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Role of Doping Copper Oxide on the structure and Bioactivity of some Silicate glasses

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The generation of a crystallized layer of hydroxycarbonate apatite (HCA) on the surface of the bioglasses after immersion in an SBF is thought to be the explanation of the bioactivity phenomena. We observed that adding minimal amounts of copper improves the performance of bioactive glasses. The aim of this study was to investigate the effect of the addition of CuO (0.2 - 4%) on the in vitro bioactivity behavior of bioactive silicate glasses. By soaking the prepared glasses in simulated bodily fluid (SBF) for one week, the behavior of the glass's bioactivity was examined. Then, different measurement methods, including scanning (SEM), FTIR spectra, and X-ray techniques, were used to evaluate the appearance of hydroxyapatite (HA). We observe the appearance of an IR band with two peaks at 560 and 604 cm^{-1} after soaking in simulated body fluid solution (SBF) in glasses with the addition of CuO (0.2 - 1%), which indicates the generation of a crystalline calcium phosphate phase that results in the formation of hydroxyapatite. SEM analyses reveal the distinctively distinctive crystals of hydroxyapatite that are supported by the FTIR results. The presence of the hydroxyapatite phase was detected through X-ray diffraction analysis. In the present research, we observed that adding minimal amounts of copper up to 1% improves the performance of bioactive studied glasses and increases the formation of a hydroxyapatite phase on the glass surface.

Keywords: Silicate; Bio glasses; CuO; FTIR; XRD; SEM.

INTRODUCTION

Over the following few decades, injuries are anticipated to be a major source of death and disability (Murray and Lopez, 1996): Injury-related deaths and morbidities in developing nations made for 11% of the global burden in 2001, placing them 11th overall in terms of causes of mortality and morbidity (Lopez et al. 2006), Road traffic accidents were the biggest cause of death for adolescents in 2012, with around 330 deaths per day, according to a major report by WHO titled "Health for the world's adolescents" which was released in May 2014. In underdeveloped countries, fractures are the main cause of trauma. The biology of fracture healing is a planned and intricate process that rebuilds bone and restores skeletal integrity. In 5–10% of cases, it is predicted that healing will be slowed down or impeded (Wegman, 1996) Over the past 50 years, there have been tremendous improvements in the management of fractures and orthopedic trauma care. Our capacity to treat musculoskeletal injuries has been impacted by recent advances in fixation devices, soft tissue treatment, and the biology and biomechanics of the musculoskeletal system. The efforts to enhance fracture care should concentrate on cost-effectiveness in addition to creating innovative remedies, particularly in developing nations (Peden et al. 2002). Because they permit changing their composition and, consequently, their properties, bioactive glasses (BGs) are a promising class of biomaterials for applications ranging from bone regeneration to soft tissue engineering (Hench, et al 1971), (Miguez et al. 2015) and (Schumacher et al 2021) Hench et al. first introduced the idea of bioactive glassy material in 1971 (Hench et al 1971) Since then, a wide variety of new glass compositions, systems, and glass ceramics have been added to the field of bioactive glasses and glass ceramics (Hench, 1991) and (Holland, 2002). When the bioactive silicate glasses like Hench patent react with body fluids or simulated body fluid (SBF), they frequently slowly and imperfectly transform into surface materials like amorphous calcium phosphate or hydroxyapatite (HA), and the presence of silicon ions that remain is doubtful (Peden et al. 2002) . Specific ions, like silver, copper, and strontium, were used to dope the bioglasses, and they were found to encourage angiogenesis, bone remineralization, and antimicrobial activity (Gupta et al. 2018), (Kargozar et al. 2021) and (Dai et al. 2021) Copper is a necessary trace element that

is crucial for maintaining the health of human cells and works well as an inorganic antibacterial agent with little cytotoxicity. According to studies, copper may effectively suppress both gram-positive and gram-negative bacteria. The copper-induced enhancement of osteogenesis has been widely studied (Wu et al. 2021), (Wang et al 2021) and (Yang et al. 2021).Additionally, copper ions have been shown to promote vascular endothelial cell proliferation, which enhances the angiogenic process, a crucial component of bone regeneration (Wang et al. 2021). Previous research has demonstrated that the copper ions assist the cross-linking of collagen and elastin in bones and increase the activity of a number of enzymes (Dai at al, 2021). However, a high level of locally accessible copper may produce free radicals that could affect bone metabolism (Malekirad et al. 2021). The type of nearby cells and the release kinetics of copper from the host material determine the threshold concentration at which copper ions in a biomaterial may be deemed to be cytotoxic (Tian et al. 2016). Therefore, varied copper concentrations and cell types must be taken into consideration when thinking of materials that demonstrate improved osteogenesis and angiogenesis when evaluating copper-doped bioglasses. The first goal of this work is to describe the bioactivity (or bone-bonding capacity) of prepared silicate glasses doped with CuO after immersion in SBF for one week. SEM and X-ray diffraction (XRD) are used to complement the overall investigation, which consists of FT infrared absorption measurements before and after immersion (SEM). To examine their impacts on the bioactivity of the silicate glasses, CuO (0.2, 1, 2, 4%) was added.

MATERIALS AND METHODS

2.1. Preparation of Bioactive Glasses.

Table 1 reports the nominal composition of the investigated bioactive silicate glasses (GSiCu0, GSiCu1, GSiCU2, GSiCu3, and GSiCu4). Fine-grained quartz (SiO_2), calcium carbonate ($CaCO_3$), sodium carbonate (Na_2CO_3), copper oxide (CuO), and ammonium dihydrogen orthophosphate ($NH_4H_2PO_4$) are all chemicals that are used in the manufacturing of glass. These ingredients are all chosen for their high purity. Silica (SiO_2) comes from quartz. Both Na_2O and CaO were introduced as their corresponding anhydrous carbonates. Phosphorus pentoxide (P_2O_5) was added in the form of ammonium dihydrogen orthophosphate ($NH_4H_2PO_4$) (Hyunh et al. 2021) We will melt the batches for 3 hours in platinum 2% rhodium crucibles. According to the composition of the bioactive glass, melting will be done in an electric furnace at 1400–1500 °C. To achieve homogeneity, the melts will be rotated several times at 30-minute intervals. The homogenized melts will be poured into a rectangular, preheated stainless steel mold with dimensions of 1 cm x 4 cm and a 1 cm depth. The finished bioactive glass samples will be annealed at 470 °C in a controlled muffle furnace. At a rate of 30 Ch−1, we leave the muffle furnace to reach room temperature. Glass samples will be polished until they are smooth with silicon carbide grit 600 until they are 2 mm thick, and then with grits 1220 and 2450 of silicon carbide and cerium oxide (Hench et al. 2004).

2.2. In Vitro Bioactivity Testing

The in vitro bioactivity of coated samples was evaluated by soaking the glass samples in simulated body fluid (SBF) for one week. SBF was prepared in accordance with Kokubo's instructions (Kokubo et al. 1991) [30], This buffer's ionic composition is comparable to that of blood plasma in humans. According to reports, SBF forms hydroxyapatite layers identical to those that would naturally develop on glass surfaces in vivo.

2.3. X-Ray Diffraction Study

We used X-ray diffractograms to confirm the amorphous nature of the glass sample and to see how it changed after soaking in SBF solution. After the glass samples were ground, a diffractometer using a Ni-filter and Cu-target was used to analyze the fine powder. The X-ray diffraction patterns were collected using a Philips PW 1390 X-ray diffractometer.

2.4. Infrared absorption measurements

We use an infrared spectrometer (type JASCO FT/IR-430, (Japan) to measure the FTIR absorption spectra of the glass samples in the 4000-400 cm^{-1} range before and after they are soaked in SBF at room temperature for one week. The samples were ground and combined with potassium bromide powder in a weight ratio of one to one hundred (0.002 g sample: 0.2 g KBr). The mixture was loaded with 5 t/cm2 in an evocable die for 2 minutes to produce clear, homogeneous discs. After making the discs, the IR absorption spectra were measured immediately.

RESULTS AND DISCUSSION

Infrared absorption spectra of bioactive silicate glass before immersion in SBF

The following factors are to be taken into consideration when interpreting the FTIR absorption results (ElBatal et al. 2003) and (Guo, 2019):

1) It is widely acknowledged that glass is a non-crystalline solid.

2) FTIR measurements of the absorption of different kinds of glasses reveal distinctive absorption bands that serve as identifiers of the presence of structural building blocks.

3) The base's FTIR In Hench's bioglass, phosphate and some silicate network units are present. A characteristic absorption in the middle of the infrared spectrum (400-2000 cm^{-1}) is known to be produced by such combined units.

4) Absorption bands in the Near-Infrared region (2,000-4000 cm^{-1}) associated with hydroxyl groups, water, or silanol (SiOH) groups are anticipated to also be present in the FTIR spectrum.

5) The broad bands at 3300-3,600 cm^{-1} are related to molecular water, OH, or silanol group (Guo et al. 2091) and (Odusote et al. 2019). While the absorption bands at 460-520 cm^{-1} are associated with the Si-O-Si and O-Si-O bending modes; the bands at 740-820 cm^{-1} can be associated with the Si-O-Si symmetric stretching of bridging oxygens bands at 1,060– 1,100 cm^{-1} are correlated with Si-O-Si antisymmetric stretching of bridging oxygens within the tetrahedral; the bands at 930-970 cm^{-1} are associated with the Si-O stretching with non-bridging oxygen (Nematidil et al. 2021)

6) Despite the fact that P_2O_5 is present in low concentrations (6%), some authors (Gupta et al. 2018) and (Kargozar et al. 2021) assume that phosphate groups are shared in the Infrared vibrations seen in these bioactive glasses, leading to the following hypotheses:

a) Both Si-O and P-O stretching vibrations can be related to the intense band at 900-1,200 cm^{-1}.

b) The vibrations of the Si-O-Si, Si-O-P, and P-O-P linkages have been combined to explain the bands at about 780-810 cm^{-1}.

c) P-O vibrations or the phosphate tetrahedral group have been linked to the bands in the 570–650 cm^{-1} range.

d) The O-Si-O and O-P-O bands have been identified as the 460–480 cm^{-1} bands.

The following statements summarize the FTIR results of the base bioactive silicate glass (GSiCu0) prior to immersion in SBF according to the prior considerations (ElBadry et al. 2000)

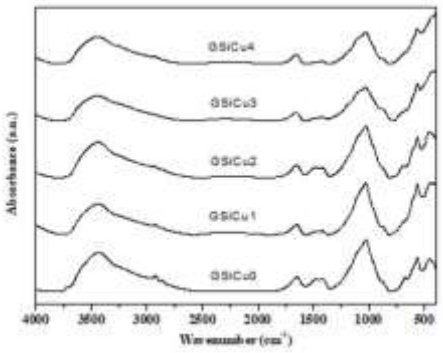

Figure1: Infrared reflection spectra of the studied samples glass before immersion in SBF

Fig. 1 Infrared reflection spectra of the studied glass samples before immersion in SBF show the following IR spectral characteristics.

1- The basic undoped glass's chemical composition leads us to believe that the network is composed primarily of silicate structural units, along with phosphate structural units, and that these two units exhibit their vibrational bands in the middle region, which range from 400 to 1600 cm^{-1}. We also noted absorption peaks caused by the vibrations of carbonate, SiOH, and water in the wavenumber range from 1420 to 4000 cm^{-1}.

2- The appearance of two peaks at 420 and 563 cm^{-1}, which are related to the Si-O-Si and O-Si-O bending modes, additionally sharing of bending among the phosphate network's oxygen atoms that act as bridges.

3- The remaining examined glasses' IR spectra (GSiCu1-GSiCu4) show similarities in all spectral aspects, particularly the location and number of the vibrational bands.

3.2. FTIR spectra of bioactive silicate glasses after immersion in SBF.

Fig. 2 shows the infrared reflection spectra of the studied glass samples after immersion in SBF for one week and the following IR spectral characteristics.

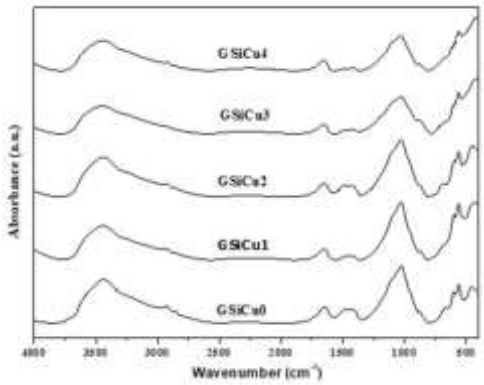

Figure 2: Infrared reflection spectra of the studied samples glass after immersion for one week

i) Two far-IR peaks, with the last one having the lowest intensity, may be seen in the glasses GSiCuO0 and GSiCuO1 at 560 and 604 cm-1. It's possible that hydroxyapatite is produced because of the creation of a crystalline calcium phosphate phase. However, the peak at 604cm^{-1} does not show in the glasses GSiCu3 and GSiCu4, which may be a sign that hydroxyapatite is not being formed.

ii) A high-intensity, wide band with a sharp peak at 1025 cm-1 exists between 800 and 1350 cm^{-1}, which is related to the coexistence of a gel layer rich in SiO2 and vibrations brought on by P-O stretching.

iii) A sharp band peak at 1652 cm^{-1} was caused by the water's adsorbed OH bending signal being absorbed.

iv) The OH, water, or SiOH groups are correlated with the bands at approximately 1424 and 3450 cm^{-1}.

3.3. X-Ray diffraction analysis

By using X-ray diffraction, we can investigate the structure of the prepared glass samples. Hench [45] was the first to propose a series of reactions that would result in the formation of a Ca-P rich layer at the surface of silica-based bioactive glasses submerged in SBF for one

week. The prepared silicate glass XRD patterns are shown in Fig. 3(a) and (b), respectively,

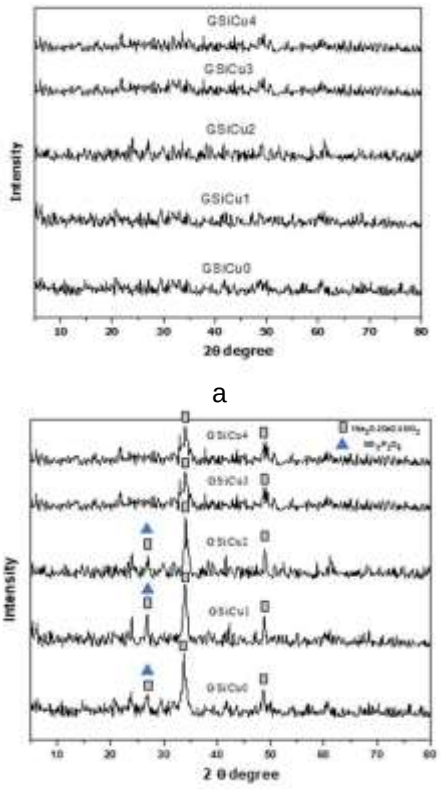

Figure 3: X-ray diffraction of the glass samples (a) before and (b) after immersion in SBF for one week

before and after one week of immersion in an SBF. According to these patterns, all glassy material that has been prepared is amorphous by nature and eventually transforms into a substance that contains the main soda-lime silicate phase ($1Na_2O.2CaO.3SiO_2$) and hydroxyapatite phases. The added dopant x-ray results show no additional crystalline phases beyond those found in the glass base sample and the undoped studied glasses. This suggests that the dopants that were added only served as nucleating agents, changing the percentage of the two crystalline phases only slightly. After immersing the prepared glasses in SBF solution for one week, we observed the appearance of HCA phases only in the glass samples GSiCu0, GSiCu1, and GSiCu2, with three sharp peaks in (2Θ = 26), (2Θ = 33), and (2Θ = 49) that are connected to the lattice planes of HA [46–47]. But when increasing the concentration of CuO in the composition of glass by more than 1% (GSiCu3 and GSiCu4), we observed that disappeared the peak at (2Θ = 26). The previous results may be related to the copper ions in low concentrations of up to 1% acting as network modifiers in the glass structure, so the addition of copper resulted in a higher number of non-bridging oxygens at the expense of birding oxygen (Mokhtari et al 2017) [46]. This resulted in a more disrupted network in copper-containing glasses, which in turn could increase the solubility and bioactivity of the glass in SBF solution. But when increasing the concertation of CuO by more than 1%, the copper ions act as a network former, leading to a decrease in the number of non-bridging oxygens, which decreases the solubility and bioactivity of the glass in SBF solution (Tian et al. 2016)[27].

3.4. Structural analysis using scanning electron microscopy

The scanning electron microscope is one of the most popular tools for examining the composition of biomaterials and how they interact with biological tissues. After being submerged in SBF for one week, the glasses' FTIR data show the appearance of a band that is indicative of the formation of crystalline hydroxyapatite. The micrographs of the undoped glass (GSiCu0) and the sample containing CuO dopants (GSiCu2) are shown in Figs. 4(a) and fig. 5(a), respectively.

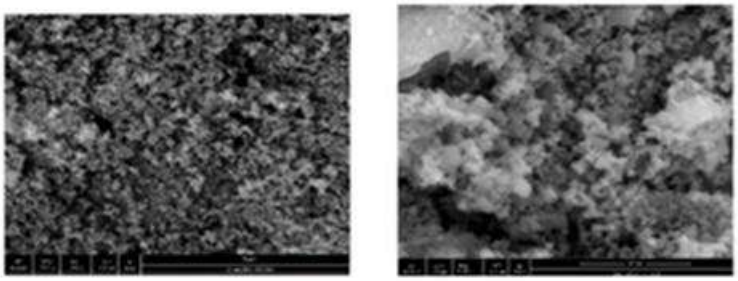

Figure 4: SEM of the glass GSiCu0 before and (b) after immersion in SBF for one week

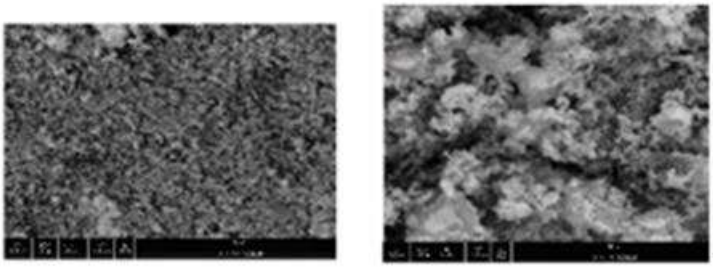

Figure 5: SEM of the glass GSiCu2 (a) before and (b) after immersion in SBF for one week.

The glass is completely smooth before immersion. However, after immersing the glass in the SBF solution, the figs. 4(b) and fig. 5(b) present some undefined grains with gradually appearing cotton-like growths or nodules on the surface of the sample. According to X-ray diffraction analysis, these cotton-like growths or nodules are caused by the formation of apatite crystals (Abdelghany, 2013) [47].

CONCLUSION

A few bioactive silicate glassy samples that are based on Hench's patented bioglasses and doped with CuO have been prepared, and their bioactivity behavior has been assessed. X-ray examination demonstrates the separation of sodium calcium silicate ($Na_2O.2CaO.3SiO_2$)

with the second phase of silicon phosphate and the crystalline phases. In the present research, we observed that adding minimal amounts of copper oxide up to 1% improved the performance of the bioactive glasses studied and increased the formation of a hydroxyapatite phase on the glass surface. FTIR spectra show distinctive vibrational bands caused by the main silicate groups and the sharing of some phosphate groups. The FTIR spectra of glasses after immersion in SBF solution for one week exhibit additional characteristics for IR peaks due to the formation of a hydroxyapatite layer. SEM analyses of the prepared glasses are completely smooth before immersion, but after immersion, we show some undefined grains with gradually appearing cotton-like growths or nodules on the surface of the sample. According to X-ray diffraction analysis, these cotton-like growths, or nodules, are caused by the formation of apatite crystals.

CONFLICT OF INTEREST

The authors declared that the present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

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