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Study on the preparation and properties of silver-doped phosphate antibacterial glasses (Part I)

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ABSTRACT

Silver-doped phosphate antibacterial glasses were prepared by the melting method. The antibacterial effects of some undoped and silver–doped glasses of compositions $65P_2O_5$–10CaO–$(25-x)$ Na_2O, $70P_2O_5$–20CaO–$(10-x)$ $Na_2O$and $(70-x)$ P_2O_5–30CaO, (where $x = 0, 0.5, 1.2$ Ag_2O), against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* micro-organisms using agar disk-diffusion assays were investigated. The structures of some glasses were studied by XRD, FT-IR, and UV–VIS spectroscopy. The variation of pH with dissolution rate was studied. The tested silver-free and silver-doped glasses demonstrated different antibacterial effects against the tested micro-organisms. For silver-free glasses, an increase in inhibition zone diameter (zone of no bacterial growth) was seen with the decrease in water pH. Silver-doped glasses showed an increase in inhibition zone diameter with increasing Ag_2O content. The low pH produced by glass dissolution was certainly a critical factor for glass antibacterial effect. The more the phosphate ions released the lower is the pH and the greater the antibacterial effect.

1. Introduction

Glasses in the P_2O_5–CaO–Na_2O system have a chemical composition similar to that of the inorganic phase of bone. These glasses consist of PO_4 tetrahedra, which can be attached to a maximum of three neighboring tetrahedra forming a three dimensional network as in vitreous P_2O_5 [1]. Adding metal oxides to the glass leads to a depolymerization of the network, with the breaking of P–O–P linkages and the creation of non-bridging oxygens. The modifying cations can provide ionic cross-linking between the non-bridging oxygens of two phosphate chains, thus increasing the bond strength of this ionic cross-link and improving the mechanical strength and chemical durability of the glasses [2]. These phosphate-based glasses are a unique class of materials in that they are completely degradable; whereas silica-based glasses are relatively stable to hydrolysis. Furthermore, the degradation of phosphate-based glasses can be tailored to suit the end application and the rate at which they hydrolyze can vary quite considerably [3].

Various types of silver-doped inorganic antibacterial materials have been developed e.g. zeolites, calcium phosphate, silica gel, and borosilicate glass and some of them are now in commercial use. Silver ions are effective against a broad range of micro-organisms including G- bacteria e.g., *Pseudomonas aeruginosa*, yeast e.g., *Candida albicans*, and G+ bacteria e.g. *Staphylococcus aureus* [4,5]. Therefore, silver ions have been commercially used to take advantage of its antibacterial properties e.g. silver nitrate, colloidal silver, and certain other silver compounds are among the most generally used bactericidal agents. A large number of healthcare products now contain silver ions, principally due to its low toxicity to human cells and high antibacterial effect. Such products include silver-coated catheters, and wound dressings [6]. Phosphate-based glasses are materials of technological importance due to their superior physical properties compared to silicate glasses e.g., low melting temperatures, low glass transition and low softening temperatures, and high thermal expansion coefficients [7,8]. Thus PBGs can be prepared and processed easily at lower temperatures. In addition, phosphate-based glasses enjoy a range of compositional and structural possibilities (ultra, meta, pyro, and ortho) that facilitate tailoring chemical and physical properties of interest for specific technological applications. Controlled-release glasses (CRGs) were first developed in the 1970s primarily for use in food production industries [9]. Drake and Allen [10] found that PBGs with a suitable composition would dissolve in water with zero-order rate constant, and by controlling the composition it was possible to produce glasses which would completely degrade in water from hours to years thus can, over a prolonged period, release

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any additional constituents incorporated into them. Hence it has been possible to use pellets of CRGs containing metal ions such as copper and cobalt, as pesticides, fungicides and in animal feeds. CRGs are manufactured in a similar way to conventional soda-lime silica glasses in that the constituents are heated to temperatures above 1000 °C, then cast into various forms such as solid blocks, powder, granules, tubes, fiber or wool. The incorporation of well-known silver, copper or zinc antibacterial metal ions in several glass systems has a proven negative influence on the growth of bacteria and fungi [11,12]. Where in presence of an aqueous medium or moisture, the glass will gradually dissolve and at the same time, silver, copper, or zinc ions are released during its dissolution to provide an antibacterial effect. Generally, antibacterial glasses can be manufactured either by addition of an antibacterial agent to the glass batch prior to their manufacture or by post-treatment processes e.g. ion-exchange or surface coating. This work is an attempting to prepare and study the antibacterial effect of high dissolution silver-doped phosphate glasses.

2. Experimental

2.1. Glass preparation

All batches were prepared from chemically pure grade chemicals in the powder form. P_2O_5 was introduced as ($NH_4H_2PO_4$) (99.0% Merck), Calcium oxide (CaO) as Calcium carbonate ($CaCO_3$) (99.5% SRL) Sodium oxide (Na_2O) as sodium carbonate (Na_2CO_3) (99.5% s.d. fine-chem), and silver oxide (Ag_2O) as silver nitrate ($AgNO_3$) (99.9% SRL).The appropriate amounts of the starting materials of each batch equivalent to 50 g glass were accurately weighed, thoroughly mixed and then transferred to porcelain crucibles. Before melting, the batches were calcined slowly in an electric muffle furnace at a temperature in the range of 350–550 °C in order to get rid of the gaseous decomposition products of the batch materials, e.g. H_2O, NH_3, NO_2, and CO_2 and to minimize the evaporation tendency of P_2O_5. Calcination was continued until the decomposition of the batch materials and evolution of gaseous products came to an end. All the batches were melted in disposable porcelain crucibles inside an electrically heated furnace in the range 800–1200 °C. The melting time was continued for 1 h to 2 h depending upon the chemical composition. During melting, the melt was stirred manually by swirling about several times to ensure homogeneity and to get ride of gas bubbles. The melt was then cast on a preheated stainless steel plate in the form of rectangular slabs which subsequently annealed in a muffle furnace maintained at a temperature in the range 200–450 °C for 20 min. The muffle furnace was then switched off and the glass samples were left overnight to cool slowly to room temperature. The visible characteristics e.g. color; transparency, and homogeneity, of all samples prepared in this work were investigated using the normal visual observations.

2.2. XRD measurements

To ensure the glassy state, some selected samples were characterized with powder X-ray diffraction technique which is commonly used to verify the amorphous state of glassy materials. In the XRD spectra of glassy materials a halo is seen instead of diffraction peaks. the samples were finely ground in an agate mortar and X-ray diffraction spectra were obtained using a Bruker D8 Advance X-ray diffractometer at room temperature with Ni-filtered Cu K_α radiation ($\lambda = 0.15418$ nm), generated at 40 kV and 40 mA. Scans were performed with a step size of 0.02° and a step time of 0.4 s over an angular range 2θ from 4° to 70°.

2.3. FT-IR absorption measurements

The FT-IR absorption spectra of some selected undoped and silver-doped glasses (0, 0.5, 1 and 2 mol% Ag_2O) were recorded at room temperature in the frequency range 400–4000 cm^{-1} using an infrared spectrometer (Jasco FT-IR 6100). The measurements were made by the KBr disc technique in which discs were prepared by mixing and grinding a small amount of glass powder with spectroscopic grade anhydrous KBr powder and then pressed under vacuum and pressure of 6 ton/cm^2 into clear disks (1.2 cm in diameter and about 0.5 mm in thickness). All measurements were recorded with a resolution of 4 cm^{-1}.

2.4. UV–VIS absorption measurements

UV–VIS absorption spectra were measured for some undoped and silver-doped glasses (0, 0.5, 1 and 2 mol% Ag_2O). Polished glass samples having dimensions 3 cm × 1 cm and of the same thickness (2 mm) were scanned in the range from 200 to 1000 nm using a UV–VIS spectrometer (T80+, PG instruments Ltd.).

2.5. pH measurements

The pH changes of the distilled water during the dissolution of some undoped and silver-doped glasses were measured at every hour and up to 6 h using IQ 140 pH-meter (IQ Inc. USA). The pH electrode was calibrated using pH calibration standards (Colourkey Buffer Solutions BDH, UK).

2.6. Antibacterial activity test

The antibacterial activities of undoped and silver-doped P_2O_5–CaO–Na_2O glasses were tested against bacterial species of American Type Culture Collection (ATCC); *S. aureus* (ATCC, 25923), *E. coli* (ATCC, 25922), and *P. aeruginosa* (ATCC, 27853) using the agar disk-diffusion assays.

3. Results

3.1. Glass forming region (GFR)

The glass forming regions and the compositions prepared in the systems P_2O_5–CaO–Na_2O–*x* Ag_2O and P_2O_5–CaO-*x* Ag_2O, $x = 0.5, 1$ and 2 mol % are illustrated in Figs. 1–3. Clear circles denote homogeneous, transparent and colorless glasses as confirmed by XRD. Black circles denote samples that showed metallic silver particles precipitation. Fig. 1 and Fig. 2 showed that the compositions containing ≥ 60 mol% of P_2O_5 in the quaternary system P_2O_5–CaO–Na_2O–*x* Ag_2O, $x = 0.5$ and 1 mol%, formed homogeneous, transparent and colorless silver-doped glasses, whereas it was not possible to obtain homogeneous glasses for the compositions containing ≤ 55 mol% of P_2O_5 since these compositions showed precipitations of metallic silver particles. Also it can be seen from Fig. 3 that the compositions containing ≥ 65 mol% of P_2O_5 in the quaternary system P_2O_5–CaO–Na_2O–$2Ag_2O$ formed homogeneous, transparent and colorless silver-doped glasses. Nevertheless, among compositions containing 60 mol% of P_2O_5, only three compositions which contain 10, 15 and 20 mol% of Na_2O formed homogeneous, transparent and colorless silver-doped glasses. For other compositions containing 5, 25, 30 and 35 mol % Na_2O, homogenous silver-doped glasses could not be obtained since these compositions showed precipitations of metallic silver particles. Overall, A glass forming region containing ≥ 60 mol% of P_2O_5 was observed in the quaternary system P_2O_5–CaO–Na_2O–*x* Ag_2O, $x = 0.5$, 1 and 2 mol%.