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Structure and Physical Properties of Silver Borate Bioactive Glasses.

H Kamal*

Department of Physics, Faculty of Science, Mansoura University, P.O. Box 35516, Mansoura, Egypt.

ABSTRACT

The in vitro behavior and characterization of a bioactive borate glasses containing Ag₂O were prepared and investigated by full replacement of silica with borate in patented Hench's bioglass. In order to estimate and explore the biodegradability and bioactivity, the samples were soaked in simulated body fluid (SBF) at 37 °C for fixed periods of time up to 21 days. The solubility testing for glassy samples was performed and the change in pH of the leaching solution were measured and evaluated then compared with a semi-empirical equation proposed to calculate the normalized dissolution rates. Calcium and silvers ions released from the glass were explored by means of x-ray diffraction (XRD), Fourier transform infrared analysis (FTIR) and scanning electron microscope (SEM). All results support the formation of hydroxyapatite (HA) layer after immersion in SBF which support the assumption that the released silver ions are inhibited after formation of HA layer. The overall combined spectral and solubility data evaluation gives evidences that the suitability and eligibility of the prepared glass make them a good candidate in the field of bone bonding and antimicrobial applications.

Keywords: Bio-based materials, biocompatibility, physical properties and Glass based composites

*Corresponding author



INTRODUCTION

Bioglasses are one of the most attractive synthetic materials that are used biomedical applications for different reasons; the most important one of them is its chemical similarity to natural bone minerals [1-3]. These glasses react with simulated body fluid (SBF) which is a solution prepared in laboratory and have ionic concentration similar to human plasma and can form a layer of hydroxyapatite (HA) on its surface of bioactive glasses [4-5].

Hydroxyapatite (HA) is the main inorganic phase of human bones and teeth which consist of nearly 72 wt% and 96 wt% apatitic materials, respectively. Calcium to phosphate ratio of HA within bone is approximately 1.67 [6-7]. Development of new categories of glass ceramics has recently concentrated on SiO₂ free glass [8-9]. Calcium phosphate-based bioceramics, such as HA, $Ca_{10}(PO_4)_6(OH)_2$, β -tricalcium phosphate (β -TCP), $Ca_3(PO_4)_2$, and biphasic calcium phosphate (BCP), a mixture of HA and β -TCP, composed of the same ions as bone, are the inorganic materials which have received most attention for bone repair applications [10-12].

Recent studies have been conducted on borate glasses found that the hydroxyapatite layer is formed faster than that with silica glass content proposed by Hench [13]. Borate glass was recently found to support the growth and differentiation of human mesenchymal stem cells [8].

Addition of fluorides, for example LiF, ZnF_2 , NaF and CaF_2 were studied previously by full replacement of silica by borate in patented Hench's bioglass [14]. Borate glasses have been used in various biomedical applications despite the fact of their low chemical durability [15]. Borate composition as a biomedical material when immersed in phosphate solutions, particles of borate glass were generally dissolved and a layer of hydroxyapatite is precipitated [9].

Silver (Ag) and its ions exhibit an oligodynamic effect with a broad spectrum of antimicrobial activities against bacteria, viruses, algae and fungi [16-17]. The in vitro antibacterial action of silver doped in the system $SiO_2-CaO-P_2O_5-Ag_2O$ was studied previously [18] In this paper, a new type of bioactive glass contain silver ions was prepared and their activity was studied, these glasses possesses a controllable degree of degradation and compatibility which make them suitable to be used in the field of bone bonding and antimicrobial applications.

EXPERIMENTAL

Preparation of bioactive glasses

Glass samples were prepared by full replacement of silica by borate in the patented Hench's bioglass using chemically pure H_3BO_3 , $CaCO_3$, Na_2CO_3 and NH_4H_2PO4 . Using nominal composition (48.63 B_2O_3 , 22.92 Na_2O , 22.86 CaO, 5.59 P_2O_5) wt%, some bioglasses were prepared with addition of 1, 2, 4 and 10 wt% Ag_2O from pure AgNO₃, each one was added separately and given codes (BG1%AgO₂, BG2% AgO₂ BG4% AgO₂ and BG10% AgO₂) respectively. The batches were accurately weighed out and then melted in porcelain crucibles using an electric furnace at 1150 °C for 2 h and the melts were shacked well several times to achieve homogeneity except BG10% Ag_2O which show clusters of Ag metal inside bulk glass. Upon complete melting and homogenizing, the melts were cast into a preheated stainless steel mould of the dimensions required.

Preparation of Simulated body Fluid (SBF)

Simulated body fluid was prepared by the chemical which give ionic concentrations which are represented in table 1 and are compared with that of human plasma.

| | Na⁺ | K^{+} | Mg ²⁺ | Ca ²⁺ | Cl | HCO ₃ ⁻ | HPO4 ²⁻ | SO4 ⁻² |
|--------|-------|---------|------------------|------------------|-------|-------------------------------|--------------------|-------------------|
| Plasma | 142.0 | 5.0 | 1.5 | 2.5 | 103.0 | 27.0 | 1.0 | 0.5 |
| SBF | 142.0 | 5.0 | 1.5 | 2.5 | 147.8 | 4.2 | 1.0 | 0.5 |

Table 1: Ionic concentration (mM) of simulated body fluid (SBF) and human plasma



X-ray diffraction analysis

The identity of the crystalline phases formed with in the samples before and after immersion was analyzed by X-ray diffraction technique in order to identify the structural changes. The samples were ground and the fine powder was examined using a diffractometer adopting Ni-filter and Cu-target. The X-ray diffraction patterns were obtained using a Philips PW1390 X-ray diffractometer.

Solubility testing

The solubility of glasses was evaluated by the measurement of weight loss in simulated body fluid (SBF) at 37 °C in the incubator. Samples were polished with a 600 grit polishing paper, washed by acetone ultrasonically for a few minutes, and then placed in polyethylene beaker containing pre-calculated volume of SBF solution so the ratio between geometric area of the glass samples and volume of the solution was fixed as 0.075 cm⁻¹ in all cases for comparison and to avoid defects resulting from volumetric differences. Samples were removed and excess moisture was removed by tissue paper at various time intervals and then reweighed.

The percent changes of weight loss were correlated to glass solubility or corrosion. Solubility measurements were carried out in triplicate for obtaining acceptable weight loss measurements.

pH measurements

Changes in solution pH are very important to analyze when bioactive glasses are dissolved in simulated body fluids. pH values are easily measured with a pH-meter (Jenway-3150 Premier Portable pH/mV/ Temperature Meter with an accuracy of 0.01). The calibration of the electrode against buffer solution was performed at an interval of 24 h.

FTIR measurements

Fourier transform infrared absorption signals of the studied borate glasses were measured at room temperature (20 $^{\circ}$ C) in the wavelength range 4000-400 cm⁻¹ using a computerized recording FTIR spectrometer (Mattson5000, USA).Fine powdered samples were mixed with KBr in the ratio 1:100 for quantitative analysis and the weighed mixtures were subjected to a load of 5t/cm² in an evocable i.e to produce clear homogenous discs. Then, the IR absorption spectra were immediately measured after preparing the discs to avoid moisture attack. The FTIR measurements were repeated after the samples were immersed in SBF.

Surface structural analysis using scanning electron microscopy

Scanning electron microscopic (SEM) investigations were performed on glass samples at room temperature using an SEM model Philips XL30 attached with EDX unit, with accelerating voltage 30kV, magnification up to 400,000. All surfaces of studied samples were coated with gold for morphological investigations.

RESULTS AND DISCUSSION

Solubility and pH measurements

Solubility is one of the most important physicochemical parameters that plays an outstanding role that affects the stability of a material which could be used as implant materials in the human body to repair and replace diseased or damaged bone [19-20].

Biodegradation or resorption of a material in vivo has been reported to be a very complex process, this is because this process includes some physicochemical mechanisms which in some way interfere with other biological mechanisms and this complication may be controlled by solubility measurements [8]. In our studied glasses, borate glasses containing different concentration of silver oxide Ag_2O were prepared by full replacement of SiO_2 by B_2O_3 in patented Hench's bioglass.



Fig (1) represents the weight loss measurements of the prepared samples after soaking in SBF about 21 days at 37 $^{\circ}$ C; we note that all different concentration of Ag₂O additives may vary the degree of weight loss for the prepared glass. The glass containing a greater concentration of Ag₂O was found to be the most durable glass among those free of Ag₂O additives. Generally it can be seen that addition of different concentrations of Ag₂O to blank composition helps to increase the durability of prepared glass. The dissolution rate is affected by several factors including the reaction product and also the stability of the bioglass during the in vivo reaction [21].



Figure 1: Weight losses of glass samples soaked in simulated body fluid solution at 37 ºC as a function of time.

A similar behavior of weight loss plots may be related to that of our prepared in which glass continued to dissolve until the reaction with SBF is finished by formation of a crystalline hydroxyapatite layer on the surface of bioglass sample. In order to estimate the changes occurred on the surface of investigated glass samples in SBF, a sample surface was analyzed after different immersion times using scanning electron microscope (SEM) in fig 2.

Addition of sodium to borate glasses was reported to increases the chemical durability since it decreases the weight loss also, addition of P_2O_5 into glass serve to decrease its durability [22]. The prepared glasses possesses a considerable range of degradability, this is because the prepared samples experience a weight loss approximately 3.5% in about 10 day of immersion in SBF. All results in weight loss measurements agree well with that obtained of other well-accepted soluble bioactive glass compositions [14].



Figure 2: Scanning electron microscope of glass BG 4%Ag₂O after immersion in simulated body fluid for 21 days at 37 °C.



Solubility validation using a semi empirical formula

In order to calculate the normalized i-th element dissolution rate, the experimental data were plotted again after using a semi empirical equation:-

$$NR_{i} = \beta_{i} exp\left(-\frac{t}{\tau_{i}}\right) + W_{i}\left(1 - exp\left(-\frac{t}{\tau_{i}}\right)\right)$$
(1)

Where β_i is the i-th component initial dissolution rate, W_i is the i-th component final dissolution rate, and τ_i is i-th component time constant [14]. From this equation it can be assumed that the dissolution of each component of prepared glass is identical if the value of i-th component final dissolution rate W_i has the same value. The normalized i-th element mass release can be calculated by integration of the previous semi empirical equation (1) and this gives:

$$NL_{i} = \tau_{i}(\beta_{i} - W_{i})\left[1 - exp\left(-\frac{t}{\tau_{i}}\right)\right] + W_{i}t^{\dots}$$

Where *NL_i* is the normalized i-th element mass release

The parameter β_i which is tangent of the first linear portion of dissolution curve, Wi is the tangent of second linear portion of curve of dissolution. Finally τ_i is the transition time which means that the time needed for conversion from the initial stage to the final stage of dissolution.

From fig.1, the parameters β_i Wi and τ_i are calculated and are given in table 2. These parameters are then used to calculate the normalized i-th element dissolution rate (NR_i) and normalized i-th element mass release (NL_i). In table 2, one can note that our calculated values for W_i are nearly the same of each type of additives \cong 0.011.

| Bioglass | β_i | Wi | $	au_{i}$ | |
|--------------------------|-----------|--------|-----------|--|
| BG 0% Ag ₂ O | 0.041 | 0.0117 | 194 | |
| BG 1% Ag ₂ O | 0.049 | 0.0115 | 190 | |
| BG 2% Ag ₂ O | 0.055 | 0.0116 | 192 | |
| BG 4% Ag ₂ O | 0.064 | 0.0110 | 191 | |
| BG 10% Ag ₂ O | 0.066 | 0.0113 | 190 | |

Table 2: Calculated values of initial and final dissolution rates in addition to the time constant for each glass.

A plot of normalized i-th element dissolution rate (NRi) and time is shown in fig. 3. The calculated value of NR_i decreases drastically with increasing the time of immersion and varies in a manner that differs with the additive of Ag₂O. Also a plot of calculated normalized i-th element mass release (NL_i) and time is given is represented in fig. 4 and behaves inversely with that obtained with calculated values of NR_i. The time dependence NR_{i(t)} indicates that dissolution of prepared glass is ideal dissolution, which occurs when the NR_{i(t)} values for each element are nearly equal.

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Figure 3. Plot of normalized i-th element dissolution rate (NRi) and time.

Figure 4: Plot of normalized i-th element mass release (NLi) and time.

As can be seen in Fig. 5, the change in pH of the simulated body fluid (SBF) at different time intervals after the glassy powder have been soaked in it. The time dependence of pH illustrates an analogue behavior for all prepared samples. The SBF solutions become more alkaline owing to glass soaking. The graph shows that the pH value of solution starts from value of 7.42 and reaches a constant value that depends on the concentration of Ag_2O added to the glass matrix. The increase in pH occurring in the earliest period of the dissolution was seen for all the different glass samples. The increase in pH may be due to the leaching of cations out of the glass, which are further exchanged sodium ions with that of H+ ions from the solution [23].



Figure 5. Changes in pH values of the simulated body fluid at different time intervals.

The greatest increase in pH, both in rate and magnitude, is observed for BG10% Ag₂O glass composition as soon as bioglass powders are immersed in SBF solution, the pH immediately rises from ~7.42 to ~8.3 in the first 10 days of immersion. Then, it increases slightly nearly another pH ~8.45 in the following 11 days. In BG 0% Ag₂O glass, an increase of rises from ~7.40 to ~8 in the first 10 days followed by increase from ~8 to ~8.1 in the other following days of immersion.

The amount of silver oxide and calcium oxide strongly influences the acid/base properties of the bioactive glass surface. Also, the amount of Ca plays an important role in interaction of glass and simulated body fluid [24]. Molar adsorption heats developed by the interaction of simulated body fluid and with bioactive glasses indicate the possibility of cooperative effects among Ca cations, when the latter are present

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in relatively high amounts. In our prepared glass, calcium constitutes about 24.4 mol%, so during reaction with SBF, a molar adsorption heats developed by interaction indicates the possibility of cooperative effects among Ca cation as the glass contains a relatively high amount of calcium. The anions in SBF are usually have at least one lone pair of electrons (ligands) and a structure containing a metal (usually metal ion) like calcium bonded to a group of surrounding molecules and form coordination complex. The ligand uses the lone pair electrons (Lewis base) to bind to a metal ion (Lewis acid). A Lewis base is a molecule or ion that accepts a lone pair of electrons to make a bond. Ca²⁺ ions are coordinated mainly by oxygen atoms. Calcium demonstrates a lot of variety coordination numbers 7 to 9 being the most ordinary coordination numbers. Ca²⁺ is also considered as a hard ion and prefers hard oxygen-containing ligans that have a low polarizability [25]. Gradual changes of glass surface features caused by the introduction of increasing amounts of Ca species are also monitored by a gradual decrease of specific surface area, due to cation bridging between glass particles, and by quantitative as well as qualitative spectral changes of stable surface carbonate-like species. Measurements of change in pH during immersion in SBF suggests that OH^{-} , H^{+} and $H_{3}O^{-}$ species from SBF attack the glass network and are exchanged Na⁺ and Ca²⁺ ions from the glass network, this process helps these ions to leach to the solution leading to increase in pH of the solution. Similar results were reported for silicate bioactive glasses and the increase in pH is considered to contribute to the partial dissolution of the outmost layer and the subsequent apatite precipitation [26].

In order to estimate the changes occurred in investigated glass samples in SBF, SBF solution was analyzed before and after different immersion times. The composition of SBF was checked with respect to silver ions content. Silver concentration increased for all compositions in the 10 days of immersion. The relative changes for all samples are comparatively shown using the same scale in Fig. 6.



Figure 6: Changes in silver ions concentration in simulated body fluid at different time intervals.

During the first period of immersion, silver ions migrate from the glass surface into SBF and the silver ion concentration increases in the analyzed SBF solution. The highest record silver ion release is 2.2 mcg/ml and is observed for the sample BG4%Ag₂O. Calcium and silver ions are extracted from SBF and gradually selfassembled on glass surface. These modifications can be shown in SEM in fig. 2 after 21 days of immersion. This behavior could be accepted since the concentration of calcium in all samples is the same so the dissolution should be the same but amount of dissolved silver oxide into the glassy matrix plays an important role in changing the dissolution rates since the composition BG4%Ag₂O is more disposed to cation release. At the same time it has to be considered that with increasing Ag₂O content in glasses increases the tendency to silver segregation in metallic particles.

The electrical conductivity measurements support the assumption of silver ions release since the electrical conductivity of SBF was measured after several soaking periods of in SBF. As illustrated in fig. 7, all cations existing in SBF solutions contribute to their conductivity. One observes that after glass immersion in SBF the conductivity increases in the first immersion days then reach a nearly constant value. This process may be related to the release of silver ions into solution. The release of silver ions into solution may be obstructed after formation of hydroxyapatite layer and there is further increase in conductivity.

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Figure 7: Changes in electrical conductivity of simulated body fluid at different time intervals.

Interpretation of the FTIR spectra of the studied borate glasses before immersion in SBF

Fig. 8 illustrates the FTIR spectra of the prepared borate glasses before and after immersion in SBF. In borate glasses, the structure of vitreous B_2O_3 consists of triangular borate (BO₃) units arranged mostly in boroxol groups [27]. The addition of alkali oxide (e.g.Na₂O) or alkaline earth oxide (e.g. CaO) changes some of the triangular borate units into tetrahedral (BO_4) units [28]. After certain limit, the formation of more BO_4 groups ceases and the surplus modifier oxide forms non-bridging oxygens [14]. The prepared bioactive borate glasses are expected to contain both triangular (BO₃) and tetrahedral (BO₄) units with ratio depends on the composition of glass constituents. The absorption bands at wavenumber range of $1250-1600 \text{ cm}^{-1}$ are attributed to the bending vibration and stretching vibration of B–O–B in [BO₃] triangles. Absorption band between 900 and 1200 cm⁻¹ was observed due to B–O stretching vibration of tetrahedral [BO₄] units. The band in the range of 650–800 cm⁻¹ is due to bending vibrations of B–O–B groups in [BO₃] triangles. The IR absorption bands recorded from the samples surface after 21 days immersion in SBF (Fig. 8) are consistent with the spectra for apatite compositions. The FTIR characteristic peaks of phosphate groups appear in the form of a strongest band at 1035 cm⁻¹. This peak actually attributed to P–O stretching vibration and that recorded in the spectral range 620–560 cm⁻¹ arises from P–O bending vibration. The bands around 1630 cm⁻¹ are attributed to vibration of the hydroxyl groups. A broad spectral band between the ranges from 3200 to 3600 cm⁻¹ is attributed to water molecules [14].



Figure 8: Fourier transform infrared signals of the BG 0%Ag₂O glasses before and after immersion in simulated body fluid.

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Interpretation of the IR spectra of the studied silver borate glasses before immersion in SBF

Fig. 9 illustrates that the IR spectra for the studied borate glasses before immersion in SBF solution. The graph represents some of observed changes. The strong resolution and presence of the band at 1050 cm⁻¹ is accompanied with the observed decrease of the intensity of the bands in the range of 1200–1500 cm-1 which may indicates the decrease of the BO₃ groups and the persistence of the BO₄ groups. This behavior is evident that it is well related to dissolution of the BO3 bearing phases which are very weak in comparison with the strong BO₄ groups in which each boron atom is attached from the four directions by alkali or alkaline earth cation to cause neutrality. Also, the IR spectra reveal the appearance of a new band with two split peaks at about 560 and 620 cm⁻¹. This specific split is attributed to the presence or precipitation of calcium phosphate or hydroxyapatite. This band is an indication of the potential bioactivity of the studied borate glasses. The peak at 1640 cm⁻¹ is correlated with hydroxyl groups (OH)⁻.



Figure 9: Fourier transform infrared signals of the prepared silver borate glasses before immersion in simulated body fluid.

The introduction of silver oxide by different proportions causes a well separation of previously assigned BO_3 and BO_4 units, theses variations are in the position or intensities of some bands but the contribution of the main characteristic bands due to BO_3 or BO_4 groups remain the same. The intensities of BO_3 units may greater before the units present in blank one.

Interpretation of the IR spectra of the studied silver borate glasses after immersion in SBF

The growth of a hydroxyapatite type layer on the investigated silver containing glasses was confirmed and extended by infrared spectroscopic analysis of blank sample and that with silver oxide additions Fig. 10. Samples with different concentrations of Ag_2O are similar and consist of well resolved absorption bands. Bands of phosphate groups are than that of the blank and are assigned to P–O bending and stretching vibrations [29]. The width of the absorption band is related to the crystallinity of self-assembled surface layer and this reflects disorder degree of the hydroxyapatite type layer that formed on surface of samples.





Figure 10: Fourier transform infrared signals of the prepared glasses after immersion in SBF for 21 day at 37 ºC.

X-ray Diffraction

XRD patterns of the prepared glass samples before and after immersion in the SBF for 21 days are shown in fig. 11 and 12. All glass samples appears to contain the following crystalline phases, calcium borate CaB₂O₄ (JCPDS 22-0141), calcium phosphate Ca₃(PO₄)₂ (JCPDS 70-0364), and sodium phosphate oxide Na₅P₃O₁₀ (JCPDS 02-0923) with different ratios in addition to other minor phases depending on the glass composition. Examination of the previous crystalline phases which are formed during immersion of glassy samples in SBF indicates a considerable crystallinity of all the prepared glasses. In Fig. 12 XRD patterns of the glasses soaked in SBF show the same crystalline phases in addition to hydroxyapatite Ca₁₀(PO₄)₆(OH)₂ (JCPDS 72-1243) phase. In powder diffraction technique, the small concentrations of hydroxyapatite in sample were not easily be detected because this technique is not sensitive to minor concentrations of HA. The obtained patterns were broader and less intense if we compare it with standard HA. This may be an indication that the formed hydroxyapatite is poorly crystallized and is considered to be in amorphous form or it may be in a nano scale size. Our results based on FTIR supports well the formation of HA in our prepared glass sample since the bending and starching vibrations of P-O is clearly seen since FTIR technique is considered to be a more sensitive technique than the powder XRD technique for monitoring the formation of HA on the surfaces of the glass particles. It is well known that calcium and phosphorus ions are arranged into the hydroxyapatite layer which is formed on the bulk glass surface.



Figure 11: X ray diffraction patterns of the prepared glasses before immersion in simulated body fluid.



Figure 12: X ray diffraction patterns of the prepared glasses after immersion in simulated body fluid for 21 day at 37 °C.



CONCLUSIONS

Solubility and pH measurements for the prepared glasses reveal that addition of Ag₂O to the prepared glasses affect the rate of ion released to SBF. In the first stage of soaking, the cations release from the coated glasses and after formation of hydroxyapatite bioactive layer, the release of silver ions diminished. According to silver ions concentration determined in SBF after different soaking times the growth of the bioactive layer on the surface of investigated glass system impresses after ten days of immersion. Conductivity data support the assumption that the silver ions released in SBF are obstructed by the growth of the bioactive layer on the glass surface. The microstructure of these materials was probed by X-ray diffraction (XRD), Fourier transform infrared absorption spectroscopy (FTIR) and scanning electron microscope (SEM) and the results suggest that these glasses are a good candidate to be used in the field of bone repair because of its bioactivity and compatibility. Moreover, silver containing glasses have a potential to be an antimicrobial materials due to silver ions released in the first SBF soaking stage and there in after they can contribute to enhancement of bone formation due to self-assembled bioactive layer of hydroxyapatite type on their surface.

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