Juárez and CONACYT for supporting this investigation.

Conflicts of Interest: The authors declare that there are no conflict of interest regarding the publication of this paper.

Data Availability: The data used to support the findings of this study are available from the corresponding author upon request.

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