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The study of some TiO₂ aerogels

PhD Thesis Summary



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Introduction

Aerogels are complex nanostructured materials with extremely porous structure, low density, very large surface area and big pores.¹ The highly porous materials have generally complex morphology and internal structure and their porous network consist in open and close pores with size distribution more or less sharp. In addition, a variety of shapes and connectivities of the pores, which defines the porous network can coexist within the same specimen, as well as a large diversity of ordering degrees. These characteristics are of utmost importance for developing materials with specific properties, the understanding of the morphological peculiarities having as primary consequence their efficient use for target applications.

This special class of strong cross-linked structures drew attention owing to their applications, such as gas sensors, manufacture of thermal isolators with exceptional properties, the photodegradation of polluting organic substances, detection of Cherenkov radiation and electronic devices.^{2,3,4} Because of the wide range of applications, the interest for studying nanostructures with high porosity is continuously increasing and therefore, the understanding of the structural particularities at nanoscale might enable the improvement of their performances. In the particular case of TiO₂ aerogels three crystalline phases exist, i.e. rutile, anatase and brookite. Anatase phase is of special interest due to its efficient involvement in applications like photocatalysis, electronic devices, bactericidal potential.⁵ Raman spectroscopy was found to be an excellent tool to investigate the TiO₂ anatase nano-crystallites size dependent properties. The tetragonal structure of anatase belongs to the space group D_{4h} and has two formula units per primitive cell, leading thus to six Raman active phonons: $3E_g (144, 196, and 638 \text{ cm}^{-1})$, $2B_{1g} (398 \text{ and }519 \text{ cm}^{-1})$, and $1A_{1g} (513 \text{ cm}^{-1})$.⁶

In the first section of my work special attention was generally focused on the way in which the position and full width at half maximum (FWHM) of the most intense Raman band located around 144 cm⁻¹ are influenced by crystallites dimension.^{5,6,7} It is well-known that this peak shifts to higher wavenumbers and its FWHM broadens as nanoparticle size becomes smaller. The influence of TiO₂ anatase nanosizes on the other Raman modes was relatively poorly investigated.⁶ However, one should emphasize that no investigation reports on the asymmetry of a certain TiO₂ anatase Raman band although it is fully accepted that this spectral parameter is affected by the nanocrystallites dimension.

Since the fundamental properties of nanomaterials depend on the nanometric dimension of the crystallites, identifying the critical size, where these properties are significantly modified, is extremely useful. In the case of TiO_2 anatase, nano-size effects like phonon confinement, phonon coupling, stoichiometry defects or strain, simultaneously contribute to the Raman signal,⁶ and finally, give rise to a certain critical size from which no variation of the spectral characteristics occurs. Typically, the value of the critical size for cumulative nano-size effects is less than 20 nm.⁷ To our knowledge, no study reports about the experimental analysis of the individual influence of the nano-size effects of TiO_2 anatase on the Raman spectral features, and principally, on the evaluation of the phonon confinement contribution alone.

Having in mind the above mentioned size dependent properties of TiO₂ nanocrystallites and their potential influence on various applications, the aim of the present study is to analyze several individual nano-size effects in TiO₂ anatase aerogels by using Raman spectroscopy and additional data provided by X-ray diffraction (XRD) measurements. Our particular interest is also focused on developing an experimental approach that can be applied only for the assessment of the phonon confinement contribution, when a symmetric size distribution exists. The investigations were carried out not only on the strongest 144 cm⁻¹ E_g mode, but also on the lowest B_{1g} and the highest E_g modes located at 398 and 638 cm⁻¹, respectively. In order to complete the information derived from the experimental analysis, a theoretical approach related to the phonon confinement was also applied by taking into consideration the most representative band located around 144 cm⁻¹.

Because during the last years TiO_2 based highly porous materials it was also extensively used in more complex structures, in particular as support in nanocomposites for different types of nanoparticles in the second section of my work it was investigated some TiO_2 -Ag aerogels. In this context, it was recently demonstrated that the presence of silver species, i.e. Ag^+ , Ag^0 , as well-defined nanoparticles or atomic clusters inside a TiO_2 porous structure improves and/or extends their properties of interest in potential applications^{8,9}. For example, these nanocomposites based on TiO_2 and Ag can simultaneously exhibit excellent photocatalytic performances, great ability for sensing minute concentration of chemical pollutants from contaminated water^{10,11} and antimicrobial effect, minimizing thus the risk of microbial contamination.^{12,13,14} Taking into consideration that the most important trends and expected breakthroughs in the manufacturing of advanced materials is the development of multifunctional structures, the understanding of the structural and morphological characteristics and ordering degree for such porous TiO_2 -Ag nanoarchitectures is crucial.¹⁵

Besides a relevant modification of the subsequent properties of the nanostructures made of Ag and TiO₂ compared to that of pure TiO₂, it was previously observed that the addition of Ag, even in small content, can also considerably influence the morphology and the microstructure of the TiO₂ porous material¹⁶. Among the different parameters sensitive to the insertion of Ag, one can mention the type of crystalline structure, the mean size of aggregates, the specific surface area, the type and the size distribution of the pores, their connectivity etc. In addition, the relative amount of the Ag species is one of the key parameters which govern the characteristics of the interface between the TiO₂ support and Ag nanoparticles, as well as the fraction of accessible (non-encapsulated) Ag particles. It is worth to mention that all these parameters are directly responsible for the most of the properties of interest of these porous nanostructured materials.

Generally, the transmission electron microscopy (TEM) techniques, applied in their 2D traditional modes (acquisition of an image, a diffraction pattern or a spectrum) have been extensively used to characterize the structure and morphology of nanomaterials at atomic scale. In the case of porous materials used generally as support for the insertion of a second phase in order to develop a particular function of the as-obtained composite, the crucial parameters are the 3D characteristics which describe their spatial organization, morphology and porous network. They are very difficult to obtain by analyzing simple 2D images, where all the details of the object are superimposed on the observation plane and the results is a thickness-integrated 2D projection. In this respect, 3D-TEM (or electron tomography) technique was found to be a powerful investigation tool in order to precise solve the mentioned parameters in the three directions of the space. It consists in the volume reconstruction of an object from a tilt series recorded in one of the various modes which exist in transmission electron microscopy: bright field (BF) conventional TEM, energy filtered TEM (EFTEM) imaging or high angle annular dark field (HAADF) in scanning TEM (STEM) mode.^{17,18} Basically, the fundamental benefit of the application of the tomographic techniques to the study of porous materials is obviously related to their ability to provide direct information at the nanoscale on the characteristics of the porous network (pore shape and accessibility, their size distribution) as well as on the 3D packing of the aggregates which compose the macroscopic material.¹⁹

In this context, to study the probable influence of the silver deposit on the 3D characteristics of the porous TiO₂ support, several samples with different content of silver were analyzed in the context of antibacterial effect. Moreover, the samples were submitted afterwards to a thermal treatment and the thermally-induced changes in morphology and structure of the initial aggregates were further proposed to be precisely determined. In order to provide a complete analysis and obtain reliable information at different scales, several complementary techniques have been used: on the one hand, the traditional selected area electron diffraction (SAED), high resolution transmission electron microscopy (HRTEM) and X-ray diffraction (XRD) which are able to furnish either local or more global structural information; on the other hand, the more specific 3D-TEM and N₂-sorption characterization tools, which gives access to the porous characteritstics of the TiO₂ support. Particular attention was paid to a consistent use of the electron tomography in a quantitative way, in order to fully characterize the porous network of the TiO₂ aerogel and to determine the characteristics of the Ag NPs (in terms of size and spatial localization within the support), as well as of the interface between the two components. In this framework, attempts to correlate the morphological and structural information with antibacterial effect of these samples tested tested on two types of bacteria: E. *Coli* and *Salmonella*. Depending on the results of these tests were selected for investigation by electronic tomography and nitrogen sorption four samples, considered representative. Because the results of these investigations were not relevant for interpreting test results antibacterial, investigations have been UV-Vis spectroscopy to identify the species of silver, STEM respectively to highlight atomic silver clusters and silver diffusion by heat treatment.

1. Experimental assessment of the phonon confinement

1.1. Samples preparation

TiO₂ gels were prepared by sol-gel method using titanium isopropoxide (TIP), HNO₃ as catalyst, EtOH and H₂O with 1/0.08/21/3.675 molar ratio. The gels were allowed to age for six weeks and supercritical dried with LCO₂ (T > 38° C and p = 1350 psi) using Tousimis Autosamdri 815 equipment. TiO₂ aerogels were subjected to a thermal treatment at 400, 425, 450, 485, 500, 512, 525 and 550 °C for 2h, and at 500 °C for 36h.

1.2. Sample measurements and data evaluation

1.2.1. XRD measurements and lattice parameters calculus

The TiO₂ nanocrystals were characterized by XRD for the phase identification and the estimation of their mean size. XRD patterns were obtained by using the CuK_{α} ($\lambda_{CuK\alpha}$ = 1.54178Å) radiation of a standard DRON-3M powder diffractometer, working at 40 kV and 30 mA.

The nanocrystallites diameter D was calculated by using the Scherrer formula:²⁰

$$D = \frac{0.94\lambda_{CuK\alpha}}{\beta\cos\theta_B},\tag{1}$$

were θ_{B} is the angle between the reflecting lattice planes and the incident beam. The parameter β was obtained from the equation:

$$\beta = \sqrt{B^2 - b^2} \,, \tag{2}$$

were *B* is the FWHM of the diffraction peak and b = 0.12 rad is the correction parameter. The lattice parameters *a* and *c* were calculated by using the angular position of the most intense diffraction peak (see Fig. 1) and Bragg's law:

$$n\lambda = 2d_{hkl}\sin\theta,\tag{3}$$

where d_{hkl} is the interplanar distance, *n* is the diffraction order, and θ is the angle between incident X-ray and atomic planes.



Fig. 1 Selected XRD diffractograms for the annealed TiO_2 aerogels: $400^{\circ}C$ 2h a); $450^{\circ}C$ 2h b); $500^{\circ}C$ 2h c); $550^{\circ}C$ 2h d); $500^{\circ}C$ 36h e).

The applied thermal treatment induces, as expected, some changes to the lattice parameters. The following equation was used to calculate the *c* parameter:

$$c = \frac{l}{\sqrt{\frac{1}{d^2} - \frac{h^2 + k^2}{a^2}}}$$
(4)

where the interplanar distance d and a were derived from the Bragg's equation and experimental data, respectively. The XRD data obtained for the annealed samples are presented in Table 1.

Table 1. The temperature and time interval of the applied thermal treatment together with the XRD derived data (the lattice parameters *a*, *c* and the crystallites mean size D_{XRD}) and the crystallites mean size obtained from TEM measurements (D_{TEM}) for the annealed TiO₂ aerogels.

Thermal treatment	Duration	<u>2</u> θ	a	ç	D _{XRD}	D _{TEM}
temperature (°C)	(h)	(")	(A)	(A)	(nm)	(nm)
400	2	25.323	3.785	9.432	7.62	6.42
425	2	25.18	3.785	9.833	7.78	7.86
450	2	25.074	3.785	10.164	8.12	8.02
512	2	25.12	3.785	10.017	9.68	8.9
485	2	25.093	3.785	10.101	9.16	9.85
500	2	25.171	3.785	9.859	11.12	10.35
525	2	25.041	3.785	10.272	12.87	12.02
550	2	25.202	3.785	9.768	14.51	12.72
500	36	25.042	3.785	10.269	15.72	13.89

The rising of the treatment temperature had as consequence the increase of the ${\rm TiO_2}$ crystallites dimension.

1.2.2 Transmission electron microscopy measurements

The specimens morphology was examined by transmission electron microscopy (TEM) on a Jeol 2100F (FEG) microscope operating at 200 kV, equipped with a corrector for the spherical aberrations, with a point-to-point resolution of 2 Å. The samples were dispersed by ultrasounds in an ethanol solution during 5 minutes and a drop of solution was deposited on carbon-membrane copper-grid. Selected TEM images are illustrated in Fig. 2 and show the existence of a symmetrical size distribution. The values of the crystallites size determined by TEM measurements (see Table 1) were further used to plot the size dependent spectral parameters.



b)

Fig. 2 Selected TEM images and the size distribution of the anatase crystallites of the annealed TiO_2 aerogel samples: $450^{\circ}C$ 2h a); $500^{\circ}C$ 2h b).

1.2.3 Raman measurements and asymmetry calculus

FT-Raman spectra were recorded using a Bruker Equinox 55 spectrometer with an integrated FRA 106 Raman module. A radiation of 1064 nm, a power ranging between 16 and 300 mW incident on sample and a spectral resolution of 1 cm⁻¹ were employed for recording the spectra of the annealed TiO₂ aerogels. The Raman measurements were carried out at -110 and - 180 0 C by using a Specac variable temperature cell attachable to the Bruker spectrometer.

To evaluate the band asymmetry, spectral analyses with the Origin software were done. The Raman peak was fixed with Data Selector and differentiated. A horizontal line was drawn from zero point. Due to the intense noise exhibited by the differentiated Raman band a 25 adjacent averaging was used for a good estimation of right and left intersection with the horizontal line. Special attention was paid to implement the adjacent averaging procedure in order to ensure both the reproducibility and the correct assessment of the data. A measure of the asymmetry was obtained by using the formula:

$$\frac{\Gamma_r}{\Gamma_l} = \frac{X_r - X_c}{X_c - X_l},\tag{5}$$

where X_r , X_c , and X_l are the points where the right, central and left sides of the differentiated band intersect the constructed line (Fig. 3).



Fig. 3 Differentiated Raman 144 cm^{-1} band and representative coordinates X_c, X_l, X_r .

The peak position was determined from the intersection of the differentiated spectrum with the y = 0 line, while the FWHM was evaluated by using a Lorentz fit procedure (Fig. 4).



Fig. 4 Raman band 398 cm⁻¹ smoothing using a Lorentz function.

The fit of the experimental points, derived from the Raman analysis of the three bands, and plotted as a function of crystallites size was performed by using the Allometric power and linear functions for the crystallites size with dimensions bellow and above critical size, respectively. One should mention that a few measurements were performed on each investigated sample in order to ensure a more precise evaluation of the derived data.

1.3 Results and discussion

In the case of TiO₂ the main phase transformations take place around 360 0 C, where the amorphous structure is transformed into anatase phase, and around 600 0 C, where the anatase to rutile phase transformation begins.²¹ However, the transition temperatures are sensitive to the preparation method.²² One should mention that the porous samples under investigation were annealed according to the differential thermal analysis data, in the 400-550 0 C temperature range, where only the TiO₂ anatase phase was found.

1.3.1 Peak position and FWHM evaluation

Raman spectra recorded on the annealed aerogel samples are presented in Fig. 5 and show well-defined bands located around 144, 197, 398, 515 and 638 cm⁻¹, which are typical for the fundamental vibrational modes of the anatase phase.^{23,24,25,26,27} A close analysis of the spectra reveals a few differences. Although the interest was usually focused on the most intense Raman band located around 144 cm⁻¹, currently, we propose to take into consideration two other important bands located around 398 and 638 cm⁻¹. One can see that an increase in intensity, a

shift to lower wavenumbers and a narrowing of these bands occur as the thermal treatment temperature increases.

Fig. 5 Selected Raman spectra of the annealed TiO₂ aerogels.

These spectral modifications could be associated with changes in the nanocrystallites size and can be discussed in terms of contributions of several associated effects like phonon confinement, phonon coupling, strain, defects, or intrinsic anharmonicity.⁶

The position and FWHM of the Raman bands located around 144, 398 and 638 cm⁻¹ as a function of crystallites mean size are displayed in Figs. 6 and 7, respectively. One observes that these spectral parameters are significantly changed, as the TiO_2 nanocrystalites mean size decreases, until a certain value, i.e. critical size. The shift of the peak position behaves similarly for all Raman bands (see Fig. 6).

Fig. 6 Peak position shift with crystallites mean size for the selected Raman bands situated around: 144 cm⁻¹ a); 398 cm⁻¹ b); 638 cm⁻¹ c).

The critical size for the peak position displacement is around 13.5 nm. Surprisingly, the better discrimination for peak position in the dimensional domain up to 13.5 nm is obtained for the 398 cm⁻¹ band (see Fig. 6b), and a relatively similar discrimination is provided for the 144 and 638 cm⁻¹ bands, e.g for crystallites with the mean size of 8.14 and 10.2 nm the peaks changed their positions from 144.32 to 143.37 cm⁻¹ and from 399.85 to 397.76 cm⁻¹ for the 144 and 398 cm⁻¹ band, respectively. One can see that the better discrimination for FWHM is obtained for 638 cm⁻¹ Raman band (see Fig. 7c).

Fig. 7 FWHM dependence with crystallites mean size for the selected Raman bands situated around: 144 cm⁻¹ a); 398 cm⁻¹ b); 638 cm⁻¹ c).

The critical size for FWHM is around 12.5 nm for all bands, excepting that at 398 cm⁻¹ for which the critical size is around 11 nm.

Besides the nanometer-size effects the temperature can also drastically influence the shape of the Raman characteristics. Therefore, the influence of the local heating on the spectral features (FWHM and peak position) due to the focused laser beam was evaluated by using the 144 cm⁻¹ Raman band and several laser powers ranging from 16 t 300 mW and it was found that no significant local heating effect is induced even on the smallest TiO₂ crystallites.

1.3.2 Local heating influence

In order to evaluate the temperature influence on the Raman band width and peak position the most intense peak located around 144 cm^{-1} and several laser powers ranging from 16 to 300 mW were used. Since the importance of the local heating effect is more pronounced for crystallites with very small sizes,⁶ the smallest grains with the average size of 6.4 nm, were considered in this analysis.

The local heating-temperature of the nanoparticles for different laser powers was estimated from the Stokes to anti-Stokes intensity ratio formula of the first order Raman spectra. The Stokes to anti-Stokes intensity ratio is given by:⁶

$$\frac{I_{s}}{I_{AS}} = \left(\frac{\widetilde{v}_{0} + \widetilde{v}_{k}}{\widetilde{v}_{0} - \widetilde{v}_{k}}\right)^{4} e^{\left(\frac{\hbar\omega_{k}}{k_{B}T}\right)}$$
(6)

where $I_{\rm S}$ and $I_{\rm AS}$ are the Stokes and anti-Stokes intensity, $\tilde{v}_0 = \frac{1}{\lambda_0} = 144 \,\mathrm{cm}^{-1}$, \hbar is Planck constant rationalized, $k_{\rm B}$ is Boltzmann constant, and $\omega_k = 2\pi c \tilde{v}_k$. Because $\tilde{v}_0 \succ \tilde{v}_k$, the ratio $\left(\frac{\tilde{v}_0 + \tilde{v}_k}{\tilde{v}_0 - \tilde{v}_k}\right) \approx 1$. Fig .8a shows the variation of estimated Raman temperature as a function of the

incident laser power. The local temperature is approximately constant because in the present study the laser wavelength of 1064 nm was focused by a lens on a large sample volume. A much consistent local heating occurs when visible wavelength is focused by a microscope objective. For example, a drastical increase of the Raman temperature, of about 500 and 350 K, respectively, was evidenced when laser line with the wavelength of 532 nm and powers ranging from 5 to 200 mW were employed for measuring nanostructures with mean sizes of 5.5 and 8 nm, respectively.⁶ The result obtained in the current investigations was confirmed by plotting the variation of FWHM and peak position of the Raman signal that appears around 144 cm⁻¹ as a function of laser power. Fig. 8b and c shows the constant dependence of these two spectral parameters with the laser power. Thus, it can be concluded that no significant local heating effect is induced.

Fig. 8 Calculated local Raman temperature versus incident laser power, and FWHM a) and peak position of the 144 $cm^{-1} E_g$ Raman mode as a function of the incident laser power b).

1.3.3 Average strain, phonon coupling and stoichiometry defects

Changes in the lattice parameters with crystallites size can affect the Raman peak position.²⁸ This effect is called average strain and is more pronounced as crystallites become smaller. By inspecting the data presented in Table 2 no relevant modification of the *c* lattice parameter for different TiO_2 crystallites mean size can be observed. Therefore, one can infer that no considerable contribution of the average strain effect occurs.

Phonon coupling describes the anharmonic coupling between phonons. If improved phonon coupling is answerable for lineshape changes, then, no significant differences between peak position and FWHM for representative nanocrystallites with marginal dimensions in the

investigated range (6.4 and 13.9 nm), at low temperatures, are expected.^{29,30} The Raman data, peak position and FWHM of the bands located around 144 cm⁻¹, 398 cm⁻¹, and 638 cm⁻¹, obtained at different temperatures, for the 6.4 and 13.9 nm nanocrystallites are presented in Table 2.

Table 2. Spectral parameters, peak position and FWHM, obtained for the analyzed Raman bands located around 144, 398 and 638 cm⁻¹ from the spectra recorded at room temperature (RT), -110 and -180° C.

Crystallites Measuremen mean size temperatur		P	eak position (cm ⁻¹)	on	FWHM (cm ⁻¹)		
(nm)	(⁰ C)	144	398	638	144	398	638
6.4	RT	144.93	399.87	636.72	20.08	24.18	33.18
	-110	143.06	400.19	641.18	14.34	26.24	25.80
	-180	141.96	400.35	642.33	12.17	23.05	23.92
13.9	RT	143.01	396.4	637.81	10.83	18.15	24.38
	-110	140.61	396.74	641.01	5.942	13.57	16.15
	-180	139.4	396.14	641.34	4.46	11.18	12.72

By looking at the data one can see that at low temperatures the values derived from the Raman spectra analyses of the smallest crystallites are different from those obtained for the largest ones. Moreover, similar differences between the values of the spectral characteristics obtained for the 6.4 and 13.9 nm nanocrystallites at room temperature and those derived at low temperatures, respectively, were achieved. These results suggest an insignificant phonon coupling contribution.

As mentioned before, besides temperature, strain, and phonon coupling the Raman features can be influenced by stoichiometry defects and phonon confinement.^{6,12} It was reported that stoichiometry defects can be correlated with the broadening and the blue shift of Raman bands and the samples color changes from white to dark for a modification of oxygen stoichiometry from 2 to 1.89.⁶ Because all our samples have the same white color one can anticipate that the presence of vacancies, associated with the change of stoichiometry does not modify the spectral characteristics.⁷ In order to validate these findings electron paramagnetic resonance (EPR) measurements were performed in our laboratories, but no signal, associated with the vacancies presence, was obtained.

1.3.4 Phonon confinement and asymmetry evaluation

In the Raman scattering process of bulk materials the photons wavenumber values is very small in comparison with that of the phonon wave vector, because $\lambda \gg a$, where λ is the wavelength of the photons, and a is the lattice constant. This means that only phonons with $k \approx 0$ contribute to the Raman scattering process and to the Brillouin scattering. In crystalline materials, the conservation of k results in a single phonon line with Lorentzian shape and narrow FWHM.^{13,14} In the case of nanosized materials the wave vector selection rules are changed for optical processes as long as dimensions are comparable with phonon wavelength. An uncertainty in wave vectors and phonons with $k \neq 0$ can be involved in Raman scattering, because a wave packet has a spatial dimension comparable to the crystallite size.^{13,15} Since the wave packet becomes more localized in real space, the size diminution increases and consequently, the phonons become more dispersed. Generally, the phonon dispersion leads to an asymmetric broadening and a shift of the Raman bands.^{31,32,33} The negative dispersion of the phonon branch is important if the nanocrystallites size is less than 10 nm.^{7,21} The nature of the spectral changes and the nanocrystallite size at which these changes take place depend on the dispersion curve of the material, and also on the intrinsic phonon lifetime.¹³ Normally, the smaller the size is, the stronger spectral change of the Raman band asymmetry occurs.⁶

The contribution of all above mentioned factors (temperature, average strain, phonon coupling and stoichiometry defects), excepting the inhomogeneous strain and confinement effect, give rise to a symmetrical broadening of the Raman bands. Therefore, the asymmetry evaluation should offer valuable information concerning the contribution of the confinement effect, when a symmetrical size distribution of the TiO₂ anatase crystallites exists.^{6,17} As can be seen from Fig. 9 the size reduction causes indeed the appearance of Raman band asymmetry.

Fig. 9 Asymmetry vs. crystallites mean size for the selected Raman bands located around: 144 cm⁻¹ a); 398 cm⁻¹ b); 638 cm⁻¹ c).

The asymmetry of the 144 cm⁻¹ and 638 cm⁻¹ peaks behaves similarly, i.e. decreasing tendency as crystallites size increases, and it has an opposite trend for the 398 cm⁻¹ band. The critical size for the asymmetry evaluation was found to be around 10.5 nm. Similarly to the FWHM assessment a poor quality for the asymmetry curve of the 398 cm⁻¹ band was obtained. The existence of a symmetrical size distribution was proved by TEM images.

In order to establish if the entire contribution of the nanosize effects can be associated with the phonon confinement the most representative Raman band of TiO_2 anatase located around 144 cm⁻¹ was further computed by applying the well known phonon confinement model.^{6,7,34,35,36,37,38} In the last years, numerous theoretical approaches were employed to investigate the consequences of the confinement effect on phonon spectra. Richter et al. proposed

a Gaussian confinement model for spherical particles that takes into consideration the contribution of phonons away from the zone-center. This model was further extended and used to explain the Raman lineshape of various nanocrystals.^{39,40,41} According to this model, for a spherical particle of diameter D, the Raman intensity profile can be approximated by:

$$I(\omega, D) \propto \int_{BZ} \frac{|C(q, D)|^2}{[\omega - \omega(q)]^2 + (\Gamma_0/2)^2} d^3q$$
(7)

where C(q,D) is the Fourier transformation of the confinement function, $\omega(q)$ is the phonon dispersion curve and Γ is the natural line width of the zone-center optical phonon in bulk anatase. To handle the Eq. (7) a very simple form of this expression is required, and, therefore, an isotropic dispersion in a spherical Brillouin zone (BZ) is considered, the wave function $\omega(q)$ representing in fact an average dispersion curve. In the present evaluation, we calculated the Raman intensity profile for two Fourier coefficients and two dispersion curves of the phonon branch in the Γ -X direction. Thus, for the calculation of both series of Raman intensities, peak shifts, and FWHMs, the Bersani³⁸ and Ivanda³⁶ models were employed. For the first theoretical approach the following coefficient and dispersion curve were used:³⁸

$$C(q,D) = \exp\left(-\frac{q^2 D^2}{16\pi^2}\right) \text{ and } \omega(q) = \omega_0 + \Delta_1 (1 - \cos(qa)), \tag{5}$$

while, for the second one, the bellow expressions were employed:³⁶

$$C(q,D) = \exp\left(-\frac{q^2 D^2}{4a^2}\right) \text{ and } \omega(q) = \omega_0 + \Delta_2 \sin(q\pi/x).$$
(6)

In our calculations we have used $\omega_0 = 142.57 \text{ cm}^{-1}$, $\Gamma_0 = 9.94 \text{ cm}^{-1}$, and for the other parameters, $\Delta_1 = 20$,³⁸ $\Delta_2 = 164$ and x = 1.51779.³⁶ One should also mention that the Raman band calculation was performed by taking into consideration a three dimensional phonon confinement model that is appropriate for zero dimensional objects such as powders, quantum dots, etc., where the infinitesimal volume element from Eq. (7) can be written as $d^3q = q^2dq$.⁴² It is well known that the use of this type of confinement results in pronounced broadening of the Raman features.⁴³

A discrepancy between the data derived from the recorded and computed data can be clearly seen in Figs. 10a and 10b, where the variations of the peak position and FWHM with the crystallites size are presented.

Fig. 10 Dependences of the experimental and simulated peak position shift a), FWHM b) and asymmetry of the Raman band located around 144 cm⁻¹ with the crystallites mean size c).

A discrepancy between the data derived from the recorded and computed data can be clearly seen in Figs. 10a and 10b, where the variations of the peak position and FWHM with the crystallites size are presented. More pronounced differences between experimental and theoretical data can be seen in the peak position assessment (see Fig. 10a), where an overestimation of the experimental data can be observed. The FWHM values derived from the simulated Raman bands underestimate and overestimate the experimental data when Bersani and Ivanda models, respectively, are applied. These discrepancies are clearly evidenced for small crystallites dimensions. Similar differences have been also reported in other studies, where the confinement effect was evaluated. ^{25,29,37} The main difference between the data presented in this study and those previously reported^{6, 8, 13} consist in using a smaller value for Γ_0 parameter, around 7 cm⁻¹, that consequently decide about the peak position and FWHM values obtained for crystallites dimensions higher than the critical size. The data derived from the Bersani model seem to fit better the experimental values obtained by plotting the variation of the peak position and FWHM with the crystallites size when compared to the data obtained by applying the Ivanda model (Fig. 10a and b).

In order to establish if the Bersani and Ivanda phonon confinment approaches fit the derived experimental data, the asymmetry of the 144 cm⁻¹ Raman band was also evaluated (Fig. 10c). The first important remark is that the decreasing rates derived from the experimental and theoretical data are in fairly good agreement. However, one can observe that the critical size value of the crystallites up to that the phonons confinement plays an important role is substantially higher for the simulated Raman band in comparison with that experimentally obtained. Nevertheless, the asymmetry data derived from Bersani's model agree better with the experimental ones. This result clearly indicates the necessity of developing an improved phonon confinement in anatase TiO_2 structures when 144 cm⁻¹ is involved, which is closely related to the spectral resolution used in the recording of the Raman spectra, must be done. Thus, since the peak position and FWHM values of this band have relatively small values, even for crystallites higher than the critical size, it is extremely important for the asymmetry assessment that the spectral resolution to be as small as possible, but much smaller than the FWHM value corresponding to the critical size.

Most of the studies reporting about the nano-size effects in TiO_2 reveal the existence of a strong phonon confinement based on a theoretical approach, but there is no study that experimentally evaluates its contribution. Because the critical size obtained from the peak position and FWHM dependency as a function of crystallites size is around 13.5 and 12.5 nm, respectively, and that obtained from asymmetry evaluation it was found to be around 10.5 nm, it can be concluded that phonon confinement contribution to the overall nano-size effects is at least 75%. The difference until 100% could be caused by other undetectable size effects.

1.4 Conclusions

The nano-size effects were evaluated for several TiO₂ porous nanostructures with dimensions between 6.4 and 13.9 nm by analyzing the 144, 398 and 638 cm⁻¹ Raman bands. Generally, the spectral modifications could be associated with several effects like phonon confinement, phonon coupling, average strain, inhomogeneous strain, defects, and temperature. All these factors, excepting the inhomogeneous strain and the phonon confinement effect, give rise to a symmetrical broadening of the Raman band. In this framework the assessment of the critical size value from the band asymmetry evaluation was further carried out. Despite the previously reported studies, when TiO₂ was investigated, it was found that a better discrimination of the FWHM with the nanocrystallites mean size exists for the 638 cm⁻¹ band in comparison with that obtained for the 144 cm⁻¹ peak. Moreover, an unexpected good discrimination for the peak position and asymmetry was found for the 398 cm⁻¹ band. The critical size value obtained from the peak position and FWHM analysis was determined to be between 12.7 and 13.1 nm, while that achieved from the asymmetry evaluation was between 10 and 11 nm. Based on previous findings it was shown that, if the size distribution of the TiO₂ anatase crystallites is symmetric, the obtained variation of the asymmetry could be considered a measure of the phonon confinement effect. Therefore, it was concluded that the phonon confinement contribution to the overall size effects is more than 75% for anatase TiO₂ crystallites with dimensions between 6.4 and 13.9 nm.⁴⁴ No direct evidence of other potential influences such as temperature, phonon coupling and vacancies, on the Raman spectral characteristics was determined. The information derived from the experimental analysis was compared with that obtained from a theoretical approach related to the phonon confinement model by considering the most representative band located around 144 cm⁻¹. The obtained results clearly indicate the necessity of developing an improved phonon confinement model. The experimental approach used for the asymmetry assessment of the Raman band can be successfully applied for a large variety of nanostructures with symmetric size distribution, as a measure of the confinement effect.

2. The study of TiO₂-Ag aerogels

2.1 Sample preparation

The TiO₂ gels were prepared by sol-gel method using TIP, HNO₃, Et-OH and H₂O (1/0.08/21/3.675 molar ratio) and afterwards putted down to age four weeks. The gels were then introduced for one day in AgNO₃ solutions with concentration of 5.10^{-5} , 10^{-4} , ..., and 5.10^{-2} mol/dm³ and then were supercritical dried with LCO₂ (T> 38⁰C and p=1350 psi). The prepared samples were further denoted as A, B, ..., H (Table 3).

Table 3. Molar concentrations of as-prepared TiO₂-Ag samples

Sample	Α	В	С	D	E	F	G	Н
Concentration [mol/dm ³]	5.10-5	10 ⁻⁴	5 [.] 10 ⁻⁴ b	5 [.] 10 ⁻⁴ a	10 ⁻³	5.10-3	10 ⁻²	5.10-2

Silver doped TiO₂ aerogels were subjected to a thermal treatment at 500° C for 1h and the samples were denoted A1, B1, ..., H1.

2.2 The results of the antibacterial tests

The antibacterial effects of TiO_2 -Ag aerogels on two strains bacteria *E. Coli* and *Salmonella* it can be see in Figs. 11 and 12.

Fig. 11. The antibacterial effect of the non-annealed samples.

Fig. 12. The antibacterial effect of the annealed samples.

These results relevs a high antibacterial activity of the as prepared TiO_2 -Ag aerogels and a non antibacterial effect for the annealed TiO_2 -Ag samples.

2.3 Sample measurements

2.3.1 XRD measurements

The XRD patterns of the four samples were obtained by using the Cu_K (λ_{CuK} =1.54178 Å) radiation of a standard DRON-3M powder diffractometer working at 45 kV and 30 mA.

Fig. 13 The XRD patterns measured for the non-annealed samples (left) and the annealed samples (right).

The presence of the crystallites within the four specimens was confirmed also by the XRD analyses. The XRD patterns are presented on Fig. 13 and their comparative analysis confirms a strong increasing of the crystallization degree after the thermal treatment. More precisely, the broad diffraction peaks and the high diffusion background of the non-annealed samples indicate a small relative amount of the crystallized phases which are generally made of small crystalline grains. On the contrary, the well-defined and narrow diffractions peaks characteristics of the annealed specimens clearly reveal the presence of some crystallized phases in major proportion, made of large grains.

2.3.2 Nitrogen sorption measurements

The specific surface area, the porous volume and the diameters of the pores within the studied samples were estimated from N₂-adsorption-desorption isotherms which were systematically measured using a Sorptomatic 1990 equipment. More specifically, the specific surface area was obtained by the Barrett–Emmett–Teller (BET) method,⁴⁵ whereas the pore volume and the pore size distribution were determined by the Barrett–Joyner–Halenda (BJH) method.⁴⁶ Before the experiment, the masses of analyzed powders were accurately measured and a pretreatment at 120 °C for a few hours was conducted to remove moisture and contaminants.

2.3.3 TEM, HRTEM, SAED and 3D-ET measurements

The high resolution TEM images, selected area diffraction patterns and tomographic measurements were carried out on a JEOL 2100F electron microscope operating at 200 kV, with a field emission gun and a point-to-point resolution in TEM mode of 0.21 nm, equipped with an EELS spectrometer, EDX detector and a spherical aberration probe corrector. Before the observation, the samples were ultrasonically dispersed in ethanol for 5 min and a drop of suspension was deposited on a carbon membrane grid. The HRTEM images were analyzed in the Digital Micrograph software, whereas the JEMS software was used for indexing the diffraction patterns.

2.4 Results and discussion

2.4.1 Structural characterization

Electron diffraction patterns in TEM mode were recorded for all aggregates. Two typical patterns are displayed on Fig. 14 for to the F and F1 specimens. In the case of non-annealed samples, the analysis of the corresponding diffraction patterns indicate the existence of structures with pronounced amorphous character. However, some well-defined diffraction spots are present, which indicates the presence of some small crystallites after the supercritical drying process. The analysis of the corresponding distances has allowed us to assign a classical TiO₂ anatase phase for this weakly crystallized phase. It is obvious that the presence of a strong diffuse background in the diffraction patterns of non-annealed specimens indicates the small relative amount of the crystalline phase with respect to the amorphous one. The structural homogeneity inside the samples was systematically tested by comparing diffraction patterns taken on selected areas with different diameters; excepting the absolute values of the intensity, no visible difference was observed. In addition, no significant structural difference between the samples F and H, characterized by different Ag amounts, can be observed in first approximation.

As expected, the thermal treatment leads to a relevant increase of the crystalline phase fraction. Indeed, compared to the non-annealed samples, the diffraction patterns acquired on F1 and H1 reveal a strong increase of the number of individual crystals which compose a unique or several polycrystalline phases. A classical indexing procedure has indicated the presence of both anatase and brookite TiO_2 phases, with the anatase in major proportion compared to the brookite. Once again, the structural homogeneity within the analysed specimens was checked by comparing the diffraction patterns recorded on selected areas with different sizes, and the same type of information was achieved.

Fig. 14 Left: TEM images of typical areas of F (top) and H (bottom) specimens selected for the electron diffraction experiments. Middle: the corresponding SAED patterns. Right: the indexing procedure of the SAED pattern, performed with the JEMS software.

The structural characteristics obtained by electron diffraction were confirmed by the analysis of the high resolution images (Fig. 15). It is worth to note the more local character of the HRTEM analysis compared to the SAED measurements. In addition, in HRTEM the observations are performed generally in the areas where a typical contrast characteristic of the presence of crystalline planes is visible. On the image of non-annealed sample, a crystalline grain with a size of about 10 nm was observed in the center of a bigger amorphous aggregate; a direct

measuring of the periodical distances allowed us to assign it to the anatase phase. On the same image, one cas also observe the presence of some dark field areas, with a size between 2 and 5 nm, associated to the Ag nanoparticles. Note that some smaller Ag particles can be also present, but their observation in the high resolution TEM mode (which is less sensitive to the mean atomic number compared to STEM-HAADF) is difficult, especially for the thicker specimens, as in this case. By inspecting the image of the annealed sample, one can also observe the presence of some dark areas. A direct measurement of the interreticular distances allowed us to assign them to the Ag nanoparticles. Their size seems to be larger than that of the nanoparticles in the non-annealed sample; this observation will be confirmed by the tomographic measurement which will be presented in the next section. On the other hand, one can observe that the major part of the aggregate (the bright areas on the image), corresponding obviously to the TiO₂ support, exhibits a rather homogenous contrast illustrating in all areas the presence of a crystallographic network. This observation suggests a small amount of the amorphous phase and confirms thus the previous result obtained by SAED. The values obtained for the crystallographic distances are summarized in Table 4, together with the corresponding distances deduced by SAED. Both indicates that the anatase is in a major proportion, but do not exclude the presence of the brookite phase. Indeed, as can be observed in the Table 4, some experimental distances measured by HRTEM or SAED could be assigned to the brookite phase. Note that the presence in the specimens of this second phase is less interesting for the applications of interest. This was the reason for what the conditions of the thermal treatment were chosen in order to promote the formation of the anatase phase.

Fig. 15 HRTEM images taken on two typical aggregates representative of the specimens which contain relative amounts of silver of about 0,1 before annealing (sample F, left) and after annealing (sample F1 right).

Table 4. Interplanar crystallographic distances for the four specimens deduced by the analysis of the high resolution TEM images and selected area diffraction pattern and their assignment to the anatase and brookite phases or to silver nanoparticles.

		HRTEM		SAED			
Sample	d	Crystalline	Miller	d	Crystalline	Miller indices	
		phase	indices		phase	(h, k, l)	
	(Å)		(h, k, l)	(Å)			
	3.52	Anatase	(1, 0, 1)	3.52	Anatase	(1, 0, 1)	
F	3.50	Brookite	(1, 2, 0)	3.46	Brookite	(1, 1, 1)	
	2.36	Anatase	(0, 0, 4)	1.66	Anatase	(2, 1, 1)	
Н	2.20	Ag	(1, 1, 1)	3.52	Anatase	(1, 0, 1)	
	2.47	Brookite	(0, 1, 2)	3.46	Brookite	(1, 1, 1)	
	3.53	Anatase	(1, 0, 1)	1.66	Anatase	(2, 1, 1)	
	2,00	Ag	(2, 0, 0)	3.52	Anatase	(1, 0, 1)	
F1	1,86	Anatasa	(2, 0, 0)	3.46	Brookite	(1, 1, 1)	
	1,88	Anatasa	(2, 0, 0)	1.66	Anatase	(2, 1, 1)	
	3,53	Anatasa	(1, 0, 1)	3.52	Anatase	(1, 0, 1)	
H1	2,41	Brookite	(2, 0, 1)	3.46	Brookite	(1, 1, 1)	
	2,35	Ag	(1, 1, 1)	1.66	Anatase	(2, 1, 1)	

2.4.2 Analysis of the morphology and porous structure

For the tomographic analysis, a drop of a solution containing fiducially markers (gold nanoparticles with a diameter of 5 nm) was deposited on the grid to facilitate the alignment step of the tomographic data treatment. The acquisition of the tilt series used to calculate the reconstructed volume was performed in bright field TEM using the automatic acquisition procedure of the Gatan Digital Micrograph, with images recorded on a CCD Ultrascan array detector. The angular interval was generally between -72° and 72° , with and angular step of 2° at 0° tilt and varying at the higher angles according to the Saxton algorithm⁴⁷. Basically, the number of images within each tilt series was about 101. After acquisition, the individual images were aligned in the IMOD software⁴⁸, using a rough alignment based on a cross-correlation procedure followed by a finer alignment using the geometrical positions of the fiducial markers. The reconstructed volumes were calculated using the iterative methods of the TomoJ plugin^{49,50} implemented in the ImageJ software. The 3D representation and analysis of the reconstructed volumes was performed using the 3D Slicer⁵¹ software and Chimera⁵². To obtain relevant 3D models of the analyzed aggregates, a segmentation procedure based on a grey-level intensity of the voxels was systematically performed for all the analysed samples. Using the analytical formula proposed by Frank⁵³, the spatial resolution in the reconstructions was estimated to be about 0.22 nm, with an elongated factor in the direction of the electron beam lower than 1.1.

As pointed out in the introduction, to obtain precise information on the external morphology of the grains, but principally on the porous network inside a specific grain and the location of Ag NPs within this porosity, several tomographic analyses were performed on all the samples of interest, i.e. F, F1, H and H1. The typical procedure of data analysis is illustrated on Fig. 16 for a representative aggregate of the H specimen. In the first step, once the reconstructed volume calculated, it can be analyzed slice by slice along any chosen direction (examples of three orthogonal slices are presented on Fig. 16 b), c) and d). In the second one, it can be subsequently used to determine a 3D modeling of the studied aggregates where the different components (here the titania and the silver) are separated and can be analyzed individually (for illustration, they are represented with different colors on Fig. 16 e) and f). The equivalent 3D modeling for the other three specimens, obtained from their reconstructed volumes by data segmentation, are shown on Fig. 17. As can be observed, they provide a real 3D visualization of the architecture of the analyzed grains. For a better visualization of the spatial distribution of the

Ag NPs within these grains, one can choose to represent only their corresponding parts from the whole 3D modeling (bottom part of Fig. 17). It is worth to note that, in addition to providing 3D representations of the analysed specimens, the principal benefit of the modeling procedure is that it allows us to perform quantitative 3D analyses. Such an analysis yields to a precise determination of parameters such as the volume and specific area of each component as well as the characteristics of the interface between them.

Fig. 16. a) Typical TEM image from the tilt series used to reconstruct the volume of the analyzed aggregate of the H specimen. b), c) and d) Three orthogonal slices extracted from the reconstruction; the z-axis represents the electron beam direction at 0° tilt; the yellow arrows indicates the localization of the Ag nanoparticles. e) 3D tomographic modeling of the analyzed aggregate, with the TiO₂ in white and Ag in blue. f) Cross-sectional view through the 3D modeling, showing the internal architecture of the analyzed grain.

Fig. 17. Top: 3D modeling of the analyzed grains chosen T-models for the samples F, H, F1 and H1; bottom: 3D representations of the same aggregates where only the Ag nanoparticles are represented.

By analyzing qualitatively the 3D positions of the Ag NPs with respect to the external surface of the grains, similar spatial distributions can be observed within the series of four samples. More precisely, the Ag 3D distribution is rather homogenous inside the grains of samples H and H1, whereas in the case of the F and F1 samples the density of nanoparticles increases from the core of the aggregate towards its external surface, yielding thus to a heterogeneous distribution. This dissimilarity in Ag spatial distribution could be explained by the difference in the relative Ag amount contained in the four samples, as well as by a preferential growth of Ag nanoparticles at the surface of the grains in the first stage of the deposit. Therefore, for the samples H and H1, the silver amount is large enough assuring thus a complete penetration of the Ag deposit inside the individual aggregates, whereas in the case of F and F1 samples, it is ten times lower leading to a global deposit located rather at the vicinity of the surface.

From quantitatively point of view, the porous characteristics of the TiO_2 support deduced by analyzing the 3D modeling are summarized on Table 5 and compared to them obtained by

nitrogen sorption. For illustration, Fig. 18 presents the nitrogen sorption isotherms for two specimens. All the measured nitrogen sorption isotherms are characteristics of a mesostructured material with a type IV isotherm. The hysteresis loops proves a delayed condensation, because the adsorption and desorption branches are relatively slight. The capillary condensation depends by the pore size distribution and the diminution of the pore size can be correlated with the presence of a lower relative pressure. These hysteresis loops are as well characteristics for porous materials with a well defined pore structure and a relatively uniform mesopore distribution (Fig. 19). A significant difference can be observed between the values of the specific surface area obtained by the two methods, especially for the non-annealed samples. To explain this difference, it is well known that the electron tomography technique has not sufficient spatial resolution to evidence the micropores and some of mesopores (lower than 2 nm), if it is not applied in high resolution mode. Indeed, several similar studies published in this field clearly indicate that the values of the specific surface area are one order of magnitude smaller when electron tomography is employed comparing to the data obtained from the sorption measurements⁵⁴. The difference in the specific surface areas deduced from tomography and BET for the non-annealed samples is thus logical, giving the high contribution of the microporosity to the surface area in such a samples. On the contrary, this difference is significantly reduced for the annealed samples, giving that the thermal treatment lead generally to a significant reduction of the porosity inside the specimen, generally attributed to a vanishing of a high number of micropores. However, the direct comparison between the tomographic and BET values of surface area for the F1 and H1 specimens suggests that in H1 the microporosity has completely vanished due to thermal treatement, whereas for the F1 sample its contribution remains significant. Concerning the mesopores, one consider generally that their mean values deduced by analyzing the nitrogen sorption isotherms are much more relevant compared to the local measures which can be obtained from the tomographic reconstructions. We have obtained thus a mean value of 10 nm for the sample F, while for the H one (with higher Ag amount) the corresponding value is slightly higher, about 13 nm (Fig. 20a). The annealing of the samples induces a different and unexpected behavior. Thus, in comparison with the prepared samples a minor increase of the median pore diameter for the sample F1, to 10.5 nm, was observed, while for the sample H1 a decrease of the mean value, to around 11.5 nm, occurred.

Table 5. Data derived from the adsor	rption-desorption and 3D-ET measurements
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G 1		а :с	G .C	G G G		G 1.4	0 1.4	14	10
Sample	Ag	Specific	Specific	Surface of	Accessible	Cumulative	Cumulative	Mesopores	Micropores
	concentration	surface area	surface	Ag (from the	Ag surface	mesopores	micropores	fraction	fraction
	(from EDX)	(from	area	total	(from the	area (from	area (from	(%)	(%)
	(%)	tomography)	(from	aggregate	total Ag	BIH)	BIH)		
	(70)	2 2	DET)	aggregate	curfoco	2	2		
		(m /g)	DE1)	surface,	surface	(m /g)	(m /g)		
			(m/g)	deduced by	deduced by				
			× <i>Cr</i>	tomography)	tomography)				
				(%)	(%)				
F	0.1	48	474.8	3	15	494.3	358.77	58	42
п	2.4	51	550.2	7	15	719 65	242.02	69	22
п	2.4	51	550.5	/	15	/18.05	542.95	00	32
F1	0.2	79	129.7	6	30	203.37	94.953	68	32
H1	2.1	72	58.6	0.4	1	164.45	44.768	79	21

Fig. 18 Nitrogen adsorption isotherms for the non-annealed H a) and annealed H1 b) specimens.

Fig. 19 Pores distribution for the non-annealed H a) and annealed H1 b) specimens.

Once the modeling of the analyzed aggregate deduced, cross-sectional views at different depths and orientations can be realized in order to visualize the interface between the Ag nanoparticles and the TiO₂ matrix. Such typical 3D views for the four specimens are represented on Fig. 21 and illustrated first of all the characteristics of the TiO₂ pores. The presence of some close pores was thus evidenced, but however the most of them are open and directly connected with the outside part of the grain. In addition, by analyzing these 3D representations in cross-section, precise information on the spatial localization of chosen NPs within the porous network as well as on their immediate area has been obtained. The most surprising result is that, besides some nanoparticles located at the surface of the pores, the majority of nanoparticles are encapsulated in the TiO₂ material. It is worth to mention here that this result concerns only the Ag nanoparticles observable in the tomographic reconstruction (with a diameter generally higher than 1.5 - 2 nm) and excludes the Ag atomic clusters eventually present in this nanocomposite.⁵⁵

By considering only the part of the 3D modeling associated to the silver, a quantitative individual analysis of the sizes of all the nanoparticles was finally possible. Because of the significant number of the analysed NPs (of the order of 600), the results can be interpreted in a statistical way and could be considered relevant at a macroscopic scale. The size histogram are represented on Fig. 20 for the samples with the higher amount of Ag, the non-annealed H and the annealed H1 ones, respectively. As expected, the mean diameter of Ag nanoparticles increases after annealing while their number decreases, due to the combined effect of nanoparticles diffusion and coalescence, or to the Ostwald ripening process. On the contrary, a rather unexpected finding concerns the higher value of the mean size of Ag NPs in the sample F (small amount of Ag) compared to the other non-anneales sample, H. A tentative explanation of this difference in mean size is the smaller diameter of the mesopores for the sample F, which can be considered as responsible for a lower penetration of the Ag precursor solution inside the porous network. The nucleation centers of the NPs are thus localized preferentially towards the external surface and consequently the NPs growth in this limited area. That yields to bigger NPs compared to the H sample where the distribution of the nucleation centers is much more homogenous. Once again, it is worth to mention that these conclusions exclude completely the presence of Ag atomic clusters which cannot be resolved in our reconstruction, given our experimental conditions used for acquisition. Finally, a significant difference between the mean

diameters of the annealed samples F1 and H1 was thus observed, having probably at its origin the initial difference between the mean diameters of the non-annealed samples.

Fig. 20. Cross-sectional views through the 3D modeling of the aggregates chosen for tomographic analyses which compose the four specimens: a) sample F, b) sample H, c) sample F1, sample H1. Such 3D representations allow visualizing the internal porous network as well as the localization of the Ag nanoparticles within this porosity, on the external surface of the grains or inside the TiO_2 material.

In the case of the applications based on the antimicrobial effect of Ag, one of the key parameters is the accessible Ag surface.^{56,57} As observed previously, some nanoparticles are encapsulated in the TiO_2 pores and consequently the atoms of Ag located at their surface are not accessible. By comparing the data presented in Table 5 for the non-annealed F and H samples, one can observe that the total specific surface area of the Ag nanoparticles for the H sample is only two times larger compared to F, while the amount of Ag is ten times higher. Knowing that the mean diameter of NPs of the H sample is smaller (and that generally leads to an increasing of the specific surface area), one can here conclude that for this Ag-rich sample the number of encapsulated nanoparticles is higher. However, note that for both samples, the relative fractions of the effective accessible surface, calculated from the total Ag surface, are very similar, about 15%.

The effects of the thermal treatment on the effective accessible surface area of Ag are opposite for the two samples. In the case of the sample F (with small amount of Ag), the contribution of Ag to the total surface of the grain on the one hand, and the accessible Ag surface on the other hand, are two times higher comparing to the initial values. That indicates a considerable increasing of the number of NPs located at the pore surface which become in addition less encapsulated in TiO₂. On the contrary, for the sample H the contribution of Ag to the total surface area of the grain becomes insignificant after annealing, as well as the fraction of this area which remains accessible from outside. That corresponds to a strong increasing of the encapsulation degree for the sample. A possible hypothesis which we can put forward to explain this completely different behavior is that the micropores are still present in the F1 sample, while they are completely flat, favoring the diffusion of different species present at the surface and leading therefore to more encapsulated nanoparticles.

As a final point, the main conclusion is that the most interesting sample for the applications where the effective accessible surface area of Ag is crucial seems to be F1 and not H1, as we expected. Indeed, even if the total Ag amount is ten times lower compared to the samples H, it can be characterized by a relative high surface specific area of TiO_2 as well as by a high value of the effective accessible surface of Ag nanoparticles.

Fig. 21 The histograms silver particle distribution for the investigated samples: H a); H1 b).

A close analysis reveals that the ratio between the silver surface (relative to the aggregate surface) and accessible silver surface (from the total silver surface) is approximately 1:5 for the samples F and F1, and 1:2 for the samples H and H1, respectively. This result indicates that no diffusion of the silver located on the aggregate surface towards the accessible silver surface takes place as a result of the annealing.

The diminution of the mesopores diameter with 28.5 % together with the increase of the silver content clarify the observed behavior concerning the reduce accessibility of the silver particles for sample H1 in comparison with the sample F1.

The sample H has abundant silver atoms that via the applied thermal treatment grown up forming silver particles, unlike the structural environmental behavior of the silver for the sample F, where the silver atoms are well dispersed even after annealing.

2.4.3 The identification of silver species by UV-Vis spectroscopy

The UV-Vis absorbtion spectra of the all as-prepared samples A, B, ...,H reveal specific electronic transition like Ag^+ (200-230 nm), Ag^0 (250-330 nm), and plasmons resonances (400-500 nm).⁵⁸ If annealing samples A1, B1, ..., H1, the signal due to species specific electronic transition Ag^+ (200-230 nm) decreases in intensity, which shows, in terms of quality, reduce the number of Ag ions in these samples (Fig. 22).

Fig. 22 Selected UV-Vis absorbtion spectra and specific electronic transition for: as-prepared samples a); annealing samples b).

Thus, different results can be explained partly as a result of the antibacterial tests obtained on the two types of samples, untreated and heat treated.

2.4.4 STEM results

High-resolution image of the same samples (Fig. 23) reveals that around silver nanoparticles are nanometer-sized atomic clusters of silver dispersed in TiO_2 matrix.

b)

Fig. 23 STEM_BF image of H sample a); STEM_DF image of H sample and atomic clusters around Ag NPc b).

The atomic clusters "vanish" with the treatment because the atoms diffuse and larger NPc (Fig.24).

Fig. 24 In- situ study of silver diffusion HAADF-STEM mode.

The progressive in-situ thermal treatment up to 500°C allowed one to identify diffusion mechanism of NPs formation.⁵⁹

2.4.5 Conclusion

Using several characterization techniques and a multi-scale approach, we have report here a complete analysis of a heterogeneous nanostructure made of porous TiO_2 and Ag nanoparticles, a composite of high interest in the last years due to its several potential applications, such as its antimicrobial properties. We have observed thus that increasing the Ag amount does not necessarily lead to better characteristics in terms of accessibility to the Ag species: the incorporated amount is higher, the spatial distribution of the Ag nanoparticles is more homogenous, but with the price of a less accessible Ag surface and consequently a moderate antimicrobial activity. On the other hand, we have shown that the effect of a thermal treatment applied to such porous nanocomposites is relatively complex: it leads to a increasing of the mean diameters of NPs and thus a diminution of the surface contribution with respect to the bulk, but it modifies also the fraction of the encapsulated NPs. This last effect seems to be strongly dependent on the morphological characteristics of the samples: if the thermal treatment induces a vanishing of the microporosity, the surface diffusion of the present species is favored leading to more encapsulated and thus less accessible Ag NPs.

From a general point of view, this work underlines once again the impressive potential of the electron tomography technique when it is applied to the analysis of such multicomposite specimens which present a 3D nanostructured architecture. However, it is worth to note that the complete analysis of heterogenous nanostructures requires the use of different characterization tools with different selectivity, able to provide local and global information concerning the crystallographic structure, the morphology or the chemical composition.

The identification results of silver species by UV-Vis spectroscopy and The *ex-situ* thermal treatment leads on one hand to support crystallization in anathase (a highly ordered TiO_2 phase) and to NPs embedding. This change is most probably the main factor responsible for the reduced Antibacterial activity.

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