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Research paper

Characterization and antibacterial activity of chlorhexidine loaded silver-kaolinite

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ABSTRACT

Chlorhexidine acetate-loaded silver-kaolinite (CA-Ag-Kaol) was prepared and characterised, and its application as an antibacterial agent was studied. CA-Ag-Kaol was prepared by the adsorption of chlorhexidine acetate (CA) (0.5 mmol/L) on Ag (50% of the Cation Exchange Capacity (CEC) of kaolinite) on kaolinite. Kaolinite (Kaol), silver-kaolinite (Ag-Kaol), CA-modified kaolinite (CA-Kaol) and CA-Ag-Kaol were characterised by X-ray diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, field-emission scanning-electron microscopy (FESEM), energy dispersive X-ray (EDX) spectroscopy, zeta potential analysis and dispersion behavior measurements. The modification of kaolinite with cationic silver and chlorhexidine ions did not change the structure of kaolinite, and the characterization of the kaolinite samples revealed the successful loading of cationic silver and chlorhexidine ions on the kaolinite. The antibacterial assay of the samples was carried out against *Escherichia coli* ATCC 11229, *Pseudomonas aeruginosa* ATCC 15442, *Staphylococcus aureus* ATCC 6538 and *Enterococcus faecalis* ATCC 29212 using the disc diffusion technique (DDT) and the minimum inhibition concentration (MIC) technique. Based on the antibacterial assay, CA-Ag-Kaol showed better antibacterial activity than Ag-Kaol and CA-Kaol, and it performed well in both distilled water and a 0.9% saline solution. Gram-positive bacteria were more susceptible to the antibacterial behavior of Ca-Ag-Kaol than Gram-negative bacteria. In conclusion, silver-kaolinite that has been loaded with chlorhexidine acetate can be used as an effective antibacterial agent because of its high antibacterial activity against wide spectrum of bacteria in solutions containing electrolytes (saline solution).

1. Introduction

Human beings are constantly exposed to various types of microorganisms, such as pathogenic bacteria and viruses. Microorganisms, such as *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*, are examples of the bacteria commonly found in hospitals and in the community (Hanim et al., 2016). They produce a wide range of diseases, and some of them can be fatal to human beings. To combat this, different types of products containing an antibacterial agent have been discovered and commercialised (Levy, 1998). However, outbreaks of infectious diseases caused by different pathogenic bacteria and the development of antibacterial-resistant properties in the bacteria itself makes treatment more complicated (Rai et al., 2009; Sharma et al., 2005). Therefore, new antibacterial agents are in great demand, and this motivates the pharmaceutical companies and researchers to develop new and better antibacterial agents.

Natural clay minerals, such as kaolinite, montmorillonite and vermiculite show no antibacterial effect, however, they could kill adsorb bacteria when some antibacterial material was intercalated in or adsorbed on them (Holešová et al., 2013). Studies on the modification of clay minerals as inorganic carriers or antibacterial agents have been extensively reported. For instance, clay minerals could be loaded with inorganic species exhibiting antibacterial properties, such as silver ions (Hundáková et al., 2013; Karel et al., 2015; Malachová et al., 2011; Shameli et al., 2010), copper ions (Hu and Xia, 2006; Malachová et al., 2011; Song et al., 2013) and zinc ions (Özdemir et al., 2010; Tan et al., 2008) or with an organic antibacterial agent such as cetylpyridinium bromide (CPB) (Malek and Ramli, 2015) and chlorhexidine acetate (CA) (Holešová et al., 2010; Meng et al., 2009a; Yang et al., 2007).

Among metal ions, silver ions have antibacterial activity against a broad spectrum of Gram-negative and Gram-positive bacteria, and they have the highest antimicrobial action against more than 650 pathogenic microorganisms (Mintova et al., 2015; Pessanha et al., 2014; Yuranova et al., 2003). Moreover, when Ag^+ ions are loaded onto a solid carrier, Ag^+ can be slowly released into the environment to maintain its antibacterial activity over a long period of time (Chernousova and Epple, 2013; Hanim et al., 2016). However, the antibacterial activity of silver diminished when in contact with electrolytes, especially chloride (Cl^-) ions. Silver ions can form the insoluble silver chloride precipitate when in contact with chloride ions. This reduces the availability of

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silver ions to interact with bacterial cells and eventually lowers the antibacterial activity (Swathy et al., 2014). Thus, the new approach presented in this study involved the adsorption of another type of antibacterial agent, namely, chlorhexidine acetate (CA), to silver-kaolinite to enhance and prolong its antibacterial activity.

Chlorhexidine is an organic salt that belongs to the bisbiguanide family with extensive antibacterial activity, being effective against Gram-positive and Gram-negative microorganisms (Holešová et al., 2010; Meng et al., 2009a,b). CA is mild to human beings and has been widely used for inflammation relief, disinfecting, and washing the surface of a wound (Yang et al., 2007). It has been documented that the antimicrobial activity of chlorhexidine can last at least forty-eight hours on the skin. This is due to its special ability to bind to the proteins present in human tissue, such as skin and mucous membranes (Hibbard, 2005). Moreover, chlorhexidine is not affected by the presence of body fluids, such as blood. Thus, chlorhexidine can still perform well even when in contact with a normal saline solution (Lim and Kam, 2008).

The purpose of this study is to prepare and characterise chlorhexidine-loaded silver-kaolinite and to determine its antibacterial activity towards two different classes of bacteria: *E. coli* and *P. aeruginosa* represent Gram-negative bacteria, and *S. aureus* and *Enterococcus faecalis* represent Gram-positive bacteria. There have been a number of valuable studies using CA and clay, such as montmorillonite (Meng et al., 2009a,b; Yang et al., 2007) and organovermiculites (Holešová et al., 2010, 2013), as antibacterial agents. To date, there has been little discussion (Karel et al., 2015; Malek and Ramli, 2015) regarding the utilization of modified kaolinite as a new antibacterial agent. Furthermore, most of the research that has been carried out tends to focus on the adsorption or intercalation of only one antibacterial agent. Montmorillonite has a higher cation exchange capacity (CEC) and a large specific surface area compared with kaolinite (Murray, 2000). However, the low swelling and high molecular stability of kaolinite (Miranda-Trevino and Coles, 2003) make the synthesis of chlorhexidine-loaded silver-kaolinite easier and less time consuming. The low swelling property of kaolinite accelerates the separation of the solid and liquid fractions after the addition of kaolinite to an aqueous solution. Furthermore, kaolinite has a lower viscosity than montmorillonite at high solid concentrations, where it can be easily dispersed in an aqueous solution (Malek and Ramli, 2015). Hence, in this study, a newly modified kaolinite (CA-Ag-Kaol) was prepared and characterised, and the antibacterial activity was evaluated against two classes of bacteria in distilled water and in a 0.9% saline solution.

2. Materials and methods

2.1. Materials

Natural kaolinite (Kaol), with the formula $Al_2Si_2O_5(OH)_4$ from Kaolin (M) Pte Ltd, Malaysia was selected as the starting material in this study. The cation exchange capacity (CEC) of natural kaolinite was determined to be 9.69 mEq/100 g, which was obtained using the technique by Ming and Dixon (1987). A similar result has been reported by Duman et al. (2012). Silver nitrate ($AgNO_3$) and chlorhexidine acetate ($C_{22}H_{30}Cl_2N_{10}.2(C_2H_4O_2)$) were purchased from Merck, Germany and Sigma-Aldrich, UK, respectively. Bacto™ yeast extract, Bacto™ tryptone and Muller Hinton agar (MHA) powder for antibacterial assays were purchased from BD, France. Sodium chloride (NaCl) was purchased from J.T Baker, USA. For antibiotics, both Penicillin G, sodium salt and streptomycin sulphate were purchased from Bio Basic Inc., Canada. All chemical reagents were of analytical grade, and all aqueous solutions were prepared with deionised water. For the antibacterial assay, *E. coli* ATCC 11229, *P. aeruginosa* ATCC 15442, *S. aureus* ATCC 6538 and *E. faecalis* ATCC 29212 bacteria were obtained from the American Type Culture Collection (Manassas, VA, USA).

2.2. Modification of kaolinite

Silver-modified kaolinite (Ag-Kaol) having an amount of Ag equivalent to 50% of the obtained CEC value for the kaolinite, was prepared by reacting an $AgNO_3$ solution (329.2 mg/L) (500 mL) with 20 g of the kaolinite. Chlorhexidine acetate loaded-kaolinite (CA-Kaol) was prepared by adding CA solution (312.8 mg/L) (500 mL) with 20 g of the kaolinite. For the loading of CA on Ag-Kaol, the preparation step was similar to that of the preparation of CA-Kaol with the replacement of kaolinite with Ag-Kaol. All of the mixtures were stirred at room temperature for 16 h, filtered with 125 mm Macherey-Nagel filter paper and dried overnight in an oven at 80 °C. All of the samples were then crushed and sieved prior to the characterization and application testing.

2.3. Analysis and characterization techniques

The characterization of the raw and modified kaolinite (Ag-Kaol, CA-Kaol, and CA-Ag-Kaol) structures was determined using X-ray diffraction (XRD) on a Bruker AXS GmbH, German machine. The powder form of the sample was placed on a polymethylmethacrylate (PMMA) sample holder (49 mm diameter) and a glass slide was used to compress the powder into the sample hole on the holder. The XRD patterns were recorded with Cu Kα radiation with $\lambda = 1.5406$ Å at 40 kV and 20 mA over a range of $2\theta = 5°$ to 50° with a scanning speed of 0.05° per second, a step size of 0.05°, a 0.5° divergence slit and a 0.5° anti-scatter slit. The samples were also characterised by Fourier transform infrared (FTIR) spectroscopy (Nicolet iS5-IR, Thermo Fisher and equipped with OMNIC™ software) using KBr pressed discs, the concentration of the sample in KBr was 0.5%. For each samples, 32 scans were recorded in a 4000–400 cm^{-1} spectral range at a resolution of 4 cm^{-1} for a duration of 3 to 4 min. The morphology of the raw and modified kaolinites was observed by a CARL ZEISS 35 VP Supra FESEM (Field-Emission Scanning Electron Microscope) with an accelerating voltage of 20 kV. The sample in a powder form was placed on a sample holder and coated with platinum (Pt) using an auto-fine coater model JFC-1600. The coated samples were then placed on a FESEM specimen holder and observed at a magnifications of 10,000 and 50,000. The FESEM instrument was equipped with energy dispersive X-ray spectroscopy (EDX) (JED-2300 analysis station), and it was used to detect the presence of silver (Ag) in the modified clays. The zeta potential of the raw and modified kaolinites was determined using a ZEECOM, ZP analyser from Microtec Co., Ltd. The samples were prepared by mixing 0.01 g of the sample with 50 mL of deionised water, and the mixture was shaken vigorously to ensure that the sample was dispersed evenly in the solution. The sample was analysed, and the data were recorded via ZEECOM software. Three replicates were done on each sample, and the values plotted in the graph are the average value of these three readings. The dispersion behaviour test of the samples was done by adding approximately 0.06 g of the sample to a mixture of distilled water (3 mL) and n-hexane (3 mL) in a 10 mL glass bottle. An image of each glass bottle was taken immediately after the addition of the sample, and the relative positions of the particles in the mixture were compared. The mixture was then shaken for 30 min, and an image was captured immediately after shaking. The bottle was then left overnight under ambient conditions. Finally, an image was captured again, and the dispersion behaviour of each sample was compared and analysed.

2.4. Antibacterial assay

The antibacterial activity of raw kaolinite and modified-kaolinite was evaluated against *E. coli* ATCC 11229, *P. aeruginosa* ATCC 15442, *S. aureus* ATCC 6538 and *E. faecalis* ATCC 29212 by the disc diffusion technique (DDT), and the minimum inhibition concentration (MIC) was determined as based on the National Committee for Clinical Laboratory Standards (Standards, 2006). DDT and MIC provide qualitative and quantitative results, respectively.