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Research paper

Preparation and characterization of sodium lauroyl sarcosinate adsorbed on cetylpyridinium-montmorillonite as a possible antibacterial agent

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ABSTRACT

An organo-montmorillonite was synthesized to generate a two-level antibacterial agent. The material (Mt-CP-SR) was prepared through the adsorption of sodium lauroyl sarcosinate (SR) on montmorillonite modified with cetylpyridinium (Mt-CP) and its structure was characterized by conducting ATR-FTIR, XRD, and SEM analyses. The results of the ATR-FTIR analyses indicated that SR on the surface could be detected when its amount exceeded the CEC of the Mt. The XRD analyses revealed that the adsorption of CP and SR causes the separation of Mt layers into smaller stacks. The adsorption and desorption study of SR onto and from 0.7 CEC Mt-CP were investigated to determine the amount of SR adsorbed at varying initial SR concentrations and the amount of SR released when diluted with water. Around 140 mg of SR could be loaded on one gram of the Mt intercalated with 170 mg of CP. The results indicated that desorption of SR from the surface is gradual and SR and CP have strong interactions on the montmorillonite (Mt) surface. The antibacterial activity of the material was tested against *E. coli, S. aureus,* and *P. aeruginosa.* Additionally, the SR solutions and CP + SR solutions in equimolar ratios were subjected to antibacterial tests for comparison purposes. SR proved to be effective against all three bacteria and the MIC values were found as 75 mg/mL for *E. coli*, 37.5 mg/mL for *S. aureus,* and 300 mg/mL for *P. aeruginosa*. The MBC was 300 mg/mL for *E. coli* and *S. aureus*. The solutions of CP + SR mixtures were ineffective against *P. aeruginosa,* whereas, they were effective against *S. aureus*. The prepared Mt-CP-SR samples were found to be effective against *S. aureus* and *E. coli*. These results indicate that the material could be used in antibacterial liquid soaps, in toothpaste formulations, personal care products, and topical applications against acne, and wounds without any negative contribution to the physico-chemical and detergency properties of the materials.

1. Introduction

In the last two decades, the well-known resistances of bacteria to antibiotics have become a bottle neck in the treatment of infectious diseases. Therefore, the work targeting the development of new antimicrobial agents is now the active area of research (Kaplancıklı et al., 2008; Bakavoli et al., 2010; Shi and Zhou, 2011). In addition to the new antibiotics, the improvement of antibacterial materials serving preventive medicine is of importance.

Their toxicity makes the heavy metal ions highly efficient antibacterial agents but it also forms a disadvantage in the applications requiring direct contact with other living organisms. Thus, instead of heavy metals, cationic surfactants like quaternary alkyl ammoniums immobilized onto a host material (mostly mineral surfaces) is a recently studied issue in literature (He et al., 2006; Malachová et al., 2009; Özdemir et al., 2010, 2013; Ke et al., 2014; Malek and Ramli, 2015; Kleyi et al., 2016; Samlikova et al., 2016). Some new investigations have introduced heavy metal ions to the quaternary ammonium immobilized mineral surfaces to increase the antibacterial effect of the material (Saad et al., 2016; Jou et al., 2016). The antibacterial activity of the quaternary ammonium cations is due to their ability to alter the permeability of the cellular membranes in such a way as to allow the intercellular ions and low molecular mass metabolites to diffuse out. This activity results in the disruption of the bacterial metabolism, inhibition of the cell growth, and finally cell death (Merianos, 1991; Hamouda and Baker, 2000; Watanabe et al., 2008).

Clay minerals with a high surface area, cation exchange capacity, chemical stability, low toxicity, and high availability are commonly used as host materials in immobilization studies (He et al., 2006; Malachová et al., 2009; Özdemir et al., 2010, 2013; Ke et al., 2014; Malek and Ramli, 2015; Kleyi et al., 2016; Samlikova et al., 2016). The exchangeable inorganic cations are replaced by organic cations, such as quaternary ammonium compounds to form organo-clays. The antibacterial activity of the organo-clays depends on the amount of the

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quaternary ammonium cation on the surface (Özdemir et al., 2010). Quaternary ammonium compounds are highly effective against microbial membranes and thus they are used as biocides, disinfectants, and fungicides (Oblak et al., 2014).

Sodium lauroyl sarcosinate is an amino acid based anionic type green surfactant and has a maximum dermatological compatibility arising from its acid-base equilibrium. Therefore, it is widely used in personal care products. Due to its anti-plaque and anti-caries properties, sodium lauroyl sarcosinate is also used in mouthwash and toothpastes (Rigano et al., 2003). Hart (1979) reported that the sarcosinates exhibit a pH-dependent antimicrobial activity and they are effective against *Staphylococcus aureus*, *Streptococcus faecalis*, *Lactobacillus acidophilus*, *Trichophyton mentagrophyles*, and *Pityrosporum ovale* at a pH 5.8. They exhibit a strong activity against the bacteria *Escherichia coli, Pseudomonas aeruginosa, Bacillus mesentericus*, and many types of fungi (Kropinski et al., 1987; Ravaoarinoro et al., 1994). The interaction of SR with both the outer cell membrane and the cytoplasmic membrane makes it a promising agent for the preparation of clay based antibacterial materials (Filip et al., 1973; Anwar et al., 1983).

CP and SR have strong interactions in solution resulting in a negative value of β interaction parameter that measures the nature and extent of the interaction between the two different surfactant molecules in the mixed micelle in aqueous solution. The interaction is strongest in equimolar mixture due to coacervation (Ghosh and Dey, 2011). In addition to the interactions between the oppositely charged head groups, they further found out that the contribution of interactions between hydrocarbon chains to the value of β parameter was also considerable. Loading of these surfactants onto montmorillonite will probably cause interactions of Mt surface and, the surfactants CP and SR, which determine the adsorption and desorption behavior of CP, and SR depending on their concentrations and thus the interlayer structure formed. Moreover, Yapar et al. (2015) reported formation of a considerable change in the interlayer structure of Mt-CP-SR compared to that of Mt-CP. They also showed that the external surface charge of the organo-montmorillonite prepared with CP and SR changes from negative to positive with the variation in pH providing a wide range of applicability.

To our knowledge there are no studies introducing anionic surfactants by adsorption onto organo-montmorillonites to be used in antibacterial applications. The adsorption of cationic and anionic surfactants together on the Mt surface is beneficial, leading to an immobile cationic quaternary ammonium layer with a long lasting antibacterial effect and a mobile anionic surfactant layer through the desorption from the surface exhibiting a higher antibacterial activity compared to the immobile surfactant layer.

In this study, the synthesis of an efficient antibacterial agent was targeted to impart a long-lasting effect induced by the immobilization of cationic and anionic surfactants on the Mt surface. The amount of SR immobilized on the Mt-CP and its release by dilution was elucidated with adsorption and desorption studies in aqueous media in equilibrium with the Mt-CP-SR matrix. The XRD analysis expresses the structure of the Mt-CP-SR and the interaction, and orientation of the cationic and anionic surfactant tails (Lee and Tiwari, 2012). For the evaluation of the Mt-CP-SR with respect to its antibacterial activity, the SR, and CP-SR mixtures in the solution were also studied for their antibacterial properties.

2. Experimental

2.1. Materials

The montmorillonite (Mt) from Middle Anatolia was purified in two steps. The inorganic impurities such as silica and iron were removed by sedimentation and the organic impurities with hydrogen peroxide. The treatment was followed by drying at 95 °C and pulverization to pass through a 595 μm sieve. The cation exchange capacity, CEC, of the Na-Mt was determined as 0.68 meq/g Mt (Özdemir et al., 2010).

The cetylpyridinium chloride monohydrate ($C_{21}H_{38}ClN{\cdot}H_2O$, MW = 358.01 g/mol) was purchased from Merck and the *N*-lauroylsarcosine sodium salt ($C_{15}H_{28}NNaO_3$, MW = 293.38 g/mol) from Sigma-Aldrich. They were analytical grade and used without further purification. The bacteria were obtained from the American Type Culture Collection (ATCC).

2.2. Adsorption and desorption studies

The adsorption of SR on Mt-CP was studied using the batch method. Previously, Mt-CP was prepared by adding CP in an amount equivalent to 0.7 times the CEC of Na-Mt to obtain single layer coverage (Özdemir et al., 2013) and shaking in PP tubes for 24 h at 175 rpm with Na-Mt in an aqueous dispersion. In the next step, the SR was added in different proportions to the Mt-CP in the aqueous mixture. The supernatants were then centrifuged and filtered with 0.45 μm PTFE syringes to prevent turbidity for the UV analysis and the initial and equilibrium concentrations of the SR were determined with a Shimadzu UV-spectrophotometer with 1 cm cell path at 222 nm. The SR concentration varied between 0.175 and 1.4 CEC of the Na-Mt.

The desorption study was conducted with samples prepared in two steps by the adsorption of 0.7 CEC CP on 1 g of Na-Mt and then 0.7 CEC SR onto the Mt-CP. The prepared Mt-CP-SR samples in the aqueous medium were subjected to a desorption study with the dilution of samples with 50, 100, 150, and 200 mL of deionized water, respectively, and no water was added to a sample which was used to determine the adsorbed amount of SR. All samples were shaken for a further 24 h and their supernatants were analyzed as explained above. Because the desorbed amount of CP from the Mt-CP was found to be very low in the previous study (Özdemir et al., 2013) desorption of CP was neglected. The adsorption and desorption experiments were accomplished in triplicate and the results were also cross-checked.

The amount of SR adsorbed was determined using mass balance:

$$Q = \frac{(C_i - C_{eq})V}{m} \quad (1)$$

where Q is the adsorbed amount in g/g, C the concentration in g/L, V the volume of the solution in L, and m the amount of Na-Mt in grams.

2.3. Preparation of Mt-CP-SR samples for antibacterial tests

The Mt-CP-SR samples were prepared in two steps. In the first step, the aqueous dispersions of Mt with a mass ratio of 1:50 was prepared by adding CP in amounts equivalent to 0.7 or 1.4 times CEC of the Na-Mt, respectively. The dispersions were then placed in polypropylene (PP) tubes and shaken for 24 h. In the second step, the SR was added in different proportions to the Mt-CP aqueous mixture and shaken for a further 24 h. The amounts of CP and SR added in the first and second steps, respectively, are given in Table 1. The Mt-CP-SR samples were separated from the supernatants by filtering with Whatman filter papers and drying at 60 °C.

2.4. ATR-FTIR and XRD analyses

ATR-FTIR analyses were carried out using a Perkin Elmer 100 FTIR

Table 1
Mt-CP-SR preparation for the antibacterial tests by immobilization of CP and SR onto the Mt.

Sample	First step	Second step
1	0.7 CEC CP	0.7 CEC SR
2	1.4 CEC CP	1.4 CEC SR
3	1.4 CEC CP	0.7 CEC SR