Synthesis of bioactive glass 60SiO$_2$-33CaO-4P$_2$O$_5$-3Ag$_2$O and its application in toothpaste

Bui Thi Hoa$^1$, Nguyen Quan Hien$^2$, Le Hong Phuc$^{2,*}$, and Bui Xuan Vuong$^3,*$

$^1$Faculty of Natural Sciences, Electric Power University, 235 Hoang Quoc Viet, Ha Noi City, Vietnam.
$^3$Faculty of Natural Sciences Education, Sai Gon University, 273 An Duong Vuong St., Ward 3, District 5, Ho Chi Minh City, Vietnam.
*Email: bxvuong@sgu.edu.vn; lhphuc@hcmip.vast.vn

Abstract. This work is related to the synthesis of silver-doped bioactive glass material 60SiO$_2$-33CaO-4P$_2$O$_5$-3Ag$_2$O by the modified sol-gel method based on hydrothermal reaction. Several physical-chemical methods such as TG-DSC, XRD, SEM, and TEM were used to evaluate the synthetic bioglass. TG-DSC shows that the processing temperature to form bioglass from dried gel is at 600 °C. XRD confirms the formation of silver crystalline phase on the amorphous phase of glass. SEM and TEM show the porous structure of the glass material containing nano-sized particles. Experiment with cells showed good biocompatibility of the bioglass material with the fibroblast L929 cell-line. Ag-doped bioglass material was mixed to make toothpaste with excellent antibacterial property. All Staphylococcus aureus and Pseudomonas aeruginosa bacteria were destroyed when exposed to toothpaste containing bioglass.

Keywords: Bioactive glass; bioactivity, hydroxyapatite; silver; biocompatibility; antibacterial.

1 Introduction

Bioactive glasses (bioglasses) are materials with bioactive property expressed through the ability to form a layer of hydroxyapatite (HA) Ca$_{10}$(PO$_4$)$_6$(OH)$_2$ mineral on their surface when they are implanted at bone defects in the human body [1-2]. The HA mineral layer is similar to the inorganic composition of human bone, so it is the bridge between artificial materials and natural bone, through which bone defect are repaired and filled. Thanks to its bioactivity, bioglasses materials are researched and applied as artificial bone materials used as components in dental filling cement; culture powder and bone graft in orthopedic surgery. Especially in recent years, they are used to add to toothpaste to help fill scratches and imperfections on the tooth surface. When first discovered, the production of bioglass with the composition of 46.1SiO$_2$ – 24.4Na$_2$O – 26.9CaO – 2.6P$_2$O$_5$ (%.mol), was performed using the melting process [3]. In this method, the precursors are melted at high temperature to form the base oxides in the glass system. After that, the glass system is annealed to stabilize at the appropriate temperature. The melting method can quickly produce glass materials with large quantities of materials. However, the synthesis process at high temperature causes the loss of volatile components such as P$_2$O$_5$. Furthermore, the synthesized bioglasses are in block and dense forms, so they are non-porous and has a low specific surface area. Recently, the solution synthesis method, often called the sol-gel method, has been used to synthesize glass materials. This method synthesizes glass from solution, so the resulting material is in powder form, porous, and has a high surface area [4-5]. The conventional sol-gel method undergoes hydrolysis of main precursors such as Si(OC$_2$H$_5$)$_4$ in an acidic or basic environment to create a sol system consisting of Si(OH)$_2$-like particles. The sol system is left for a few days to transform into a SiO$_2$·nH$_2$O gel system. The gel system is dried into powder, then calcined to create glass powder. When synthesizing bioactive glasses using sol-gel method, cations such as Ca$^{2+}$ or Na$^+$ in salts are introduced to disrupt the Si-O-Si bonds, creating an amorphous three-dimensional network structure of bioglass materials. The process of breaking order bonds occurs when processing dry gel systems at high temperatures. Some typical bioglass systems have been synthesized by the sol-gel method such as 70SiO$_2$ - 30CaO, 55SiO$_2$ - 41CaO - 4P$_2$O$_5$, 58SiO$_2$ - 38CaO - 4P$_2$O$_5$, 92SiO$_2$ - 6CaO - 2P$_2$O$_5$ (%.mol) [6-8]. To enhance the functionality of bioglass, antibacterial agents are often added to the composition of glass systems. Among them, nano silver is the leading candidate because of its antibacterial ability [7-8]. The purpose of this work is to manufacture three-component SiO$_2$ - CaO - P$_2$O$_5$ glass system doped with Ag using the modified sol-gel method. Doping Ag into glass system to create antibacterial property of glass material. The Ag-doped glass material will be mixed to make toothpaste that has both the biological activity of bioglass and antibacterial property.
2 Materials and Method

2.1 Production of Ag-doped bioactive glass

The Ag-doped bioactive glass with composition of 60SiO2-33CaO-4P2O5-3Ag2O (mol.%) was synthesized by the modified sol-gel method. The synthesis process includes three main stages as follows. Step 1: Hydrolysis of precursors including tetraethyl orthosilicate Si(OCH3)4; triethyl phosphate (C2H5)3PO; calcium nitrate tetrahydrate Ca(NO3)2·4H2O; silver nitrate AgNO3 in a hot solvent system (≈ 70 °C). The precursors were added one by one into the reaction vessel containing the solvent system H2O/C2H5OH (ratio of 1/8 by weight). The reaction vessel is connected to a reflux condenser to avoid solvent evaporation. The reaction system was magnetically stirred at 200 rpm during 2 hours. At the end of stage 1, a transparent and homogeneous sol system is obtained. Step 2: Converting the sol into a gel in a hydrothermal reactor. The reacting time of this step is 24 hours at 180 °C. Step 3: Washing the gel with distilled water and ethanol to remove by-products, dry the gel and bake the dry gel to make bioglass.

2.2 Production of toothpaste containing Ag-doped bioactive glass

The process of making toothpaste is as follows. Step 1: Stir water-soluble substances such as NaHCO3 (5g), NaF (0,2g), Sorbitol (5g), Xylitol (5g) into a glass cup containing 50 (g) of distilled water. Then, Cellulose Gum (5g) and Xanthan Gum (1g) are added to the above solution system, stirred for 15 minutes so that Cellulose Gum and Xanthan Gum completely swell to create a thick-gel system. Step 2: Add CaCO3 (10g) and Bentonite (1g) abrasive agent to the glass cup in step 1, the mixture was stirred for 10 minutes to create a homogeneous paste. Step 3: Add substances such as vitamin E (0,1g), preservative compound PE 9010 (0,1g), peppermint essential oil (0,1g), and moisturizing agent glycerin (2g) to the cup in step 2, stir well for 5 minutes. Step 4: Gently mixing sodium lauryl sulfate (3g) and coconut oil (10g) into the mixture in step 3 to create a homogeneous mixture. The toothpaste obtained is a pure white paste and stored at room temperature.

2.3 Physical-chemical evaluation

Thermal Gravimetric Analysis-Differential Scanning Calorimeter (TG-DSC) is used to determine the thermal stability of the substance, the reactions that occur during thermal decomposition, to choose the appropriate temperature and find the stability temperature to create biomedical glass systems. X-ray diffraction (XRD) method is used to determine the phase composition and crystal structure of the synthetic material. Comparing the measured diffraction pattern with the standard spectrum, we can identify the compounds present in the sample. Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) are used to verify the morphology of synthetic bioglass.

2.4 Cytotoxicity

Biocompatibility of synthetic glass with cells was evaluated according to ISO10993-5 standard referring to previous studies [9-10]. Fibroblasts (L-929) were cultured in 96 well plates (103 cells/well) under culture conditions according to prescribed standards. Extracts (100 µL) of synthetic glass at different concentrations (0, 12.5, 25, 50, and 100 %) were added to the cell plate and left for 24 h. The extract was then separated and then 20 µL of MTT solution (5 mg/mL in PBS) was added. Cell plates were incubated at 37 °C for 24 h. The medium was then added with 150 µl DMSO to dissolve the formazan crystals into a homogeneous solution. The final solution was measured for absorbance at 570 nm using a microplate reader. Four reads were performed for each sample.

2.5 Antibacterial evaluation

Two types of bacteria (staphylococcus aureus and pseudomonas aeruginosa) were selected to test the antibacterial properties of toothpaste containing Ag-doped bioglass. These are two common types of bacteria and are highly harmful to human health. The testing protocol is according to the BS EN 1040-2005 standard [11]. The toothpaste sample was diluted in distilled water and filtered to obtain the extract. P. aeruginosa and S. aureus bacteria were cultured in standard nutrient medium for 24 hours, at a temperature of 37 °C. After the culture period, the bacterial suspensions are determined using specialized equipment. Bacterial suspensions were added to the extracts and maintained at 20 °C for an exposure time of 5 min. At the end of the exposure period, an aliquot was removed and the amount of viable bacteria was tested.

3 Results and Discussion

3.1 Physical-chemical characterization of bioactive glass 60SiO2-33CaO-4P2O5-3Ag2O

Thermal analysis result of bioglass 60SiO2 - 33CaO - 4P2O5 - 3Ag2O (mol.%) show four regions of mass loss; the first is from 50 to 200 °C, followed by 200-300 °C, 300-390 °C, and finally from 390 to 600 °C (Fig. 1). The first region has an
endothermic peak at 138.73 °C with weight loss of 16.711 %, which is characteristic of the removal of physically adsorbed water or alcohol by-product from the condensation reaction that were not removed during the drying process [13]. In the next three regions of mass, exothermic peaks are appeared at 287.76 °C, 321.86 °C and 414.85 °C, accompanied by weight loss of 10.62 %, 5.905 %, and 5.993 %, respectively. These exothermic peaks are corresponding to the dehydration of ethanol remaining in the drying gel, or removal of alkoxy groups [12-13]. At about 954.98 °C, an endothermic peak without mass loss is appeared due to the melting of the glass phase (melting point) [13]. From the result of TG-DSC analysis, the processing temperature for bioglass synthesis is determined at 600 °C. At this temperature, the remaining water, and organic residues are completely removed.

![TG-DSC curves of synthetic bioglass 60SiO₂ – 33CaO – 4P₂O₅ – 3Ag₂O.](image1)

![XRD diagram of synthetic bioglass 60SiO₂ – 33CaO – 4P₂O₅ – 3Ag₂O.](image2)
XRD analysis of the bioglass material 60SiO$_2$ - 33CaO - 4P$_2$O$_5$ - 3Ag$_2$O is presented in Fig. 2. The XRD diagram shows the appearance of the Ag-crystalline phase on the glassy-amorphous phase [15-16]. The Ag-crystalline phase is characterized by sharp, pointed peaks; and the amorphous phase of bioglass is shown in a broad diffraction halo. Thus, the synthetic Ag-doped bioglass exhibits two phases: the amorphous phase of glass and the crystalline phase of Ag. This result confirms that silver was successfully added to silicate glass system.

Figure 3 shows the SEM image (A), and TEM image (B) of the synthetic bioglass 60SiO$_2$-33CaO-4P$_2$O$_5$-3Ag$_2$O. SEM image shows spherical particles, quite uniform in nano-size. These nanoparticles are connected together to form a 3D structure with pores and gaps on the surface of the material. This result is much different from previous studies, in which glasses synthesized by the conventional sol-gel method had smooth surfaces or irregular structures [11-12, 17]. In this study, the modified sol-gel method is based on hydrothermal reaction with high autogenous temperature and under high-pressure condition, so the sol/gel formation take place and end quickly, thus can produce material particles with a fairly uniform structure. The 2D image obtained by TEM method (B) demonstrates glass material at nano-particle size. Nanoparticles were observed in sizes ranging from 10 to 15 nanometer.

Fig. 3. Images SEM (A), and TEM (B) of synthetic bioglass 60SiO$_2$ – 33CaO – 4P$_2$O$_5$ – 3Ag$_2$O.

### 3.2 Biocompatibility of synthetic bioglass 60SiO$_2$-33CaO-4P$_2$O$_5$-3Ag$_2$O

Figure 4 presents the results of statistical analysis evaluating the biocompatibility of bioglass 60SiO$_2$ – 33CaO – 4P$_2$O$_5$ – 3Ag$_2$O. The result show that this material is completely biocompatible with cells. All extracts show biocompatibility of over 70% according to IUPAC regulation [11]. Thus, Ag doping to create antibacterial property of bioglass is completely non-toxic to cells.

Fig. 4. Cytotoxicity of synthetic bioglass 60SiO$_2$ – 33CaO – 4P$_2$O$_5$ – 3Ag$_2$O with fibroblast (L-929) cells.
Images of L-929 fibroblasts after exposure to the glass extract at different concentrations are shown in Fig. 5. The images show good survival of the cells when exposed to the solutions of bioglass extraction. The cytotoxicity assessment results obtained are quite similar to previous studies [11, 17], confirming the biocompatibility of Ag-incorporated bioglass.

![Images of fibroblast cells after contacting with the extracts of bioglass 60SiO$_2$ – 33CaO – 4P$_2$O$_5$ – 3Ag$_2$O](image)

**Fig. 5.** Images of fibroblast cells after contacting with the extracts of bioglass 60SiO$_2$ – 33CaO – 4P$_2$O$_5$ – 3Ag$_2$O

### 3.3 Antibacterial evaluation of toothpaste containing Ag-doped bioglass

Images of *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Staphylococcus aureus* (*S. aureus*) bacteria before and after experimental exposure to toothpaste are shown in Fig. 6 and Fig. 7. It was observed that no *P. aeruginosa* or *S. aureus* bacteria could survive after contact with toothpaste containing Ag-doped bioactive glass. This result confirms that silver-doped bioglass can be applied to toothpaste as a bioactive inorganic mineral ingredient, while also acting as an antibacterial agent for toothpaste.

![Image of *P. aeruginosa* bacteria before (concentration 10$^{-7}$) and after experiment (concentration 10$^{-1}$) with toothpaste](image)

**Fig. 6.** Image of *P. aeruginosa* bacteria before (concentration 10$^{-7}$) and after experiment (concentration 10$^{-1}$) with toothpaste
4 Conclusions

Silver-doped bioactive glass 60SiO2-33CaO-4P2O5-3Ag2O has been successfully synthesized by the modified sol-gel method, in which, thanks to the hydrothermal reaction, the sol-to-gel conversion time is shortened. The synthesized material has a porous structure, including nanoparticles with sizes ranging from 10 to 15 nanometers. The bioglass material demonstrates good biocompatibility when contacted with fibroblast cells (L-929), as shown by the cell survival rate after exposure to glass extract being over 70% according to the IUPAC standard. Silver-doped bioglass used to make toothpaste, which has excellent antibacterial property, demonstrated by its ability to completely destroy the bacteria of staphylococcus aureus and pseudomonas aeruginosa.

References