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Additive Synthesis by Sol-gel Method for its use as Antimicrobial

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Abstract

The antimicrobial activity of silver nanoparticles makes this chemical element attractive for their incorporation in different materials. Additionally, heteropolyacids (HPAs) have advantages as catalysts which make them environmentally very interesting. The objective of this work is to incorporate in a silica matrix obtained by sol-gel an HPA together with Ag, for their use as fillers in paints or adhesives. Six samples were synthesized, using tetraethoxysilane, methyltrimethoxysilane, acetic acid, molybdophosphoric acid and silver acetate. The silicas were characterized by potentiometric titration, XRD, SEM, TEM, S_{BET} and FT-IR. When HPA is used instead of acetic acid, a variation on the final acidity is produced. The specific surface area decreases when HPA is incorporated into the silica synthesized using tetraethoxysilane as precursor, noticing a slight increase when Ag is added because a different distribution of HPA in the matrix. By SEM, it was observed that the particle size is bigger when the silicas are synthesized with TEOS-HPA, but it still shows the characteristic morphology of bulk silica. Additionally, preliminary test to evaluate the antimicrobial properties of the solids were carried out with fungi as *Chaetomium globosum* and *Alternaria alternata*, isolated from contaminated paints, and the first results are encouraging to continue with the development of these materials.

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1. Introduction

α -quartz is the most abundant silica in the world and is present in a large number of rocks such as granite or sand. It can be found in pure state as rock crystal or with impurities (rose quartz, amethyst). Other less crystalline forms are chalcedony, agate and jasper. Less abundant crystalline modifications of silica are tridymite and cristobalite as well as diatomite (Maciá-Agulló et al., 1993).

Additionally, different synthetic silica exist on the market with a variety of properties and applications, such as fumed silica, which are applied in epoxy and polyurethane adhesives as fillers (Turallas et al., 2005; Martín Martínez et al., 2005; Musante et al., 2006; Vega-Baudrit et al., 2007).

Other important synthetic silica is the precipitated one, traditionally used as rubber reinforcing filler. Precipitated silica is used frequently to improve tear resistance or to reduce the heat generation by friction in rubber technical pieces. In the last years, the use of silica has been also extended to the tires of passenger cars, due to the availability of degrees of silica of high dispersion, which impart excellent wear resistance, low rolling resistance and good adhesion on wet surfaces or ice. Silica powder is natural silica obtained by sand grinding. It is used as reinforcing filler in coatings, sealants and adhesives. The incorporation of Ag nanoparticles into different materials is being widely studied due to their effective antimicrobial properties (Silver, 2003; Sharma et al., 2007; Egger et al., 2009; Duncan, 2011; Hasan et al., 2013). On the other hand, the catalytic properties of heteropolyacids (HPAs), have drawn wide attention in the last decades, especially those with Keggin structure that have several advantages as catalysts, besides being economically and environmentally attractive (Tatibouet et al., 1997; Kozhevnikov, 2007; Popa et al., 2010).

The objective of this work is to synthesize materials by sol-gel method from different alkoxide precursors, specifically tetraethoxysilane (TEOS) and methyltrimethoxysilane (MTMS), and to add a heteropolyacid together with Ag to give antimicrobial properties to the solids, which will be used as additives in hygienic paints and/or adhesives. The materials obtained were characterized by different physico-chemical techniques such as textural studies, estimation of acidic properties and morphological and structural characterization. The selection of the different compounds was always made trying to get a non-invasive environmental technology. It is also a "one pot" synthesis to comply with the principles of Green Chemistry (Anastas et al., 2008). Preliminary test to evaluate the antimicrobial properties of the solids were carried out with fungi as *Chaetomium globosum* and *Alternaria alternata*.

2. Experimental

The sol-gel method, under nitrogen atmosphere, was used to synthesize the solids based on silica. Six samples were obtained using tetraethoxysilane ($\text{Si}(\text{OCH}_3)_4$) (TEOS) and methyltrimethoxysilane ($\text{CH}_3\text{Si}(\text{OCH}_3)_3$) (MTMS) as silica precursors, in order to obtain different structures of siliceous gels. Acetic acid (AcH) and molybdophosphoric acid ($\text{H}_3[\text{P}(\text{Mo}_3\text{O}_{10})_4] \cdot x\text{H}_2\text{O}$) were used as catalyst whereas absolute ethanol ($\text{CH}_2\text{CH}_3\text{OH} - 99.9\%$) (EtOH) and distilled water were used as solvents in all the synthesis. In the synthesis with acetic acid the molar ratios of the reagents were 1:1.17:5:3.73 (precursor: catalyst: solvent: water). In the samples prepared using HPA, this reagent was added in order to obtain a final concentration of 11% w/w. The hydrolysis ratio R (water/precursor) was 3.7 for all the synthesized samples. Silver acetate (AgAc) was added in two samples in the necessary quantity to occupy the half of the acid sites of the HPA ($\text{H}_{1.5}\text{Ag}_{1.5}$).

The following experimental procedure was performed. A portion of solvent was put into a beaker with the acetic acid, the precursor was then incorporated and after, the addition of the remaining amount of ethanol proceeded. In the samples prepared with HPA, this compound was incorporated dissolved in the second amount of ethanol and, in the two samples containing silver, HPA was added together with the silver acetate. Subsequently, the slow addition of water was carried out and the mixture was stirred for 2 h. Finally, the wet gel particles were dried at room temperature and atmospheric pressure up to the complete solidification. The volumetric amounts of the reagents and the nomenclature for all the samples are shown in Table 1.

The estimation of the acidic properties of the solids was achieved by potentiometric titration with n-butylamine, carried out in a Metrohm 794 Basic Titrino titrator (made in Switzerland), with a double junction electrode. 0.025 ml/min of n-butylamine in acetonitrile (0.025 N) was added to 0.025 g of the sample previously suspended in acetonitrile (45 ml) and stirred for 3 h. The textural properties of the silicas, as the specific surface area of the solids

(S_{BET}), were determined in Micromeritics Accusorb 2100 equipment (USA), using N_2 as adsorbable gas. The X-ray diffraction (XRD) patterns were obtained in Philips (Holland) PW-1390 (channel control) and PW-1394 (motor control) equipment coupled to a scanning graphical recorder, using $Cu K_{\alpha}$ ($\lambda=1.5417 \text{ \AA}$) radiation, Ni filter, 20 mA and 40 kV in the voltage source, a 5-60 $^{\circ}2\theta$ scanning angle range, a scanning rate of 2 $^{\circ}$ /min and 2000 counts/sec for the amplitude of the vertical scale. Scanning electron microscopy (SEM) was achieved to obtain different micrographs of the solids, in Philips 505 equipment (Holland), using a voltage of 15 kV; samples were supported on graphite and metallized with a sputtered gold film. The micrographs were obtained with an ADDAII acquisition device (Soft Imaging System). FT-IR spectra were obtained using Bruker IFS 66 equipment and pellets of the sample in KBr, measuring in a range between 400 and 4000 cm^{-1} .

Table 1. Nomenclature and synthesis parameters of synthesized silica.

	ST	ST-H	ST-HAg	SM	SM-H	SM-HAg
TEOS (ml)	34	34	34	-	-	-
MTMS (ml)	-	-	-	22	22	22
EtOH (ml)	43.5	43.5	43.5	43.5	43.5	43.5
AcH (ml)	10	-	-	10	-	-
HPA (g)	-	1.5	1.5	-	1.5	1.5
AgAc (g)	-	-	0.2	-	-	0.2

3. Results and discussion

Digital photographs of the solids after one week of drying process are shown in Figure 1. It can be observed the change to yellowish color of the samples when the heteropolyacid is incorporated into the synthesis.

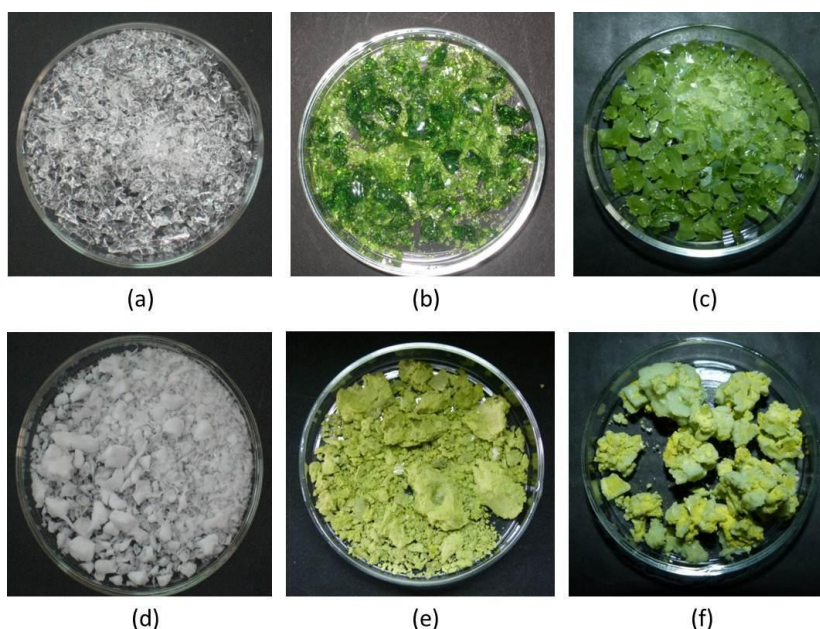


Figure 1. Digital photos of synthesized samples: (a) ST; (b) ST-H; (c) ST-HAg; (d) SM; (e) SM-H and (f) SM-HAg.

As for the estimation of the acidic properties, Figure 2 shows the potentiometric curves of the different samples and the curve corresponding to the commercial heteropolyacid. Potentiometric titration is based on the difference in the electrode potential (E) which is determined mainly by the acid environment around the membrane, therefore, the electrode potential measured is an indicator of the acidic properties of the dispersion of solid particles.

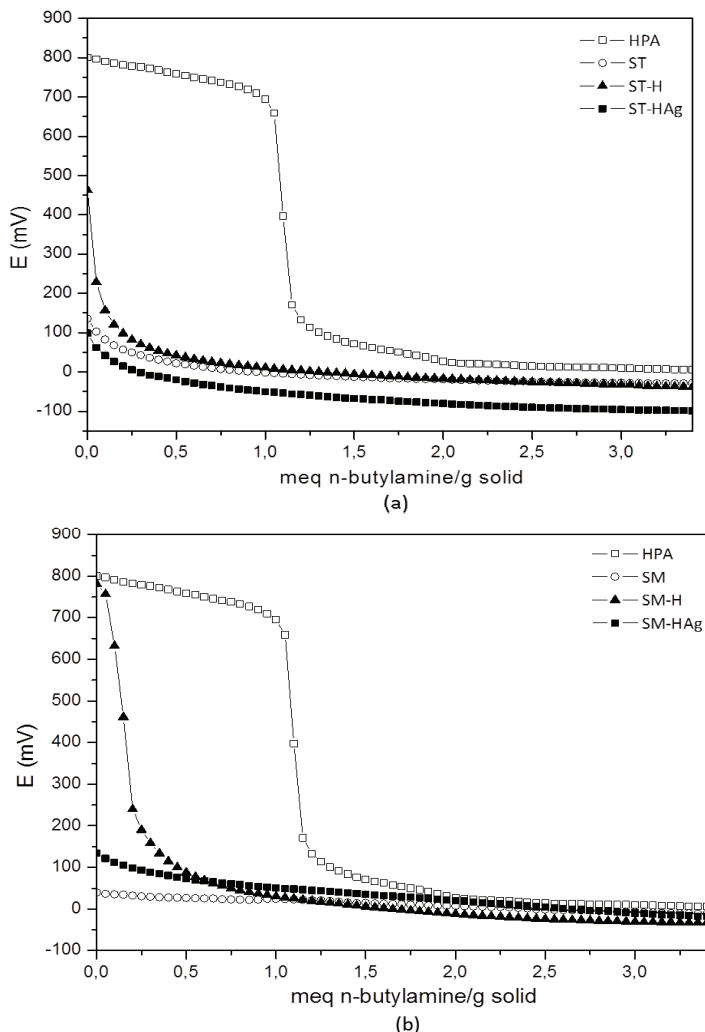


Figure 2. Potentiometric curves of the samples synthesized with: (a) TEOS and (b) MTMS as silica precursors.

The initial electrode potential (E_i) indicates the maximum acid strength of the sites and the value of meq amine/g solid, where the plateau is reached, indicates the total number of acid sites. On the other hand, the acid strength of the surface sites may be classified according to the following scale: very strong sites, $E_i > 100$ mV; strong sites, $0 < E_i < 100$ mV; weak sites, $-100 < E_i < 0$ mV; and very weak sites, $E_i < -100$ mV (Villabrille et al., 2002).

In the potentiometric curves, it is observed that the initial acid strength of the HPA is very high, producing an E_i of 800 mV. After replacing the acetic acid by the HPA as catalyst in the silica synthesis, there is an increased acidity as expected due to the properties of the latter. In the samples prepared using TEOS (Figure 2a), when Ag is added together with the HPA, the acidity of the material decreases noticeably (from 463 to 98 mV), which means that the Ag^+ salt of the $[\text{P}(\text{Mo}_3\text{O}_{10})_4]^{3-}$ anion is formed, without knowing the proportion in which the cation has replaced the protons in the acid. In the silicas obtained with MTMS as precursor (Figure 2b), the incorporation of HPA markedly increases the initial acidity of the sample, approaching the value of bulk HPA. This means that the HPA would not be included in the silica network, but it stays mainly on the surface when this precursor is used as the network forming agent. Finally, when the synthesis is performed adding Ag with the HPA, the initial acid strength decreases to 134 mV. It could be assumed that the salt formation takes place on the surface of the silica obtained with MTMS

and may be compared with the titration value obtained for the silica prepared using TEOS. This is an important point because Ag, as antimicrobial agent, must have contact with the environment and, at the same time, remaining stable in the network that contains it.

In the Figures 3a and 3b, the complete FTIR spectra of the obtained samples are shown.

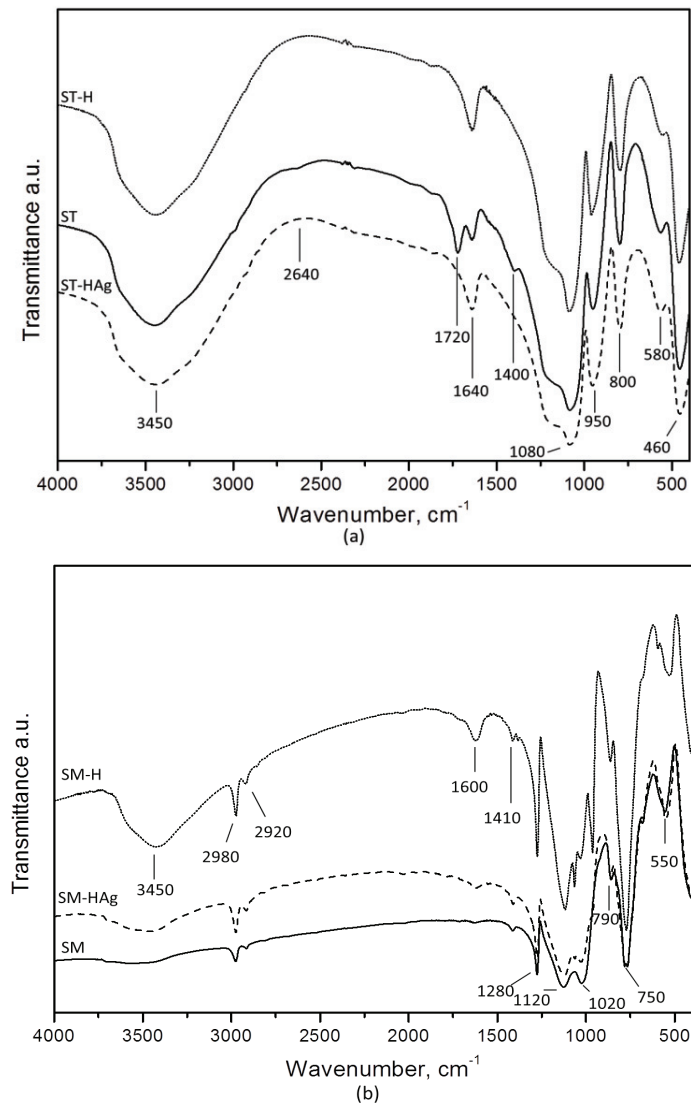


Figure 3. FT-IR spectra of the samples synthesized with (a) TEOS and (b) MTMS as precursors.

The absorption band at 1611 cm⁻¹, shown in Figure 3, is assigned to the vibration of non-protonated water molecules and the broad band between 3460 and 3540 cm⁻¹ is associated with the stretching vibrations of OH groups. In the synthesis with MTMS, two distinctive absorption bands are developed at 1410 and 1280 cm⁻¹ due to CH₃ groups provided by the organically modified precursor (Si-CH₃ bond). In order to see better and confirm the presence of the Keggin anion on the silica-HPA composites, the low-frequency FTIR spectra of the samples obtained with TEOS and MTMS were analyzed (Figures 4a and 4b). The HPA spectrum shows the characteristic bands at 1063, 962, 865 and 787 cm⁻¹. The oxygens in the Keggin anion are denoted as O_i (internal), O_t (terminal),

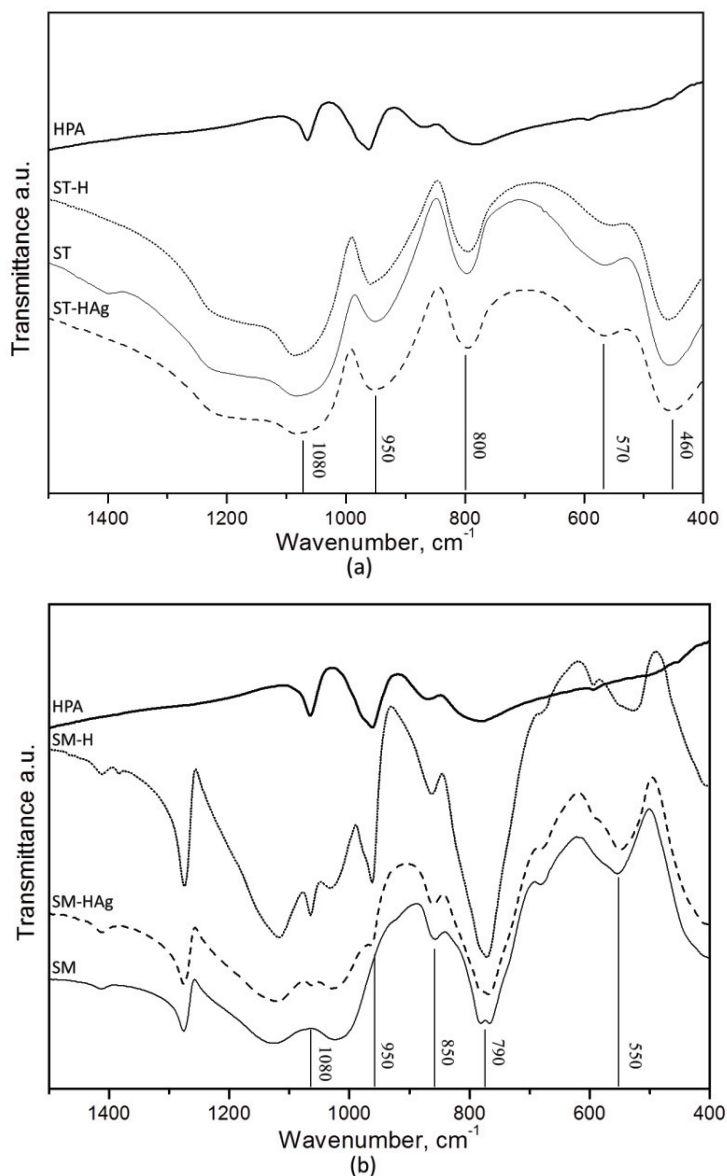


Figure 4. FT-IR spectra of the samples synthesized with (a) TEOS and (b) MTMS at low wavenumbers.

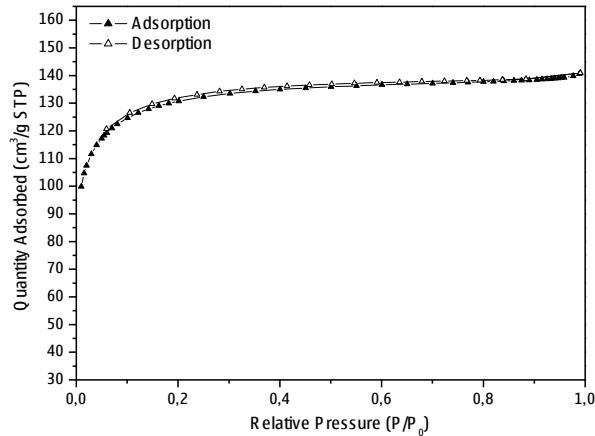
O_c (corner-shared) and O_e (edge-shared). The mentioned bands are assigned to the stretching vibrations of $P-O_i$, $Mo=O_i$, $Mo-O_c-Mo$ and $Mo-O_e-Mo$, respectively. It can be seen that the characteristic absorption bands of the HPA are preserved in samples in which it was incorporated, but they are broadened and partially overlapped with the strong absorption bands of silica (1080, 950, 800, 570, 460 cm^{-1}). Therefore, the incorporation of HPA into the silica matrix slightly affected the structure of the resulted solid.

The textural properties of the synthesized silicas are presented in Table 2. The values obtained for the samples synthesized by using MTMS cannot be reported because they are within the experimental error and the materials have the texture like an elastic gum, making it difficult to find the measurement conditions. In the samples prepared using TEOS, the specific surface area S_{BET} decreases when the HPA is included in the matrix. When it involves the formation of a silver salt of molybdophosphoric acid, the surface area increases by steric effects, as the radius of the

Table 2. Textural properties of the samples synthesized with TEOS.

Sample	S_{BET} (m^2/g)	Pore volumen (cm^3/g)
ST	464	0.22
ST-H	227	0.11
ST-HAg	316	0.02

compound included increases, i.e. acid compared to the salt, and intra-molecular spaces are greater. Particularly, the ST sample shows an adsorption isotherm corresponding to type I according to the classification proposed by Brunauer et al. (1940), characteristic of microporous solids (Figure 5).

Figure 5. N_2 adsorption-desorption isotherm of ST sample.

The X-ray diffraction patterns of the samples correspond to materials with amorphous morphology (Figure 6). The wide band with maximum placed around $23^\circ 2\theta$ is the typical diffraction of this type of silica (Pakizeh et al., 2007). It is important to note that, as an example, the patterns of the silica obtained with TEOS as precursor are presented. In the ST-H sample, it was observed the appearance of the characteristic signals of compounds with Keggin structure, in the interval between $6.5\text{--}10^\circ 2\theta$ (Popa et al., 2010), when it is incorporated into the silica.

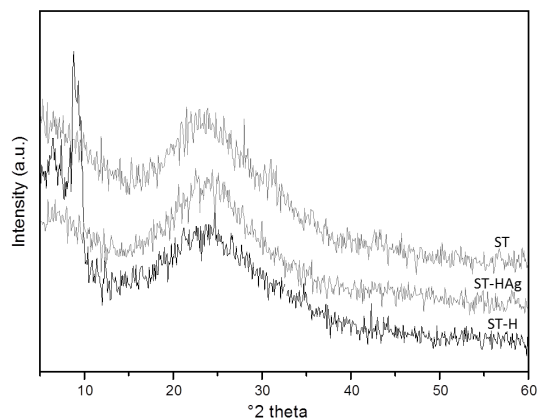


Figure 6. X-ray diffraction patterns of the solids obtained using TEOS as precursor.

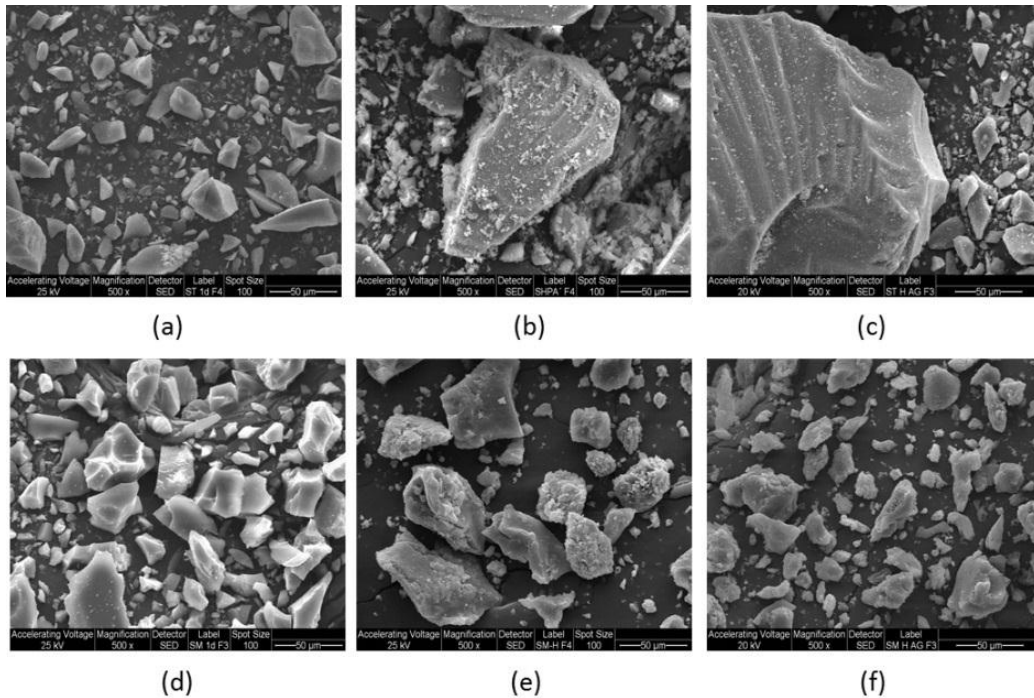


Figure 7. SEM micrographs of (a) ST; (b) ST-H; (c) ST-HAg; (d) SM; (e) SM-H and (f) SM-HAg silicas. Magnification 500x.

Following the characterization, Figure 7 shows the SEM micrographs obtained. As can be seen, the materials synthesized with TEOS maintain the laminar morphology characteristic of amorphous silica, while in the materials prepared with MTMS as precursor the presence of agglomerations is observed.

It is important to note that some antimicrobial tests were carried out with fungi as *Chaetomium globosum* and *Alternaria alternata*, isolated from contaminated paints, and the preliminary results are encouraging to continue with the development of these materials. Both fungi were assayed growing into solid culture medium (CM-agar), and different silver concentrations were kept constant into the Petri dishes (0.001%, 0.01% and 0.05% w/v). Control CM-agar without the solid was carried out at the same time. The CM-agar plates were incubated at 25 °C and after 10 days the colony diameter was recorded. Figure 8 shows, by way of example, the results obtained for ST-HAg sample (0.01%) compared with the control after 10 days at 25 °C.

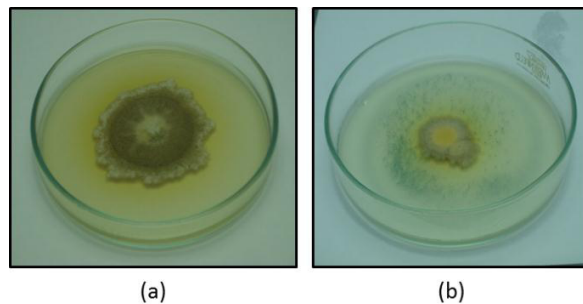


Figure 8. *Chaetomium globosum* growth on CM-agar plates after 10 days at 25 °C, (a) without and (b) with ST-HAg sample.

4. Conclusions

A homogeneous incorporation of the heteropolyacid in the silica matrix is observed when it is added in the synthesis. When acetic acid is replaced by the HPA an increase of acidity is produced due to the HPA properties. In the case of the addition of Ag, materials with lower acidity are obtained. It is observed a decrease of the specific surface area when HPA is incorporated into the silica synthesized with TEOS, but slightly increases when Ag is added due to a different distribution of the HPA into the silica matrix. For the samples prepared using MTMS, textural characteristics could not be obtained because the gummy texture of the final solids. By SEM, the particle size is higher when silicas are synthesized with TEOS-HPA, but the materials still have the characteristic morphology of bulk silica. In the samples obtained with MTMS as precursor, the presence of agglomerations is observed.

The addition of the solids into the CM-agar reduces the fungal growth. The preliminary tests of the materials provided encouraging results for the future use of these solids as additives in hygienic paints and/or adhesives.

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