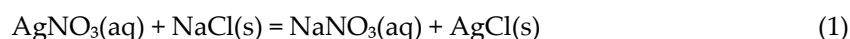


in the wavenumber range of 400 cm⁻¹ to 4000 cm⁻¹ with a resolution of 4 cm⁻¹. Thermogravimetric analysis was performed on a TGA-DSC analyzer (Thermal Analysis Instruments, SDT 2960, TA Instruments, Warszawa, Poland) in the temperature range of 20–1100 °C. The analysis was carried out at a linear heating rate of 10 °C/min in a nitrogen gas stream [36].

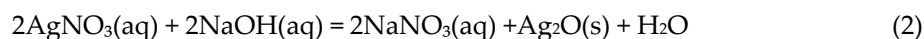
2.3. Preparation of (AgCl,Ag)NPs/Diatomite Composite

Synthesis of (AgCl, Ag)NPs/diatomite composites was carried out by the impregnation of diatomite with aqueous silver nitrate solution. An initial solution of AgNO₃ (POCH, Gliwice, Poland) with a concentration of 1000 mg/L was prepared. A natural diatomite mass of 1.0 g was added to a silver nitrate solution (100 mL) with the following Ag ion concentrations: 100 mg/L, 500 mg/L and 1000 mg/L expressed as 1, 5 and 10% in calculations of 1, 5 and 10% of silver relative to the mass of used diatomite. The obtained suspensions were stirred for 30 min and then basified with 0.1 M NaOH to pH = 9.

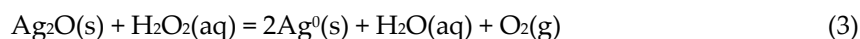
During the interaction of the silver nitrate solution with diatomite, according to the inorganic chemistry of silver [37], we assumed that the silver chloride nanoparticles (AgCl-NPs) were formed immediately as a result of the exchange reaction of silver nitrate with the halite mineral impurity (NaCl) in the diatomite:



Then, taking into consideration the fact that the silver nitrate content in the prepared slurry was in excess compared to the halite mineral impurity, the silver ion residues Ag in the alkaline medium were oxidized to silver oxides after completion of the exchange reaction of the silver nitrate with the halite mineral impurity:



Further reduction of the formed silver oxide was carried out with hydrogen peroxide. The reducing agent was added in a 1:3 molar ratio of AgNO₃/H₂O₂. The suspension was stirred for 15 min at 300 rpm until the complete reduction of the silver ions:



The addition of the reducing agent changed the pH of the solution from 9.02 to 6.88. Next, the (AgCl, Ag)NPs/diatomite composite was washed five times with deionized water, centrifugated (Centrifuge 9000, MPW-251 rpm) and dried at 110 °C.

2.4. Antimicrobial Assay of (AgCl, Ag)NPs/Diatomite Composite

The antimicrobial properties of the obtained (AgCl, Ag)NPs/diatomite composites (0.71% Ag/diatomite, 4.65% Ag/diatomite, 7.21% Ag/diatomite) were investigated by a minimum inhibitory concentration (MIC) assay. The method was performed using the Miller Hilton (MH) broth medium according to Clinical and Laboratory Standards Institute (CLSI) procedures (with suitable changes) and the resazurin-based 96-well plate microdilution method [38]. For this purpose, two different bacterial strains were used: one Gram (+) - *Staphylococcus aureus* strain and one Gram (-) - *Klebsiella pneumoniae* strain. Firstly, the bacterial cells were inoculated in MH broth media for 24 h at 37 °C. Then, in 96-well flat-bottom plates (Sigma Aldrich, Poznan, Poland), the cultured bacterial strain (1 × 10⁶ CFU/mL) and different concentrations (10 mg/mL, 5 mg/mL, 2.5 mg/mL, 1.25 mg/mL, 0.625 mg/mL, 0.312 mg/mL, 0.156 mg/mL) of the investigated formulations were mixed in a ratio of 1:1. Subsequently, to each well, 12 µL of dye from a resazurin-based in vitro toxicology assay kit (Sigma-Aldrich, St. Louis, MO, USA) was added. Once the samples had been prepared, plates were kept at 37 °C under continuous stirring for 24 h. Natural diatomite (raw material) served as a control. The MIC value was determined by changes in the indicator color from blue to pink. All the experiments have been performed in triplicate.