Applied Clay Science 158 (2018) 21–28

Contents lists available at ScienceDirect

Applied Clay Science

journal homepage: www.elsevier.com/locate/clay

Research paper

Antimicrobial activity of organoclays based on quaternary alkylammonium and alkylphosphonium surfactants and montmorillonite

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ARTICLE INFO

Keywords:
Staphylococcus
Escherichia
Surfactants
Colloids
Antibacterial
Organoclay

ABSTRACT

Organoclays are mostly materials composed of clay minerals modified with organic surfactants, predominantly of an alkylammonium type. Such hybrid materials often exhibit properties which are superior to those of their components. The main objective of this study was to characterize the antimicrobial activity and physico-chemical properties of tetrabutylammonium (TBA), dodecyltrimethylammonium (DDA), and tetrabutylphosphonium (TBP) cations, and the effectiveness of the organoclays composed of montmorillonite SWy-2 (Mt) and those cations. Their activity was tested on the Gram-positive bacteria *Staphylococcus aureus* and the Gram-negative bacteria *Escherichia coli*. Prior to the determination of their antimicrobial activity, organoclays were characterized using X-ray diffraction and infrared spectroscopy. The results confirmed the irreversible adsorption of organic cations onto the surface of Mt and the stability of the complexes in the medium used for biological experiments. The cytotoxicity of the organic cations on a HeLa cell line determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction assay proved to be dependent mainly on the type of surfactant. While DDA with long alkyl chains fully inhibited surviving HeLa cells at concentrations of 1 and 0.5 $mmol\,L^{-1}$, lower concentrations (0.1 and 0.05 $mmol\,L^{-1}$) were not toxic. On the other hand, TBA and TBP also exhibited a very low toxicity at the higher concentrations. DDA alone exhibited the highest antimicrobial properties. It inhibited surviving *S. aureus* and *E. coli* at a concentration of 1 $mmol\,L^{-1}$, estimated by the determination of colony forming units (CFU). Both TBA and TPB were only effective at a concentration of 10 $mmol\,L^{-1}$. All organoclays were prepared with a ratio of organic cation/Mt of $10^{-3}\,mol\,g^{-1}$ at a concentration $\leq 1\,mmol\,L^{-1}$. The antimicrobial effect of Mt alone was negligible and did not interfere with the effect of the organic cations. At this concentration, the most effective organoclay was based on DDA, which reduced the survival of both *S. aureus* and *E. coli* by over 93%. The organoclays based on TBA and TBP exhibited much lower antimicrobial effectiveness. The effectiveness of organic cations bound to the surface of Mt particles at a concentration of 0.1 $mmol\,L^{-1}$ was similar with *S. aureus* and lower with *E. coli* compared to that of the respective surfactant solutions. The results of this work provided new knowledge on the biological activities of the selected quaternary ammonium and phosphonium cations, which are often used in organoclays. The antimicrobial activity of the organoclays should be considered when they are applied as carriers of bioactive substances or in other applications dealing with biological systems.

1. Introduction

Organoclays are a large group of hybrid materials of various types based on clay minerals modified with organic compounds. Most common organoclays contain cationic surfactants, most often of the alkylammonium type. These materials were one of the first organoclays, introduced in the middle of the 20th century, and have achieved a broad range of industrial applications (de Paiva et al., 2008). Later on, there was a new focus of attention on organoclays as components in polymer nanocomposites (Alateyah et al., 2013), functional hybrid nanomaterials (Zhou et al., 2011), active sorbents of pollutants (Zhu and Chen, 2009), etc. Quaternary alkylammonium cations are a group of organic compounds, which are suitable for modifying the surface properties of clays. They are relatively stable and do not hydrolyze, in

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https://doi.org/10.1016/j.clay.2018.03.010
Received 14 November 2017; Received in revised form 4 March 2018; Accepted 6 March 2018

contrast to the cations derived from primary, secondary or tertiary amines. Long-chain alkylammonium cations are irreversibly adsorbed onto the surface of smectites, which makes organoclays derived from such organic cations very stable. The adsorption of alkylammonium cations significantly alters the properties of the mineral. Surface activation due to the presence of non-polar alkyl chains, the expansion of interlayer galleries and hydrophobization are the main changes that occur with modification with alkylammonium cations (Bujdák, 2015; Gamba et al., 2017). The organoclays with cationic surfactants bearing shorter alkyl chains in their molecules are less frequent than those based on their analogues with long alkyl chains (Açışlı et al., 2017). Organoclays with bulky-shaped quaternary organic cations have been introduced as efficient sorbents of some organic pollutants (Kukkadapu and Boyd, 1995; Abbas et al., 2017). Recently, they were successfully applied in the expansion of some non-swelling layered compounds, such as layered oxides, niobates, vanadates, and titanates (Ma and Sasaki, 2010; Maluangnont et al., 2013; Hashemzadeh, 2016). The method is efficient for the expansion and exfoliation of layered compounds, which are very stable solids and do not swell under standard conditions. Acid treatment to prepare H^+-forms and subsequent exchange/neutralization with tetraalkylammonium hydroxide is one of a few efficient ways to prepare finely dispersed and exfoliated colloidal systems of these compounds (Shiguihara et al., 2007). This method may have potential for the modification of non-expandable layered silicates, such as vermiculites and mica (Williams et al., 1996). Another promising type of organoclay is based on quaternary alkylphosphonium cations (Pálková et al., 2017). The structure of alkylphosphonium cations is almost the same as their alkylammonium homologues. However, a much lower polarity of C—P bond and higher thermal resistance of the phosphonium cations (Xie et al., 2002; Hedley et al., 2007) makes these organoclays promising for hybrid materials with properties that are superior to traditional organoclays (Cai et al., 2009).

In the last few decades, clay minerals have been considered as efficient carriers of active drug molecules, aiming towards applications in the medical industry. More complex systems have been investigated in terms of drug encapsulation (Delcea et al., 2011; Yapar et al., 2017), drug delivery systems (Kinninmonth et al., 2014), the formation of antimicrobial surfaces (Nigmatullin et al., 2009; Babu et al., 2018), and the release of active substances (da Silva et al., 2016). The influence of clays on drug efficiency was also considered and investigated (Ambrogi et al., 2014). Organoclays are a potential precursor of such complex systems. They are efficient sorbents of various types of drugs, such as antibiotics, photosensitizers (Donauerová et al., 2015) or disinfectants (Calabrese et al., 2016). Quaternary ammonium salts are industrially used as broad-range antimicrobial and disinfection agents (Bragg et al., 2014), determined to also exhibit activity against resistant bacteria (Yuen et al., 2015). Some organoclays have been reported to exhibit an antimicrobial effect, but there has still not been a detailed comparison with the activities of free surfactant salts (Ohashi and Oya, 1996; Hong and Rhim, 2008).

The objective of this work was to determine the antibacterial properties and physico-chemical characteristics of organoclays based on montmorillonite (Mt) and quaternary alkylammonium and phosphonium cations, both bulky molecular structures with long alkyl chains.

2. Experimental

2.1. Materials - clay mineral and surfactants

Montmorillonite (SWy-2) was purchased from the Clay standards depository of the Clay Minerals Society. It was exchanged to its Na^+-form using NaCl solution and washed with deionized water to remove excess salt solution. The fine fraction $< 2\,\mu m$ of a colloidal dispersion of the mineral was obtained by sedimentation. The sample was isolated by centrifugation, dried at 60 °C and ground to pass through a 0.2 mm sieve. Chlorides of tetrabutylammonium (TBA), tetrabutylphosphonium (TBP) and dodecyltrimethylammonium (DDA) were purchased from Sigma-Aldrich (UK) in p.a. purity and used as received.

2.2. Media, solutions, and substances used in microbiological experiments

The additives and chemicals were either of p.a. purities or designed for specific applications. Mueller-Hinton broth (M-H, Biolife, Italy) was prepared according to the supplier's instructions. It was sterilized by autoclaving (121 °C for 20 min) and maintained at 4 °C for a maximum of 4 weeks. M-H agar was prepared from broth by adding agar (Biolife, Italy, 2% w/v). Dulbecco's Modified Eagle's Medium (DMEM medium, Lonza, Belgium) was prepared as a 7% solution containing 7% fetal bovine serum (FBS, Lonza, Belgium), 1% penicillin, 1% streptomycin, 1% L-glutamine, and 0.1% gentamicin. The MTT solution contained 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma-Aldrich, UK) at a concentration of 0.5 mg mL^{-1} in phosphate-buffered saline solution (PBS; 137 mmol L^{-1} NaCl, 2.7 mmol L^{-1} KCl, 10 mmol L^{-1} Na_2HPO_4, 2 mmol L^{-1} KH_2PO_4, pH 7.4, sterilized by autoclaving). The MTT solution was sterilized by filtration (0.22 µm membrane filter, Millipore, UK) and stored in a refrigerator at 4 °C for a maximum of 2 weeks.

2.3. Tested systems

The solutions of organic salts exhibited variable antimicrobial effectiveness, and so they were tested at a specific range of concentrations. These were obtained from preliminary experiments: i) high concentrations – 1.10^{-3}–1.10^{-2} mol L^{-1} (for TBA and TBP); ii) low concentrations – 5.10^{-5}–1.10^{-3} mol L^{-1} (for DDA). Mt and organoclays were tested as aqueous colloidal suspensions. Organoclays were prepared with a constant ratio of organic cation/Mt, 10^{-3} mol g^{-1}, which is close to the cation exchange capacity of the mineral. This loading guarantees a complete adsorption of the organic cations on Mt, which was confirmed by preliminary experiments (data not shown). The standard concentration of organic cations in the organoclay suspension was 1 mmol L^{-1}, which was equivalent to an Mt concentration of 1.0 g L^{-1}. At this concentration, the antimicrobial effect of Mt alone was negligible and did not interfere with the efficacy of organic cations, whose antimicrobial effectiveness was the target of this study. Lower concentrations of organic cations in an organoclay suspension were only tested for DDA. Sterile solutions of organic salts were obtained by filtration (0.22 µm membrane filter, Millipore, UK). Colloidal suspensions of Mt and organoclays were sterilized by autoclaving (121 °C for 20 min). Solutions and suspensions were stored at 4 °C for a maximum of 2 weeks.

The characterization of Mt, organoclays, and their interactions with M-H medium was done using X-ray diffraction (XRD) and infrared (IR) spectroscopy in the middle - (MIR) and near-infrared (NIR) regions. IR spectra were obtained in a Nicolet 6700 FTIR spectrometer (Thermo Scientific, USA) using the attenuated total reflectance method in the MIR region and the diffuse reflectance infrared Fourier transform method in the NIR region. The XRD measurements were measured in scanning mode in a Panalytical Empyrean diffractometer (PANalytical, USA). The measurement was in the range of 3–15° 2θ, with scanning step 0.02° and speed 2 s/step. The source of CuKα1 radiation was adjusted to 45 kV and 40 mA. The solids specimens were isolated from aqueous colloidal suspensions of either organoclays or their complexes with the M-H medium, identical to those used in biological experiments. The isolation of solids was performed by centrifugation and washing twice with deionized water (100 mL). Wet products were dried at 60 °C on air and ground to pass through a 0.2 mm sieve. Mt was used directly in the Na^+-form. Powders were directly used for the measurements of IR and NIR spectra. For the XRD measurements, thin films were deposited from wet pastes of the specimens, which had been prepared from the respective powder mixed with a few drops of