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Effects of the sol-gel route on the structural characteristics and antibacterial activity of silica-encapsulated gentamicin

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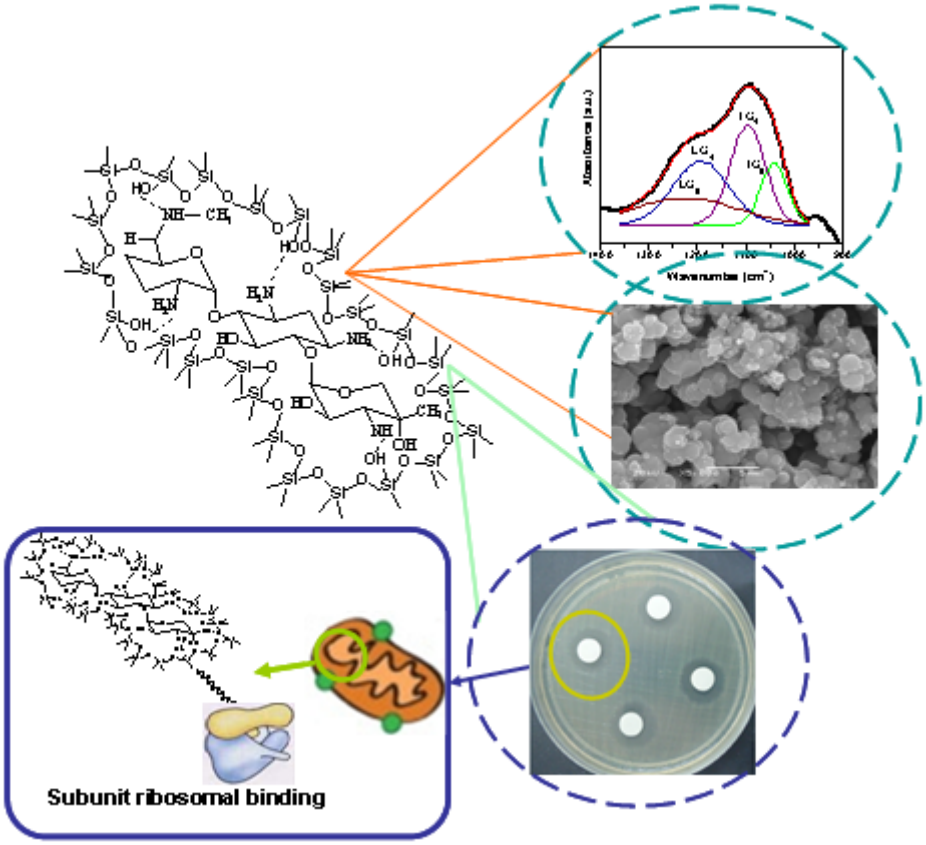
GRAPHICAL ABSTRACT



ABSTRACT

The effects of sol-gel processes, i.e., acid-catalyzed gelation, base-catalyzed gelation and base-catalyzed precipitation routes, on the encapsulation of gentamicin were investigated. The resulting xerogels were characterized using a series of complementary instrumental techniques, i.e., the adsorption/desorption of nitrogen, small-angle X-ray scattering, Fourier transform infrared spectroscopy, diffuse reflectance spectroscopy, X-ray photoelectron spectroscopy, atomic force microscopy and scanning electron microscopy. The encapsulated gentamicin samples were tested against five bacterial strains, including two Gram-positive (*Staphylococcus aureus* ATCC 25923*, Bacillus cereus* ATCC 11778) and three Gram-negative strains (*Escherichia coli* ATCC 25922, *Enterobacter aerogenes* ATCC 13048 and *Salmonella typhimurium* ATCC 14028*).* The best antimicrobial activity was observed for the encapsulated gentamicin that was prepared via the precipitation route, even in comparison with the neat antibiotic, especially in the case of the Gram-positive strain *Staphylococcus aureus.* The gentamicin concentration on the outermost surface and the zeta potential were identified as factors that affected the highest efficiency, as observed in the case of encapsulation via the base-catalyzed precipitation process.

Keywords: Sol-gel; silica; gentamicin; antibacterial activity; encapsulation.

INTRODUCTION

Encapsulation represents a technological approach that consists of enveloping a given entity, such as drugs (Guo et al., 2012); Zhang et al., 2012), catalysts (Fisch et al., 2008), pesticides (Li et al., 2012) and cells (Sakai et al., 2012), with, for instance, a coating or a shell, whose role depends on the final application. For instance, this shell may protect a given drug from deteriorating effects (e.g., vitamins from the effects of oxygen), may make manipulation easier and provide physical stability for sensors or may make transport easier and prevent the deactivation of a catalyst by a poison.

In a medical context, the main aim of drug encapsulation is the control of the rate at which a drug leaves the encapsulating medium, as in the case of the controlled delivery of drugs. Such an approach is very effective for controlling the concentration of therapeutic agents in blood and for improving their bioavailability (Ciriminna et al., 2011). Other applications of encapsulation involve the use of encapsulated molecules for imaging and diagnostic techniques (Choi et al., 2012; Lee et al., 2012).

One class of drugs that has been investigated for encapsulation methods is antibiotics. In this context, biodegradable microspheres are useful for prolonged drug release and for targeting drugs to specific infection sites. Furthermore, in some cases, the encapsulation of antibiotics in polymeric nanoparticles overcomes the problem of antibiotic deactivation because this encapsulation prevents interactions between the antibiotics and, for instance, the sputum contents in the case of inhalation. Examples of antibiotic encapsulation include levofloxacin in poly(lactic-co-glycolic acid) nanoparticles (Cheow , 2010), ciprofloxacin in alginate/pectin microspheres (Islan et al.,

2012), ofloxacin in chitosan microspheres (Sezer and Akbua, 2010) and violacein in poly-D,L-(lactide-co-glycolide) (Martins et al., 2011).

The encapsulation of gentamicin, which is an aminoglycoside antibiotic, for use in several types of bacterial infections, especially those that are provoked by Gram-negative organisms, has been investigated in the literature. Gentamicin has been encapsulated in organic matrices, such as liposomes (Lutwyche et al., 1998), Phospholipon®90G and Softisan® 154, using a solid-reversed-micellar solution for intramuscular administration (Umeyor et al., 2012) and in biodegradable polymers (polylactic acid and cellulose acetate), which are a shell material, using the coaxial electrospinning technique (Vichitchote et al., 2012).

Inorganic carriers, either with or without organic counterparts, have also been used in the encapsulation of drugs (Pang et al., 2012; Nampi et al., 2012; Qian and Bogner, 2012). Silicon-based materials are usually preferred for drug delivery systems because of their relative bio-inertness and their degradation into nontoxic silicic acid (Ciriminna et al., 2011). Furthermore, silica-based materials can easily be chemically modified, thus producing a broad range of hybrid materials (McInner, 2009). In the case of gentamicin, several studies were conducted that combined mesoporous-based silica and layer-by-layer films, such as poly(allylamine hydrochloride) and poly(styrene sulfonate) (Zhu, 2007). Other examples, including the preparation of poly(lactic-co-glycolic acid)/mesoporous silica (Xue, 2004; Xue and Lukito, 2006), a SiO_2-CaO-P_2O_5 sol-gel glass (Meseguer-Olmo et al., 2006) and silica (Wang et al., 2008); Morais et al., 2012b), have been reported. In the latter, the encapsulation of the drug was achieved using the sol-gel process of tetraethoxysilane with

hydrochloric acid, i.e., it was catalyzed by an acid. The mild conditions of the sol-gel encapsulation route are beneficial as the process can be conducted at room temperature (Schubert, 2005). In previous studies, we used the sol-gel process to develop molecular imprinting silica-based materials with pharmaceuticals as templates for environmental matrix pre-concentrations (Morais et al., 2012a; Morais et al., 2012b; Morais et al., 2012c).

In the sol-gel process, there are several routes that enable the production of silica-based materials, which, in turn, affect the structural, textural and morphological characteristics of the resulting xerogels. To the best of our knowledge, the effect of encapsulation via the sol-gel route on the biological efficacy of an antibiotic has not been reported in the literature. In the present paper, we report the effect of three sol-gel processes on the encapsulation of gentamicin: acid-catalyzed gelation, base-catalyzed gelation and the base precipitation route.

The resulting materials were characterized using a series of complementary instrumental techniques, i.e., an elemental analysis, the porosimetry from the adsorption/desorption of nitrogen (BET method), small-angle X-ray scattering (SAXS), Fourier transform infrared spectroscopy (FT-IR), diffuse reflectance spectroscopy (DRS), X-ray photoelectron spectroscopy (XPS), atomic force microscopy (AFM) and scanning electron microscopy (SEM). The encapsulated gentamicin samples were tested against five bacterial strains: two Gram-positive (*Staphylococcus aureus* ATCC 25923*, Bacillus cereus* ATCC 11778) and three Gram-negative strains (*Escherichia coli* ATCC 25922, *Enterobacter aerogenes* ATCC 13048 and *Salmonella typhimurium* ATCC 14028).

EXPERIMENTAL

Reagents and chemicals

Gentamicin (IQ Soluções Químicas SA, Santos, Brazil), tetraethylorthosilicate (TEOS) (Shinetsu, Tokyo, Japan), chloridric acid (Synth, Diadema, Brazil), ammonium hydroxide (Quimex, São Paulo, Brazil), agar MH (Oxoid, Wade Road Basingstoke, Hampshire, UK) and the gentamicin positive control disk (DME, Araçatuba, Brazil) were used as received.

Xerogel synthesis

Xerogels were synthesized via the sol-gel method using three processes: (i) the acid-catalyzed route (**A**); (ii) the base-catalyzed route (**B1**) and (iii) the base-catalyzed route by precipitation (**B2**). In route **A**, 8.6 mL of chloridric acid (0.2 M) (a catalyst) were added to a 500 mg solution of gentamicin that was dissolved in 10 mL of TEOS. The mixture was stirred for 24 h until gelation occurred. The resulting material was dried at room temperature and ground, thus producing the xerogel **SILAG**. In the base-catalyzed routes, ammonium hydroxide was used as the catalyst in different amounts. In route **B1**, 5 mL of ammonium hydroxide (2.8 %) were added to a 500 mg solution of gentamicin that was dissolved in 10 mL of TEOS. The mixture was stirred for 24 h until gelation occurred, followed by drying at room temperature and grinding, thus producing xerogel **SILBG**. In the case of route **B2**, 20 mL of ammonium hydroxide (28 %) was added to a 500 mg solution of gentamicin that was dissolved in 10 mL of TEOS. The mixture was stirred for 20 minutes until precipitation occurred. The resulting material was dried at room temperature and ground, thus producing **SILBP**. The three corresponding materials were

161 labeled SILAG, SILBG and SILBP. Their respective blanks were SILA, SILB and
162 SILP.
163
164 *Characterization of the xerogels*
165 The carbon and nitrogen contents were determined using a PerkinElmer
166 (Wellesley, MA, USA) M-CHNSO/2400 analyzer. SEM experiments were
167 conducted on a JEOL (Tokyo, Japan) JSM/6060 microscope. The samples
168 were fixed on carbon tape that was affixed to a sample stub and then coated
169 with gold using conventional sputtering techniques.
170 AFM images were obtained using a Nanoscope IIIa atomic force
171 microscope (Digital Instruments Co.) in contact mode with silicon nitride probes.
172 The WSMX 4.0 software from Nanotec Electronic S. L. was used for image
173 treatment. The surface roughness was quantitatively identified using the root-
174 mean-squared roughness (R_{rms}), which is given by the standard deviation (S.D.)
175 of the data from the AFM images, and was determined using software with the
176 standard definition shown in equation 1:

177 $$R_{rms} = \sqrt{\frac{\sum_{n=1}^{N} (z_n - \bar{z})^2}{N-1}} \quad (1)$$

178 where z_n represents the height of the nth data point, $\bar{z}$ is the mean height of z_n in
179 the AFM topography and N is the number of data points (Leprince, 2001).
180 The specific surface area was determined from the Brunauer-Emmett-
181 Teller (BET) equation (P/Po = 0.05 – 0.35), and a nitrogen adsorption isotherm
182 was measured at -196 °C in a Gemini 2375 (Micromeritics, Norcross, GA, USA).
183 The samples were previously degassed (10^{-2} mbar) for 8 h at 150 °C.

SAXS experiments were conducted on the D2A and D11A beamlines at the Brazilian Synchrotron Light Laboratory (LNLS, Campinas, Brazil) at a wavelength of 1.488 nm. The incident beam was detected at two different sample-to-detector distances (1549.8 mm and 2245.7 mm) to increase the range of the scattering vector q (q = (4π/λ) sinθ, 2θ = scattering angle). The dried samples were placed between two Kapton® foils, and the collimated X-ray beam was passed through the chamber that contained the stainless steel sample holder. All measurements were performed at room temperature. Silver behenate powder was used as a standard to calibrate the sample-to-detector distance, detector tilt and direct beam position. Transmission, dark current and Kapton® foil corrections were performed on the 2D images before further data processing. The isotropic scattering patterns were radially averaged. SAXS data analysis was performed using the Irena evaluation routine (Ilavsky, 2009), which was implemented in the IgorPro Software (WaveMetrics, Portland, USA (Kline, 2006). A multilevel unified fit was used to describe the two levels of structural organization that were evident in the scattering data. In this method, the scattering that is provided by each structural level is the sum of a Guinier exponential form and a structurally limited power-law tail. A generalized equation that represents any number of levels can be written as (Beaucage, 1995; Beaucage, 1996):

$$I(q) = \sum_{i=1}^{n} G_i \exp\left(\frac{-q^2 R_{gi}^2}{3}\right) + B_i \exp\left(\frac{-q^2 R_{g(i+1)}^2}{3}\right)\left[\frac{(\mathrm{erf}(qR_{gi}/\sqrt{6}))^3}{q}\right]^{Pi} \qquad (2)$$

where n is the number of structural levels that are observed, G is the Guinier prefactor, R_g is the radius of gyration and B is a prefactor that is specific to the power-law scattering, which is specified as the decay of the exponent P.

For the Fourier transform infrared spectroscopy (FT-IR) measurements, spectra were recorded at room temperature on a Bomem MB-102 Spectrometer; 36 scans with a resolution of 4 cm^{-1} were added together. The samples were analyzed in absorbance mode as pellets that were prepared by sample dilution in KBr. For the analysis of the xerogels using UV-Visible spectroscopy, the solids were analyzed using a diffuse reflectance spectroscopy (DRS) accessory that was equipped with a round sampling cup covered by a quartz window. The spectra were recorded at room temperature on a Varian Cary 100 UV-Visible Spectrophotometer; 32 scans in the 200 – 800 nm range were added together. X-ray photoelectron spectroscopy (XPS) measurements were performed on an Omicron-SPHERA station using Al K_α radiation (1486.6 eV). The anode was operated at 225 W (15 kV, 15 mA). Survey spectra were recorded with a 50 eV pass energy. The detection angle of the photoelectrons (Θ) with respect to the normal of the sample was fixed at 53° for all of the measurements.

Zeta potential measurements were conducted using a Zeta PALS Analyzer (Brookhaven Instruments). The Zeta Potential Analyzer version 3.18 (Brookhaven Instruments) software was used to collect the data. In a typical experiment, 50 mg of the sample was first diluted in 20 mL of MilliQ water and then filtered through VertiPure NYLON syringe filters (13 mm, 0.45 µm, 100/pk filter). Next, 1.5 mL of the filtrate was introduced into polystyrene cuvettes (square, 10 mm, 4.5 mL, four-sided clear). The instrument automatically calculated the zeta potential from the electrophoretic mobility (which is related to the ζ potential at the interface) using the Smoluchowski equation (Hunter, 1981):

$$\upsilon_E = 4\pi\varepsilon_0\varepsilon_r \frac{\zeta}{6\pi\mu}(1+\kappa r) \quad (3)$$

where ε_0 and ε_r are the dielectric constant and electrical permittivity, respectively, of a vacuum, μ is the solution viscosity, *r* is the particle radius and $\kappa = (2n0z^2e^2/\varepsilon_r\varepsilon_0 kBT)^{1/2}$ is the Debye–Hückel parameter.

Antimicrobial activity

Bacterial strains

The encapsulated gentamicin samples were tested against five bacterial strains, which included two Gram-positive and three Gram-negative strains: *Staphylococcus aureus* ATCC 25923*, Bacillus cereus* ATCC 11778*,* *Escherichia coli* ATCC 25922, *Enterobacter aerogenes* ATCC 13048 and *Salmonella typhimurium* ATCC 14028*.* All of the strains were stored at -20 °C in the appropriate medium with 10 % glycerol and were sub-cultured in tryptic soy agar (TSA) slants that were maintained at 4 °C.

Disc-diffusion assay

The detection of the inhibitory effects of the samples on the tested bacteria was conducted using the agar disc-diffusion method (NCCLS/CLSI) with the following modification: approximately 10^6 CFU mL^{-1} suspensions of the tested bacteria were used to inoculate the agar plates. Briefly, bacterial cultures from TSA slants were grown overnight at 35 °C in tryptic soy broth (TSB), and culture suspensions were adjusted to approximately 10^8 CFU mL^{-1} in a 0.9 % saline solution by visual comparison with a 0.5 McFarland standard. The adjusted suspensions were then diluted to approximately 10^6 CFU mL^{-1} in a saline solution and inoculated using a sterile swab in Mueller-Hinton agar

plates. Solutions of each tested substance, both neat gentamicin (the reference antibiotic) and encapsulated gentamicin, were diluted to 5 mg L^{-1} in distilled water. Sterile paper discs (9 mm in diameter) were impregnated with 25 µL of the diluted samples and placed on the center of the inoculated plates, which were incubated at 36 °C for 18 – 24 h. The diameters of the inhibition zones were measured in millimeters. Tests were performed in triplicate, and the results are presented as a mean (± standard deviation).

RESULTS AND DISCUSSION

In silica that is prepared by the sol-gel method and is based on silicon alkoxides, two fundamental concurrent reactions occur: hydrolysis and condensation (Brinker, 1990). In the first step, hydrolysis leads to the formation of Si – OH bonds with alcohol release. In the second step, condensation reactions produce water or alcohol, leading to the generation of siloxane (Si-O-Si) bonds, which form the bulk silica skeleton. The rate between these two reactions is affected by the catalyst: an acid catalyst promotes the hydrolysis reaction, while a base favors the condensation reaction. Thus, the nature of the sol-gel route may affect the final textural properties of the materials and, in the case of the encapsulation process, the content of the incorporated target molecule. Table 1 shows the elemental analysis of the resulting xerogels.

(insert Table 1)

As shown in Table 1, the acid route produces the lowest amount of encapsulated gentamicin. Because it favors hydrolysis, the acid route engenders the formation of weakly crosslinked gels, while the base-catalyzed route produces a hierarchical and much more complex network structure.

SILBP showed the highest gentamicin encapsulated content. These results are in agreement with the nitrogen adsorption measurements: SILBG and SILBP presented very low surface areas of 8 m^2 g^{-1} and 3 m^2 g^{-1}, respectively. A high surface area xerogel was achieved via the acid-catalyzed route: 326 m^2 g^{-1}. The highest surface area, which was measured for SILAG, may be the result of the texture that is intrinsic to such a route, i.e., a ramified structure. Therefore, the structure that is exhibited for the acid-catalyzed sol-gel route may favor the leaching of gentamicin from the forming silica network during the encapsulation process, which, in turn, may lead to the low gentamicin content.

To further investigate the effect of presence of gentamicin in the sol-gel syntheses on the structure of the silica network of the resulting hybrid materials, FT-IR analyses were performed.

According to the literature, silica-based materials have a long-range amorphous structure, which results from a random network of elementary SiO_4 units that are locally arranged into cyclosiloxanes, which mostly contain four and six Si atoms (Fidalgo, 1994). The relative proportions of these cyclic units can be obtained from the deconvolution of the $v_{as}(Si\text{-}O)_{Si\text{-}O\text{-}Si}$ infrared band. The four components that were obtained were previously assigned to the longitudinal and transverse optical doublets (LO/TO) in four-fold, $(SiO)_4$, and six-fold, $(SiO)_6$, siloxane rings (Fidalgo, 1995). The deconvoluted FT-IR profiles of three typical samples are shown in Figure 1.

(insert Figure 1)

These proportions may be estimated for each sample using the following ratio: (areas of the LO_6 + TO_6 components)/(total area of the $v_{as}(Si\text{-}O)_{Si\text{-}O\text{-}Si}$

band). Table 2 reports the wavenumber, area of each component and percentage of six-fold, $(SiO)_{6,}$ siloxane rings for the different systems.

(insert Table 2)

According to Table 2, the presence of gentamicin in the sol-gel syntheses of silica-based hybrid materials resulted in different network structures depending on the synthesis protocol. For the SILBP system, the presence of gentamicin led to an approximate 40 % decrease in the proportion of six-fold ($(SiO)_6$) siloxane rings in comparison with that observed for the SILBP system. In the case of the systems that were prepared by gelation routes, the addition of gentamicin in the sol-gel syntheses resulted in an increase in the number of $(SiO)_6$ siloxane rings in comparison with the bare systems. For SILBG, this increase was 9 %. Finally, in the case of SILAG, which was prepared under acid conditions, an increase of 24 %, in comparison with the bare silica SILAG, was observed. These results suggest that the addition of gentamicin in the synthesis of SILBP produced a more compact and denser network in comparison with that of the SILBG and SILAG systems.

The systems were further characterized by diffuse reflectance spectroscopy (DRS) in the UV-Vis region (Figure 2).

(insert Figure 2)

As shown in Figure 2, spectrum (a) show the maximum absorption bands of neat gentamicin at 206 and 254 nm. These bands correspond to the $n\rightarrow \sigma^*$ transitions (Williams, 1966) of the isolated electron pairs of oxygen and nitrogen atoms. After encapsulation within the silica matrix (spectrum b), these peaks shifted to 213 nm and 264 nm, indicating a bathochromic effect, which is most likely due to the change in medium that is caused by the silica framework. Thus,

this process caused a reduction in the energy levels of the n→ σ□ transitions of the isolated electrons from oxygen and nitrogen atoms (Williams, 1966).

An attempt to estimate the amount of encapsulated gentamicin on the outermost external surface of the grain was made using X-ray photoelectron spectroscopy (XPS). In the case of silica, the sampled measured depth was in the range of 5 nm. Table 3 lists the surface analysis expressed as N/Si, in which the amount of nitrogen is assigned to gentamicin, while that of Si is assigned to silica.

(insert Table 3)

According to Table 3, the precipitation route produces the highest encapsulated gentamicin content on the outermost external surface.

The SAXS technique was also used for the characterization of silica to elucidate the structures of the materials. The SAXS curves of these materials have a structure that is formed by organization levels that consist of a Guinier region (level 1) and a Potency Law (level 2). A typical SAXS curve for the SILAG system is shown in Figure 3.

(insert Figure 3)

According to Figure 3, the unified set of SAXS data reveals that the materials are arranged in a structure that consists of two organization levels. By adjusting level 1, which is located in the q region above 0.03 $Å^{-1}$, the radius of gyration (*Rg*) of the primary particles can be determined. Level 2, which is located in the q region below 0.01 $Å^{-1}$, provides information on the organization of these particles, i.e., on the structure of the fractal clusters (secondary particles) that result from the aggregation of the primary particles. The structure of the primary particle clusters that constitute level 2 can be obtained by

analyzing the power law exponent of the scattering curve. If the exponent of the power law ($I \alpha q^{-p}$) is between 1.0 and 3.0, the secondary particles have a mass fractal structure (Schmidt, 1991). When the exponent is between 3.0 and 4.0, the secondary particles have a fractal surface. In the case of an exponent of 4.0, the secondary particles have a dense core and a uniform surface. In the present study, all of the SAXS curve systems revealed the presence of two distinct organization levels. The results that were obtained from the unified set of SAXS curves for the silica-based mixed oxides are presented in Table 4.

(insert Table 4)

According to Table 4, the radius of gyration (R_g) of the primary particles for the investigated systems was between 2.9 and 4.4 nm. Regarding the organization of the primary particles, the values suggest the formation of secondary particles with fractal surface characteristics.

The morphology of these materials was investigated by scanning electron microscopy (SEM), as shown in Figure 4.

(insert Figure 4)

According to the SEM images, the acid route produces lamellar materials. The base-catalyzed routes by gelation and by precipitation produce particular materials with some spherical domains. The surface topography of the drug-entrapped silica that was obtained from the three sol-gel routes was measured by atomic force microscopy (AFM). Figure 5 shows the AFM micrographs and the roughness RMS that were obtained in contact mode for the drug-entrapped silica systems.

(insert Figure 5)

As shown in the AFM micrographs in Figure 5, the surface topography of drug-entrapped silica was clearly influenced by the preparation protocol of the materials. For the system that was synthesized under an acid condition (SILAG), the AFM micrograph exhibits a surface that is composed of large particles or aggregates of particles. This sol-gel route produced a silica material with a surface roughness (215 nm) that was higher than that observed for the two other systems. For the system from gelation under basic conditions (SILBG), the surface morphology revealed a surface that was formed by small particles with sizes in the range of 0.1 – 1.0 μm. The estimated surface roughness was 145 nm. A similar surface morphology was observed for SILBP, but a higher surface roughness was observed (195 nm). These differences in surface roughness values among the systems may be attributed to both the different rates of hydrolysis and silica formation condensation (the synthetic route) and to the presence of different amounts of gentamicin within the particles.

The encapsulated gentamicin was evaluated using a series of bacteria, as shown in Table 5.

(insert Table 5)

Table 5 shows the antimicrobial activity of the tested samples, as determined by the disc-diffusion test. The results for the preliminary assessment of the antibacterial activity of encapsulated gentamicin in SILBP indicate a potential effect on all of the tested microorganisms, especially against the Gram-positive strain *Staphylococcus aureus.* The higher efficacy of gentamicin on this microorganism is most likely because Gram-positive bacteria present low intrinsic resistance to antimicrobial agents because these bacteria do not

have specific membrane receptors or permeases that control the entrance of a substance into the cell, whereas Gram-negative bacteria lipopolysaccharides have an outer membrane that limits access, particularly for lipophilic substances, to cell membrane phospholipids (Russel, 1991).

According to the literature, the presence of encapsulated materials in a reaction medium does not generally affect the spread of its molecules; therefore, the effect on the microorganism (Nicholls and Dawson, 1988), i.e., the structure-activity relation, is not altered, thus preventing the effectiveness of bacterial defense and/or resistance mechanisms through enzymatic action. In the present case, we observed that the encapsulation method appeared to affect the activity of the drug, most likely due either to deactivation during synthesis or even to the textural characteristics of the final xerogel.

Another factor that may have influenced the improved efficiency of the encapsulated antibiotic from the SILBP route is the amount of gentamicin that is contained on the outermost particle surface, thus providing a controlled release of the molecule during the reaction. The amount of gentamicin that was contained on the SILBP capsule surfaces was determined from XPS analysis (N/Si = 0.49) and was higher than that in SILAG (0.08) and SILBG (0.04), which did not exhibit any noticeable inhibitory effect against the tested bacteria, most likely due to the low gentamicin concentration that was present on the surfaces. Thus, SILAG and SILBG do not exhibit any inhibition potential due the low amount of molecules on the surface, as characterized by the XPS technique.

The fact that the surface phenomenon between the antimicrobial agent and the microorganism cell is crucial for the inhibitory effect cannot be neglected. All aminoglycosides, including gentamicin, act via the same inhibition

mechanism; they exert their bactericidal effect by binding to bacterial ribosome. Therefore, it is necessary that the antibiotic molecule remains attached to the cell wall. Thus, the cell permeability is affected, enabling the transport of the active fraction of the antibiotic (Taylor et al., 2004; Donnell et al., 2010; Blanco-Príeto et al., 2002). In this sense, the roughness of SILBP (195 nm, as detected by AFM) may have influenced the antimicrobial effect, as verified by the increase in the inhibition area that was found for the microorganisms *Staphylococcus aureus, Escherichia coli, Enterobacter aerogenes* and *Bacillus cereus*. The same behavior was not observed for *Salmonella typhimurium*. The surface roughness from this treatment may facilitate contact between the antibiotic molecule and the cell membrane, thus favoring the cellular inhibition mechanism of aminoglycosides.

The zeta potential results of SILAG, SILBG, SILBP and their respective blanks are shown in Table 6.

(insert Table 6)

SILBP has the highest zeta potential, which is positive. The cell wall of Gram-positive bacteria (peptidoglycan) has a negative superficial charge due to the phosphoryl groups that are localized as teichuronic acid substituents. Because the peptidoglycan of Gram-negative bacteria is sequestered in the periplasmic space and not exposed to the extracellular environment, the negative surface charge in these microorganisms is conferred by the phosphoryl groups and carboxyl-2-ceto-3-deoxyoctonate lipopolysaccharides that are located on the outside membrane (Cheow, 2010). Therefore, the material with the highest positive zeta potential (SILBP) could have higher interactions with “tight junctions” and with the cell membrane, so the

paracellular permeation of hydrophilic compounds (Cheow, 2010), such as gentamicin, feasibly increases their antimicrobial activity.

Another factor that appears to influence the gentamicin antimicrobial activity is the N/Si ratio, which was measured by XPS. By analyzing the data from Table 3, it can be seen that the SILBP material exhibits the highest N/Si ratio, indicating that this material has a higher gentamicin concentration on the surface. This fact explains the zeta potential result, considering that the NH_2 groups from gentamicin confer a positive charge to the material surface. A correlation (R^2 = 0.9998) between the N/Si ratio and the zeta potential was observed, indicating that there is a strong correlation between the data. Therefore, pharmaceutical diffusion into the medium is easier than for other materials with lower N/Si ratio values, in which the drug was less available.

CONCLUSIONS

For the encapsulation of gentamicin within silica-based materials, it has been shown that the sol-gel route affects the elemental, structural, textural and morphological characteristics of the resulting xerogel, which, in turn, determine the antimicrobial activity. Of the evaluated routes, the route involving base-catalyzed precipitation produced the most active system, even higher than that of the bare antibiotic. The surface concentration of gentamicin and the zero potential of the xerogel appear to vary depending on the encapsulation route, which is therefore a relevant factor in the antimicrobial effect.

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Figure Captions

Figure 1. Deconvolution of the Si – O region in the FT-IR spectra of hybrid materials: (a) SILBP, (b) SILBG and (c) SILAG.

Figure 2. Diffuse reflectance spectra of (a) gentamicin and (b) SILAG.

Figure 3. Typical SAXS curve for the SILAG supports, and the curve fit from the unified model.

Figure 4. SEM images of (a) SILAG, (b) SILBG and (c) SILBP.

Figure 5. AFM images of (a) SILAG, (b) SILBG and (c) SILBP.

Tables Captions

Table 1. Elemental analysis of the produced xerogels.

Table 2. Wavenumber, area of components and percentage of six-fold, (SiO)6, siloxane rings for the different systems.

Table 3. N/Si ratio measured by XPS.

Table 4. SAXS data for silica-based mixed oxide determined from the curve fits.

Table 5. Antimicrobial activity of encapsulated gentamicin, the silica blank and gentamicin.

Table 6. Zeta potentials of SILAG, SILBG, SILBP and their respective blanks.

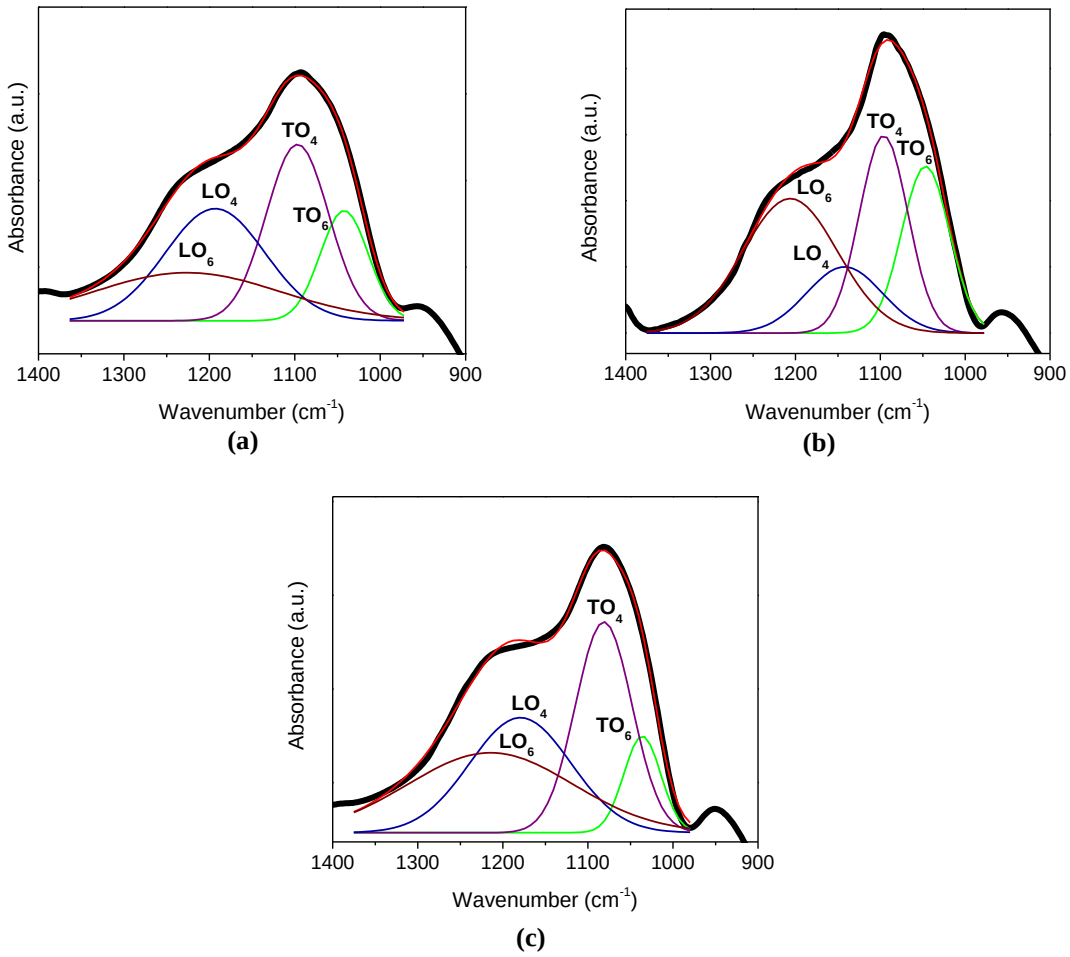


Figure 1. Deconvolution of Si – O region in the FT-IR spectrum of hybrid materials: (a) SILBP; (b) SILBG and (c) SILAG.

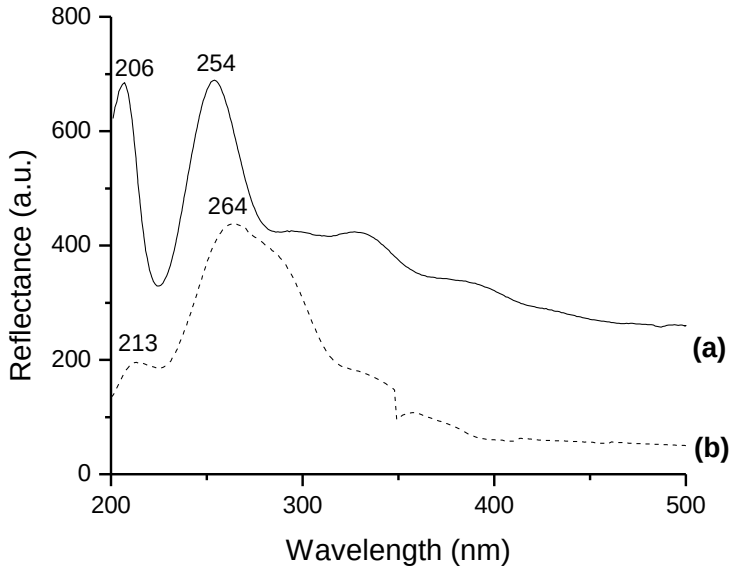


Figure 2. Diffuse reflectance spectrum of (a) gentamicin and (b) SILAG.

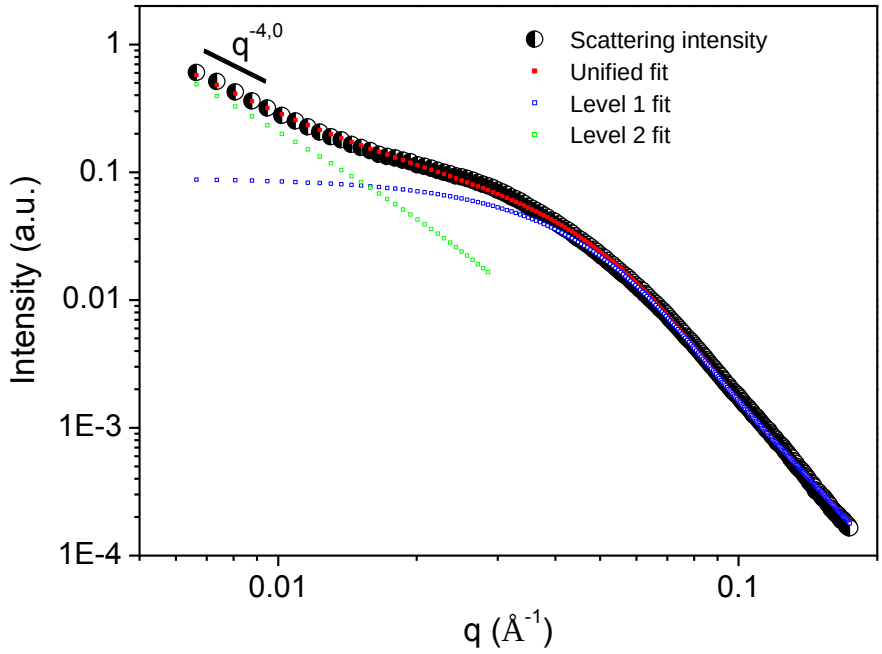
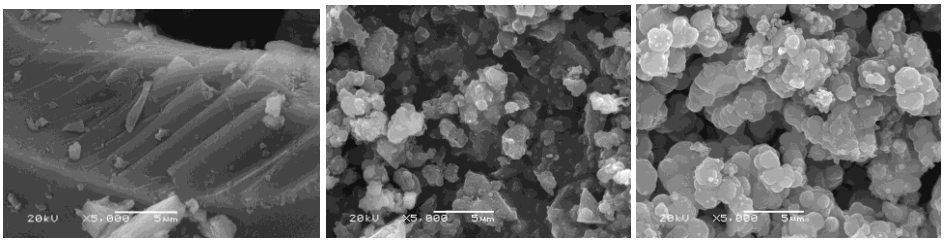


Figure 3. Typical SAXS curve for the SILAG supports and curves fit through the unified model.



(a) **(b)** **(c)**

Figure 4. SEM images of: (a) SILAG; (b) SILBG and (c) SILBP.

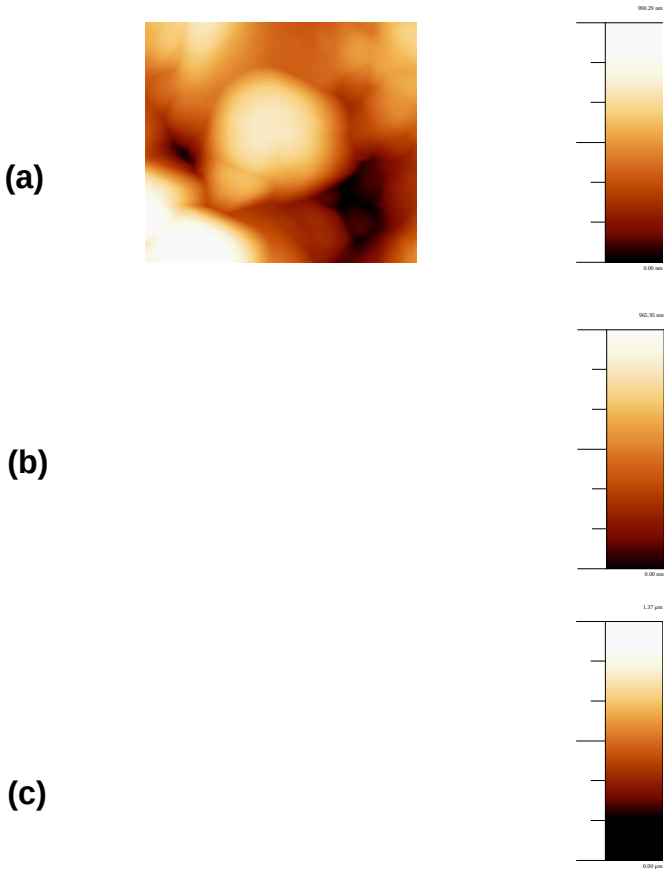


Figure 5. AFM images of: (a) SILAG; (b) SILBG and (c) SILBP.

Table 1. Elemental analysis of the produced xerogels.

Xerogels	Element content (%)		Gentamicin encapsulated ($mg.g^{-1}$)
	C	**N**	
SILAG	0.56	0.17	11.2
SILBG	0.81	0.42	27.8
SILBP	0.77	0.84	55.6

Table 2. Wavenumber, area of components and percentage of six-fold, $(SiO)_{6,}$ siloxane rings for the different systems.

	SILBP	SILP	SILBG	SILB	SILAG	SILA
LO_6	1226	1188	1206	1205	1214	1232
A	75.6	163.4	56.6	184.4	41.5	3.3
LO_4	1193	1116	1142	1128	1179	1175
A	94.6	42.1	23.4	26.4	36.5	14.8
TO_4	1097	1095	1096	1099	1081	1096
A	93.1	49.0	42.9	178.7	37.5	30.6
TO_6	1042	1054	1046	1055	1036	1024
A	44.8	37.1	35.8	54.6	11.3	20.1
$(SiO)_6$(%)	39	69	58	53	42	34

Table 3. N/Si ratio measured by XPS.

Encapsulate gentamicin	N/Si
SILAG	0.08
SILBG	0.04
SILBP	0.49

Table 4. SAXS data for silica-based mixed oxide determined from the curve fits.

System	Level 1		Level 2	
	***Rg* (nm)**	**P**	***Rg* (nm)**	**P**
SILAG	3.9	4.0	-	3.8
SILBG	2.9	4.0	-	3.9
SILBP	4.4	4.0	-	4.0

Table 5. Antimicrobial activity of encapsulated gentamicin, the silica blank and gentamicin.

	Inhibition areas (mm) [a]				
	Staphylococcus aureus	***Escherichia coli***	***Enterobacter aerogenes***	***Salmonella tiphymurium***	***Bacillus cereus***
	+	**-**	**-**	**-**	**+**
SILAG	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
SILBG	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
SILBP	9.67 ± 0.35	8.57 ± 0.29	9.41 ± 0.31	7.23 ± 0.23	8.51 ± 0.46
Silica	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Cut control	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Gentamicin	8.52 ± 0.21	8.02 ± 0.88	7.99 ± 0.57	8.00 ± 0.80	7.52 ± 0.32

[a] Inhibition area including 9 mm disc diameter expressed as the mean of four replicates ± SD.

Table 6. Zeta potential of SILAG, SILBG, SILPG and their respective blanks.

Zeta potential (mV)		
System	**Blank**	**Encapsulated**
SILAG	5.41 ± 0.320	8.80 ± 1.07
SILBG	-27.7 ± 2.26	6.67 ± 1.17
SILBP	-35.5 ± 0.36	27.8 ± 1.06

ANEXO: Normas da revista “Journal of Colloid and Interface Science”

Introduction

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