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Antimicrobial supported nanoparticles: Gold *versus* silver for the cases of *Escherichia coli* and *Salmonella typhi*

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ABSTRACT

Silver and gold nanoparticles were dispersed on TS-1 silicalite with a low titanium content. These materials were evaluated as biocides for *Escherichia coli* and *Salmonella typhi*. In the presence of TS-1, free of silver or gold, both bacteria use the silicalite's surface to reproduce rapidly. Ag- and Au-TS-1 were shown to be efficient for eliminating both *E. coli* and *S. typhi* present in a nutritive media. The efficiency of materials was related to their textural properties. In general, *E. coli* was eliminated faster than *S. typhi*.

1. Introduction

Antimicrobial agents are required to inhibit the reproduction of pathogens in several media therefore numerous antimicrobial agents have been proposed. Their efficiencies are determined by several physicochemical factors [1–3]. Many of them, however, are toxic, which makes them undesirable for applications in sensitive media such as drinking water, foods or textiles. In this sense, silver is a non-toxic, non-tolerant disinfectant that can significantly reduce many bacterial infections [4]. In fact, silver has been commonly used to treat or avoid infections since long time [5–7]. Commonly, silver is used in the form of ions, Ag^+, but this form is unwelcome for some specific applications, for instance where Ag^+ could incorporate to materials by exchange cations. This commonly occurs with non-supported silver species and for example the application in textiles is not desirable in this form. Thus, recently it was proposed that silver nanoparticles supported on zeolites could be lethal for bacteria [8]. Zeolitic supports, however, differ greatly regarding their physicochemical properties, which in turn tune the properties of the metal being supported [9–11]. Silver-supported particles have shown to be efficient inhibiting the growth of pathogens but it is also true that silver-resistant bacteria are more and more frequently reported [12,13]. The relationship between resistance and eventual bioaccumulation of silver is not clear still [14,15]. In this sense, new materials, few explored as bactericide could be an alternative for silver materials. Actually, gold complexes with sulfur have been applied in some arthritis treatments [16] and since few years gold nanoparticles have been efficacy used as catalysts in a wide variety of reactions [17,18].

We have started this work in order to explore silicalite-supported silver and gold as bactericide for *Escherichia coli* and *Salmonella*. This support was selected because it has been shown to be very desirable to stabilized small gold particles [19] and the amount of ions stabilized by exchange ionic does not occur.

2. Experimental procedure

2.1. Support's synthesis

The TS-1 sample was prepared by a hydrothermal synthesis. Tetraethyl orthosilicate (TEOS) and tetrabutyl orthotitanate (TBOT) were used as source of silicon and titanium, respectively. Briefly, to one solution tetrapropylammonium hydroxide (TPAOH, 25 wt.%) were added a mixture of TEOS and TBOT (ratio Si/Ti equal to 50) under vigorous stirring. The reagent mixture was stirred for 12 h at room temperature, and then hydrothermally treated at 453 K for 90 h. The obtained solids were dried at 383 K for 24 h, and then calcined in air at 823 K for 5.0 h. The TS-1 sample contained Ti 2 wt.%, as determined by inductively coupled plasma-atomic emission spectroscopy (ICP-AES).

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2.2. Silver containing zeolites

TS-1 sample (2 g) was shaken for 3 h in 50 mL of a 0.05 M solution of $AgNO_3$. The samples were then separated by centrifugation, washed three times with deionised water and dried at 80 °C. Later, samples were reduced at 500 °C under H_2 (5 mL/min) for 4 h. The content of silver in reduced sample (named Ag-TS-1) was 0.13% as measured by atomic absorption spectroscopy.

2.3. Gold containing zeolites

Au deposition on TS-1 was performed at room temperature as follows. First, 0.5 g of support was added to 25 mL of 10 mM $HAuCl_4$ aqueous solution and the pH regulated to 9.0 by adding aqueous NaOH. The suspension was stirred vigorously at room temperature for 3.0 h. Lastly, the solid was centrifuged, washed three times with water, dried at 323 K under nitrogen and reduced at 500 °C in hydrogen. At end of preparation the sample contained Au 0.11 wt.% and it was named Au-TS-1.

2.4. Characterization

X-ray diffraction patterns of the samples were obtained on a Siemens D500 diffractometer with a copper X-ray anode tube. Kα radiation (wavelength of 1.315 Å) was selected with a diffracted beam monochromator.

Solid-state ^{29}Si Nuclear Magnetic Resonance (NMR) single excitation experiments were performed on a Bruker Avance 400 spectrometer at the frequency of 79.4 MHz. Spectra were acquired using the combined techniques of Magic Angle Spinning (MAS) and Proton Dipolar Decoupling (HPDEC). Direct pulsed NMR excitation was used throughout, employing 90° observing pulses (3 μs) with a pulse repetition time of 40 s. Powdered samples were packed in zirconia rotors and spun at 5 kHz. Chemical shifts were referenced to TMS.

Small-Angle X-ray Scattering (SAXS) curves were acquired using a Kratky camera coupled to a copper anode tube. The SAXS data were processed with the ITP program [20–24], where the angular parameter (h) is defined as $h = 4\pi \sin\theta/\lambda$, where θ and λ are the X-ray scattering angle and wavelength, respectively. The obtained data can be described by $I(q) = \sum_i I_i(0) \exp\left[(-R_{gi}q)^2/3\right]$, where $I_i(0)$ denotes the scattering intensity at $q = 0$ of the scattering center i with the radius of gyration R_{gi} [25]. The shape of the scattering objects was estimated from the Kratky plot, *i.e.*, $h^2I(h)$ against h. The shape is determined depending on the Kratky curve profile. For instance, the scattering curve for the globular conformation follows the Porod law, where $I(h)$ is proportional to h^{-4} for large h values and to h^{-2} for moderate h values. Hence, the Kratky plot exhibits a clear peak in the case of spherical particles. If a shape can be assumed, the size distribution function may be calculated. Lastly, from the slope of the curve $\log I(h)$ vs. $\log(h)$, the fractal dimension of the scattering objects was evaluated. It is worth notice that by the Babinet principle, the small-angle X-ray scattering may be due either to dense particles in a low-density environment or to pores or low-density inclusions in a continuous high electron density medium. Then, in order to characterize only the silver or gold phase, we have substracted the SAXS data of the TS-1 sample from those of the gold or silver-loaded zeolite. This method was earlier shown to be efficient in the characterization of particles supported onto porous materials [26,27].

2.5. Bacteria experiments

Escherichia coli and *Salmonella typhi* were acquired from ENCB Mexico. For growing and maintaining the bacterial cultures tripticaseine broth medium was used. Briefly, a starter culture of each strain was inoculated with fresh colonies and incubated for 24 h in tripticaseine medium. Bacterial growth rates were determined by counting the number of surviving colonies in a selective agar. Fresh medium (18 mL) was inoculated in test tubes with the starter culture and grown at 35.5 °C with continuous agitation at 30 rpm. Ag or Au containing silicalite (0.022 g) was then added to the culture, and the colonies were measured over a time course. During all experiments treated with bacteria the material used was sterilized and zeolite used immediately after preparation.

3. Results and discussion

3.1. Materials

XRD patterns (not shown) of gold and silver loaded silicalites did not differ from the reference TS-1 sample. This was expected because the conditions to incorporate metal were soft and the amount of metal loaded is beyond the detection limits of XRD technique. Of course, these results clearly indicate that the zeolite structure was preserved after silver or gold deposition. ^{29}Si MAS NMR spectra in Fig. 1 show that no modifications occurred in the local environment of silicon atoms as a consequence of the silver or gold incorporation. The spectra are composed by a broad signal with a maximum centered at −113 ppm with a shoulder on the high-field side. This result gives clear evidence that samples are composed mainly by Q^3 (peak at −113 ppm) and Q^4 (signal as shoulder) units containing Ti into the silicalite framework [28]. It is important to emphasize that practically none difference between the three spectra were observed, the free metal TS-1 and the gold or silver loaded samples, which means that incorporation of metal occurred without changes in the silanol surface.

Fig. 2 shows a comparison of the Kratky plots of Ag-TS-1 and Au-TS-1 samples; Both scattering profiles have a region at moderate angles where the intensity is related to h^{-2}, and at higher angles, the scattering intensity tends to be proportional to h^{-1}. These profiles are typical of particles with a fibrous shape [29]. This is a relevant result showing that texture of the metallic particles is determined by the support as earlier was reported that clinoptilolite induce the formation of globular silver particles [8]. It was also reported by TEM that gold dispersed in the TS-1 support leads to formation of very small spherical gold nanoparticles. Thus, the results of TEM and SAXS are complementary and suggest that the

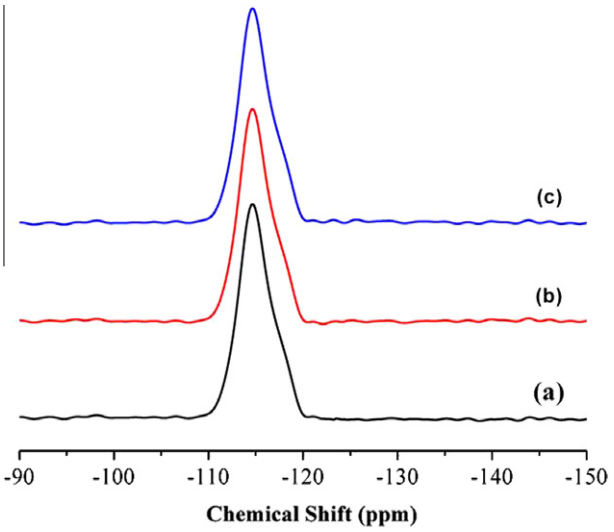

Fig. 1. ^{29}Si MAS NMR spectra of silicalite samples. TS-1 (a); Ag-TS-1 (b); Au-TS-1 (c).