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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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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 a series of Gram-positive and Gram-negative bacterial strains. The best antimicrobial activity was observed with 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 process.

1. Introduction

Encapsulation represents a technological approach that consists of enveloping a given entity, such as drugs [1,2], catalysts [3], pesticides [4] and cells [5], 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 [6]. Other applications of encapsulation involve the use of encapsulated molecules for imaging and diagnostic techniques [7,8].

One class of drugs that has been investigated with a variety of encapsulation methods is antibiotics. In this context, biodegradable microspheres are useful for prolonged drug release and for targeting drugs to specific infection sites. In some cases, the encapsulation of antibiotics in polymeric nanoparticles overcomes the problem of antibiotic deactivation because this encapsulation prevents interactions between the antibiotic and, for instance, the sputum contents in the case of inhalation. Examples of antibiotic encapsulation include levofloxacin in poly(lactic-co-glycolic acid) nanoparticles [9], ciprofloxacin in alginate/pectin microspheres [10], ofloxacin in chitosan microspheres [11] and violacein in poly-D,L-(lactide-co-glycolide) [12].

The encapsulation of gentamicin, which is an aminoglycoside antibiotic, for use in the treatment of 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 [13], Phospholipon®90G and Softisan® 154, using a solid-reversed-micellar solution for intramuscular administration [14] and in biodegradable polymers (polylactic acid and cellulose acetate), which are a shell material, using the coaxial electrospinning technique [15].

Inorganic carriers, either with or without organic counterparts, have also been used in the encapsulation of drugs [16–18]. Silicon-based materials are usually preferred for drug delivery systems because of their relative bio-inertness and their degradation into nontoxic silicic acid [6]. Furthermore, silica-based materials can easily be chemically modified, thus producing a broad range of hybrid materials [19]. In the case of gentamicin, several studies were conducted that combined mesoporous-based silica and layer-by-layer films, such as poly(allylamine hydrochloride)

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and poly(styrene sulfonate) [20]. Other examples have been reported, including the preparation of poly(lactic-co-glycolic acid)/mesoporous silica [21,22], a SiO_2-CaO-P_2O_5 sol–gel glass [23] and silica [24,25]. For the latter, the encapsulation of the drug was achieved using the sol–gel process of tetraethoxysilane with hydrochloric acid, i.e., an acid-catalyzed process. The mild conditions of the sol–gel encapsulation route are beneficial as the process can be conducted at room temperature [26]. In previous studies, we used the sol–gel process to develop molecular imprinted silica-based materials with pharmaceuticals as templates for environmental matrix pre-concentrations [25,27,28].

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 the encapsulation method on the biological efficacy of an antibiotic as a result of the choice of the sol–gel route 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., elemental analysis, 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 antimicrobial effects of encapsulated gentamicin samples were tested against fifteen bacterial strains of pharmaceutical interest.

2. Experimental

2.1. 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.

2.2. Synthesis of xerogels

Xerogels were synthesized via the sol–gel method using one of three processes: (i) an acid-catalyzed route (A), (ii) a base-catalyzed route (B1) and (iii) a base-catalyzed route by precipitation (B2). In route A, 8.6 mL of chloridric acid (0.2 M) (a catalyst) was added to a 250, 500, 750 or 1000 mg solution of gentamicin dissolved in 10 mL of TEOS. The mixture was stirred for 24 h until gelation occurred. The resulting precipitate was dried at room temperature and ground, thus producing the xerogel SILAG. In the base-catalyzed route, ammonium hydroxide was used as the catalyst in varying amounts. In route B1, 5 mL of ammonium hydroxide (2.8%) was added to a 500 mg solution of gentamicin 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 dissolved in 10 mL of TEOS. The mixture was stirred for 20 min until precipitation occurred. The resulting material was dried at room temperature and ground, thus producing SILBP. The three corresponding materials were labeled SILAG, SILBG and SILBP, and their respective blanks were SILA, SILB and SILP.

2.3. Characterization of the xerogels

The carbon and nitrogen content of the xerogels was determined using a PerkinElmer (Wellesley, MA, USA) M-CHNSO/2400 analyzer. SEM experiments were conducted on a JEOL (Tokyo, Japan) JSM/6060 microscope. The samples were fixed on carbon tape and then coated with gold using conventional sputtering techniques.

AFM images were obtained using a Nanoscope IIIa atomic force microscope (Digital Instruments Co.) in contact mode with silicon nitride probes. The WSMX 4.0 software from Nanotec Electronic S.L. was used for image treatment. The surface roughness was quantified using the root-mean-squared roughness (R_{rms}), which was obtained from the standard deviation (S.D.) of the data from the AFM images, as determined using software and the standard definition shown in equation (1):

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

where z_n represents the height of the nth data point $\bar{z}$, is the mean height of z_n in the AFM topography and N is the number of data points [29].

The specific surface area was determined from the Brunauer-Emmett-Teller (BET) equation (P/P_o = 0.05–0.35), and a nitrogen adsorption isotherm was measured at -196 °C in a Gemini 2375 (Micromeritics, Norcross, GA, USA). 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 ($q = (4\pi/\lambda)\sin\theta$, where 2θ is the 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 [30], which was implemented in the IgorPro Software (WaveMetrics, Portland, USA) [31]. A multilevel unified fit was used to describe the two levels of structural organization evident in the scattering data. In this method, the scattering 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 [32,33]:

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

where n is the number of structural levels observed, G is the Guinier prefactor, R_g is the radius of gyration and B is a prefactor 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 combined to generate the spectra. The samples were prepared by sample dilution in KBr to generate pellets and were analyzed in