CuO/LDHs and Ag/LDHs AS NANOSTRUCTURED ASSEMBLIES WITH ANTIMICROBIAL PROPERTIES

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Introduction

Ensembles of different nanostructures which are able to combine their characteristics and to organize themselves for creating materials owning complex properties have a great potential in biomedicine applications due to the particular interactions that can be established between nanoscaled architectures and biological interfaces. Nanoparticles with antibacterial activity have attracted a great deal of attention in biomedical applications. Nanoparticles of metals or metals oxides are easily aggregated; the aggregation behavior is the cause of in vitro and/or in vivo toxicity of metal nanoparticles [1]. A variety of organic solvents, stabilizers or coating agents (such as: polymers, ligands or surfactants) have been used for reducing the aggregation behavior and stabilizing them. These large organic compounds not only increase the cost of the obtained material but also strongly decrease the purity and the accessibility of the active nanosites. The assembly nanoparticles of metals or metal oxides / biocompatible inorganic matrix can improve this problem. Hydrotalcite – like anionic clays, or more generally speaking, layered double hydroxides (LDHs) are synthetic anionic clays with brucite ($Mg(OH)_2$)-like layers in which some of the divalent cations have been replaced by trivalent ions. Intense research has been carried recently on LDH anionic clays mainly due to the ability of these biocompatible inorganic matrices to incorporate and to transport various bio – and organic molecules [2]. The aim of the present work is to present nanoparticles of silver / layered double hydroxides (Ag/LDH) and nanoparticles of copper oxide / layered double hydroxides (CuO/LDH) as nanostructured assemblies with antimicrobial properties.

Experimental

MgAlLDH hydrotalcite – like anionic clay, denoted as LDH, is obtained by the coprecipitation method.
The nanoparticles of silver – LDH assembly, denoted as Ag/LDH: the "freshly" calcined clay was added to 200 mL of Ag_2SO_4 aqueous solution (1N) with stirring, under nitrogen atmosphere, at a constant pH.
The nanoparticles of copper oxide – LDH assembly, denoted as CuO/LDH: the "freshly" calcined clay was added to 200 mL of $CuSO_4$ aqueous solution (1N) with stirring, under nitrogen atmosphere, at a constant pH.
The obtained samples were aged at ambient temperature, washed, centrifuged and dried under vacuum.
Structural characteristics, crystallinity and purity information were recorded by X-ray diffraction (XRD) using a Shimadzu XRD 6100 diffractometer. TEM analysis was performed on a Hitachi H-900 instrument operating at 200 kV. The Fourier transform infrared (FTIR) spectra were collected on a Perkin Elmer Spectrum 100 spectrophotometer in the wavenumber range 450 - 4000 cm^{-1} at a resolution of 4 cm^{-1} using KBr pellets. The antibacterial activity against gram-positive bacteria, *Stphylococcus aureus* (ATCC 25923, denoted as *S. aureus*) and gram-negative bacteria, *Escherichia coli* (ATCC 35218, denoted as *E. coli*) was studied. The antibacterial activity was qualitatively and quantitatively assessed by agar diffusion tests and MIC *(minimal inhibitory concentration)* values, respectively. Tubes containing 10 ml solution of the tested sample were placed onto an agar plate seeded with 10^7 CFU/ml of microorganisms. MIC values were determined as the lowest concentration of the tested sample where the absence of growth was recorded [2].

Results and discussions

The TEM photographs of CuO/LDH (Fig.1(a)) and Ag/LDH (Fig.1(b)) show that, after the clay reconstruction in aqueous solutions of $CuSO_4$ and Ag_2SO_4 respectively, on the larger nanoparticles of LDH clay much smaller nanoparticles are supported.

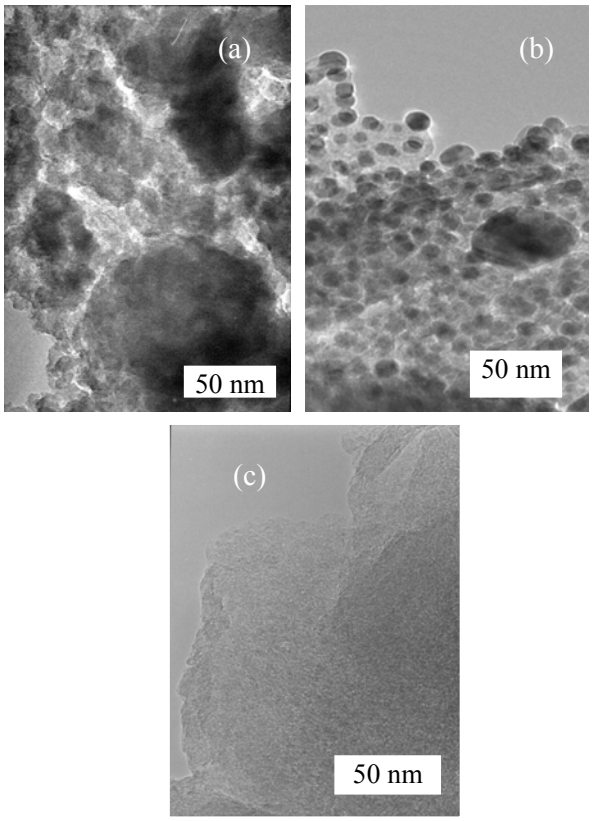

Fig. 1. The TEM photographs of (a) CuO/LDH, (b) Ag/LDH and (c) LDH.

Their average diameter is 15 nm for CuO/LDH and ~ 7 nm for Ag/LDH, respectively. The small nanoparticles are well dispersed in the case of Ag/LDH while their degree of agglomeration accentuates for CuO/LDH. On the contrary the TEM image of the calcined LDH (see Fig.1(c)) indicates a rough and clean surface of the larger nanoparticle of the clay (average diameter 90 nm).

Antibacterial effects, in the form of inhibition zones, of Ag/LDH and CuO/LDH are summarized in Table 1 and compared to that of nanoparticles of unsupported silver. The symptoms of clear zones against *S. aureus* and *E. coli* are presented in Fig. 2.

Table 1. Zone of inhibition (mm) and MIC values against the tested bacteria of the tested samples.

Sample(*)	Zone of inhibition (mm)	MIC values ($\mu g\ mL^{-1}$)
Ag /LDH	E Coli: 21.4 S. Aureus: 17.1	0.3 0.3
CuO/LDH	E Coli: 8.0 S. Aureus: 4.7	3
Ag [N]	E Coli: 14 .2 S. Aureus: 11.1	-

(*) concentration of silver and copper oxide in the tested sample is equal to 0.7 µg/ml; (obtained by ICP).
[N] unsupported nanoparticles of silver - obtained by the procedure described in [3].

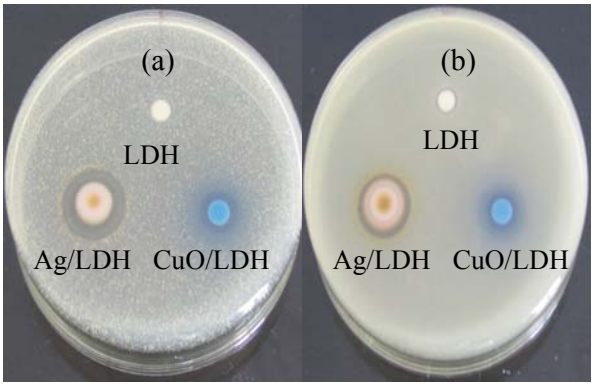

Fig. 2. Symptoms of clear zones of Ag/LDH and CuO/LDH against (a) *E. coli, (b) S. aureus*.

Enhanced antibacterial activities are characteristic to silver – clay assembly in comparison to copper oxide – clay assembly. The results (see Table 1) also indicate that silver - clay assembly shows accentuated antimicrobial properties and enhanced stability in comparison to unsupported nanoparticles of silver (Ag^N). The LDH clay alone doesn't show any antibacterial properties.

References

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