

Processing, Characterization and Application of Some Borophosphate Glasses Containing Antibacterial and Antifungal Oxides in Bioactive Demands

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A SERIES of borophosphate glasses doped individually with few antimicrobial oxides; CeO₂, ZnO and CuO were prepared by melt quenching technique. The structure and bioactivity of the prepared samples were analyzed through FTIR spectral technique and corrosion behavior before and after immersion for 2 weeks in simulated body fluid (SBF) under static condition. The microstructure of the glass was examined using SEM to confirm the bioactivity of glass through the formation of calcium phosphate (CaP) layer on the surface. FTIR spectra of the glass after immersion in SBF show characteristic peaks of calcium phosphate. Weight loss% display a difference in solubility in the SBF solution in relation to the antibacterial oxide added. SEM results show clear nodular microcrystalline calcium phosphate. The prepared glasses revealed effect against some microbes and bacteria. It was established that the addition of the antimicrobial oxides had a positive effect on the glass bioactivity and may find a part in the biomedical applications.

Keywords: Borophosphate glasses, bioactivity, FTIR, SEM, Antibacterial-antifungal glass.

Introduction

Bioactive glasses (BG) were known and applied in medicinal field especially in orthopedics, otology and dentistry, a long time ago [1]. Firstly, bioactive materials were prepared to eliminate bone deficiencies, but later, they applied in tissue engineering and therapeutic uses [1]. Dental implants, bone substantial, soft tissue renewal are the traditional applications of BG. But the interesting issue in the coming period is how to increase the antibacterial properties of BG and how to be benefit in different therapeutic uses [1].

The importance of borophosphate glasses is coming from their high valuable collective structural chemistry and uses in sorption and separation, heterogeneous catalysis photonic technologies, ion exchange and others. Borates and phosphates reveal a complicated construction which increases the tendency to create polynuclear anionic units [2]. The building of anions in both boron-oxygen tetrahedron and phosphorus-oxygen tetrahedron with several unifications, in borophosphate glasses, is of high importance

because of their uses in plasma display panel (PDP) phosphorus, and scintillation materials. The chemical durability, thermal and mechanical stability of phosphate glasses is improved by the addition of B₂O₃ to a phosphate network [3]

Living body and alkaline earth borophosphates are able to contact through biological reaction [4]. Human bone inorganic phase consists basically of calcium and phosphate. So glasses of calcium phosphate main composition were studied in therapeutic demands because they produce biologically active calcium phosphate (CaP) layer which upsurges the attachment between bone and soft tissues [5-7].

Bioactive glass doped with different antibacterial oxides (CeO₂, ZnO, CuO) which have biological features have been explored outside human body to form alloplastic materials effectively [7].

Cerium has a stable character against bacteria, consequently bioactive glasses containing cerium is valued in many applications like implantation

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into periodontal pockets, infected frontal sinuses and hypersensitive teeth [8, 9].

Bone construction and cell progression are basically independent on zinc element [10, 11]. Many enzymes based on zinc as a cofactor, preparation of protein and DNA reproduction also depend on zinc [11, 12]. A lack of zinc leads to the delay in skeleton progression and changes in ordering of bone tissue [13]. Chemical durability of many glasses containing Zn, increased by slowing down their dissolution in aqueous solutions [in vitro body fluids] and increasing the mechanical properties [14]. Copper (Cu) is a vital ion with valuable properties in the human body. Because its favorable role on endothelial cells, it performs a major part in angiogenesis and blood vessel maturation process [15].

The aim of the present work is to study the effect of addition of different oxides (CeO₂, ZnO, CuO) on the bioactivity and antimicrobial behavior of borophosphate glasses in order to develop effective bioactive materials.

Materials and methods

Preparation of glass

The glass system was prepared by mixing starting reagents [orthoboric acid for B₂O₃, ammonium dihydrogen phosphate for P₂O₅, calcium fluoride for CaF₂, sodium carbonate for Na₂O] in the calculated quantities. Cerium, zinc, copper oxides for CeO₂, ZnO, and CuO as shown in Table 1. Weighted batches were

homogeneously mixed via an agate mortar before melting for 2 hours in covered porcelain crucibles at 1200°C. The melts were rotated at intervals to attain homogeneity. The prepared glasses were transferred to a muffle furnace regulated at 280°C for annealing. The melted ingot was crushed in an agate mortar and sieved to obtain particles of size (0.3-0.6 mm)

Structural and bioactivity characterization

FTIR measurements

FT Infrared absorption spectra of the prepared glasses were measured at room temperature in the wavenumber range of 4000-400 cm⁻¹ using FTIR spectrophotometer (Type Matson 5000, USA). Fine powder of the samples was mixed with KBr in the ratio 1:100 for qualitative analysis and the mixture was subjected to a load of 5 tons/cm² in an evocable die to produce clear homogeneous discs. The measurements were taken pre and post immersion of the prepared glasses in SBF solution.

Weight loss%

1 g of glass grains (0.3-0.6 mm) was soaked in 100 ml of SBF solution for 2 weeks at 37°C. Degradation degree (weight loss, %) of the prepared glass was valued as:

$$\text{Wt loss \%} = (W_i - W_t) / W_i \times 100 \dots\dots\dots (1)$$

Where W_i is the weight of glass powder before immersion, and W_t is the weight of glass powder after immersion for a time (t) [16].

TABLE 1. Composition of prepared glasses.

Wt%	B ₂ O ₃	P ₂ O ₅	CaF ₂	Na ₂ O	CeO ₂	ZnO	CuO
Blank	30	40	5	25	---	---	---
G1	30	40	5	25	3	---	---
G2	30	40	5	25	---	3	---
G3	30	40	5	25	---	---	3

SEM

For SEM imaging, samples were sputtered with gold. The surface texture and morphology of the prepared samples were characterized using SEM [SEM Model Quanta FEG 250, Holland].

Antibacterial properties

Biology experiments

In-vitro antimicrobial activities

The antibacterial and antifungal activities of prepared glasses were determined by means of the agar diffusion method according to

Sherief et al., 2016 & El-Batal et al., 2017 [17, 18]. All samples were screened in-vitro against different pathogenic strains of Gram positive (*Bacillus subtilis* ATCC6633 and *Staphylococcus aureus* ATCC29213), Gram negative bacteria (*Escherichia coli* ATCC25922 and *Pseudomonas aeruginosa* ATCC27953), yeast (*Candida albicans* ATCC10321) and fungi (*Aspergillus niger* NRC53 and *Fusarium solani* NRC15). Bacteria and yeast strains are American Type Culture Collection, and fungal isolates were obtained from the culture collection of the Department of Chemistry of Natural and Microbial Products, National Research Center (NRC), Giza, Egypt. In antimicrobial test, the spore suspension of pathological strains was prepared and adjusted to be approximately (1×10^6 spores^{-ml} of fungi and 1×10^8 spores^{-ml} of bacteria). 1 ml of spore suspensions was inoculated into each plate containing 50 ml of sterile potato dextrose agar (PDA) and nutrient agar medium (NA), respectively. After the media had cooled and solidified, About 100 mg of the powder samples were applied on the inoculated agar plates and incubated for 24 h at 30°C for bacteria and yeast, 72 h at 28°C for fungi. The observation of the Halo zones was carried out to assess the antimicrobial activity. The antimicrobial effect was evaluated by measuring the inhibition zone diameter (IZD) around samples in (mm).

Results and Discussion

Hard work has been made to combine elements into the bioactive glass matrix [19]. So as to amend the glass functionality, bioactive and biological response of bioactive glass, replacement with different trace elements has been considered [20-22]. The glass network connectivity of un-substituted glass reduced upon the addition of CeO₂, which mainly acts as a network modifier. Particularly, the oxygen buffering capacity of ceria is well recognized [23]. Cerium oxide as redox-active rare earth ions grown wide interest in the biomedical field owing to its potential therapeutic applications in metabolic bone, cancer and infectious diseases. Whereas, ZnO doing as intermediate ions is a crucial trace element that stimulates bone formation [24, 25]. Copper oxide (CuO) is an indispensable ion in the human body aimed at its significant role in the course of angiogenesis and the maturation of blood vessels. CuO has a positive effect on the antibacterial mechanism. As a result, in common bone implant infections CuO seems to be a talented option for

aerobic bacterial inhibitor systems [26].

Infrared absorption spectra of the prepared glasses before immersion in SBF

Figure 1 shows the FTIR absorption spectra of the as-prepared glass powders. The following features are illustrated: An intense IR broad band is observed showing three peaks at 3550, 3480, 3418 cm⁻¹, two small peaks at 2925 and 2857 cm⁻¹, a kink at 2364 cm⁻¹, two peaks at 1721 and 1632 cm⁻¹, a broad band at 1400 cm⁻¹, a broad band at 1176 cm⁻¹, a band at 618 cm⁻¹. The absorption bands from 1400-1150 cm⁻¹ are typical of the vibrations of non-bridging PO₂ groups. While the bands in region of 1150-900 cm⁻¹, vibrations of terminal P-O and PO₃ groups can be found. The range of 900-700 cm⁻¹ illustrate the vibrations of bridging P-O-P groups [16]. In contrast, borate groups show three active IR spectral regions which are: Bending vibration of borate segments arises in the range of 800-600 cm⁻¹, B-O stretching vibration of triangular BO₃ units such as meta-borate chains, pyro-borate and orthoborate groups in the range of 1500 and 1200 cm⁻¹ and B-O stretching vibration of the tetrahedral BO₄ units such as tetra-borate and di-borate groups are in the range of 1200-850 cm⁻¹ [16]. Spectra reveal the absence of boroxol rings which may exist at 806 cm⁻¹.

The bands at 1400 and 1176 cm⁻¹ are caused by PO₂, BO₃, and BO₄ groups. Band at 1161 cm⁻¹ is attributable to vibrations of P-O and BO₄ groups. The vibration band at 618 cm⁻¹ is related to borate segments. Molecular H₂O or OH group is situated at 1632 cm⁻¹. The bands at 3418, 2925, 2857 cm⁻¹ are correlated to the different modes of water [OH, P-OH and B-OH] [27].

FTIR spectra of the prepared glasses after immersion in SBF solution

Figure 2 demonstrated the FTIR spectra of the surface of the prepared glass powder after soaking in SBF for 2 weeks. Accordingly, FTIR spectra illustrated increase in the sharpness of absorbance bands at 1617, 1721 cm⁻¹ and a decrease in the intensity of the two bands 2925 and 2850 cm⁻¹. The appearance of two bands at about 623 and 481 cm⁻¹ were barely detected. Such bands correspond to the vibration mode of crystalline P-O, in the (PO₄³⁻) orthophosphate groups are well anticipated to the formation of amorphous calcium phosphate (ACP) at the initial stage of conversion in SBF. ACP confirms the bioactivity of the studied glass. Apparently ACP is a precursor to the formation of the crystalline HA phase.

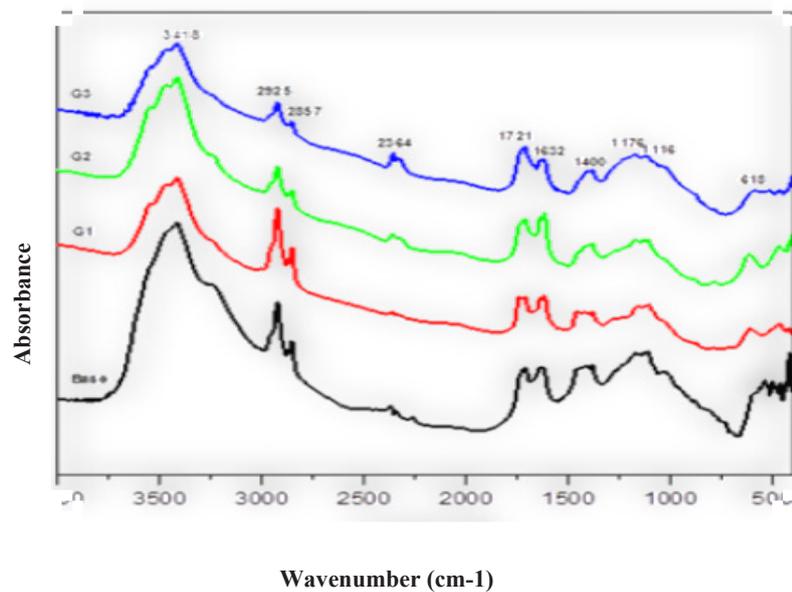


Fig.1. FTIR spectra of prepared glasses before immersion in SBF solution.

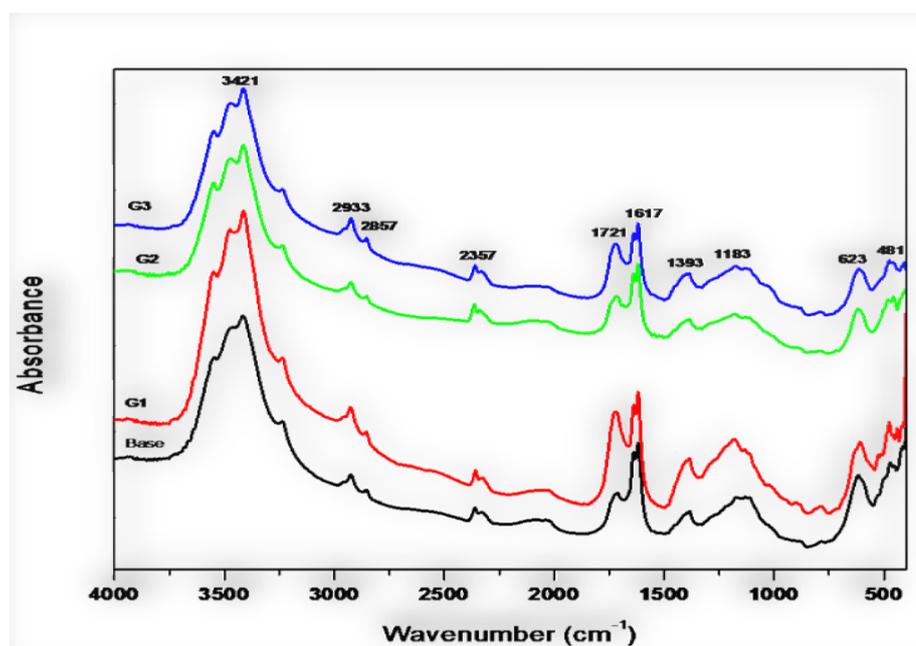


Fig.2. FTIR spectra of prepared glasses after immersion in SBF solution.

Weight loss%

The dissolution behavior of bioactive glass in SBF has a considerable effect on the antibacterial properties of the glass [1]. Particles of size of selected in this experiment because; smaller particle size can have a significant effect on the antibacterial efficiency of the material. As the reduction in size increases the active surface area and subsequently enhances the release of ions [28-30]. The dissolution behavior directly affects the microbial infections that may occur on the future implanting of the materials in the living body [31].

The corrosion behavior of borate glasses can be interpreted as follows [32]. With some variations in the degree of solubility to all constituents, borate glasses having alkali and/or alkaline earth oxides are properly soluble in aqueous solutions. The tetrahedral BO_4 borate glasses will be less soluble in aqueous solutions than glasses with high content of triangular borate (BO_3) groups. The addition of alkaline earth oxide (e.g. CaO) to sodium borate glass probably decreases the solubility of glass. This might be attributed to the introduction of doubly charged cations and their interfering in the solubility process and diffusion through the percolating channels in the glass network forming CaOH[33]. The weight loss%

data could be explained as follows: (a) Several factors control the dissolution process of borate glasses, such as: glass constituents, the duration and temperature of corrosion and the nature of leaching solution (i.e., constituent ionic species). (b) In the dissolution of soda lime borate glasses, Day et al.[33] expected a conversion process in which both sodium and borate ions dissolve in the leaching solution. However, the calcium ions from the glass will react with phosphate ions from the solution to form a calcium phosphate precipitate [34]. The formation of such precipitate was supposed to start at the glass surface before moving inward [35-37]. (c) By changing the antibacterial elements, there will be a difference in the weight loss%. This could be clarified by considering different parameters, including the differences in the ionic radii of the respective cations and/or the interfering of numerous mixed ions throughout the dissolution method. (d) Upon addition of CeO_2 , the weight loss% will increase the interaction of Ce^{2+} ions with the phosphate ions. A highly soluble product will be formed which exceeds that of both calcium and sodium ions. Calcium ions have the affinity to form a mixture of calcium phosphate. According to our results; the addition of cerium oxide is more antimicrobial than zinc and copper oxides as shown in Fig. 3.

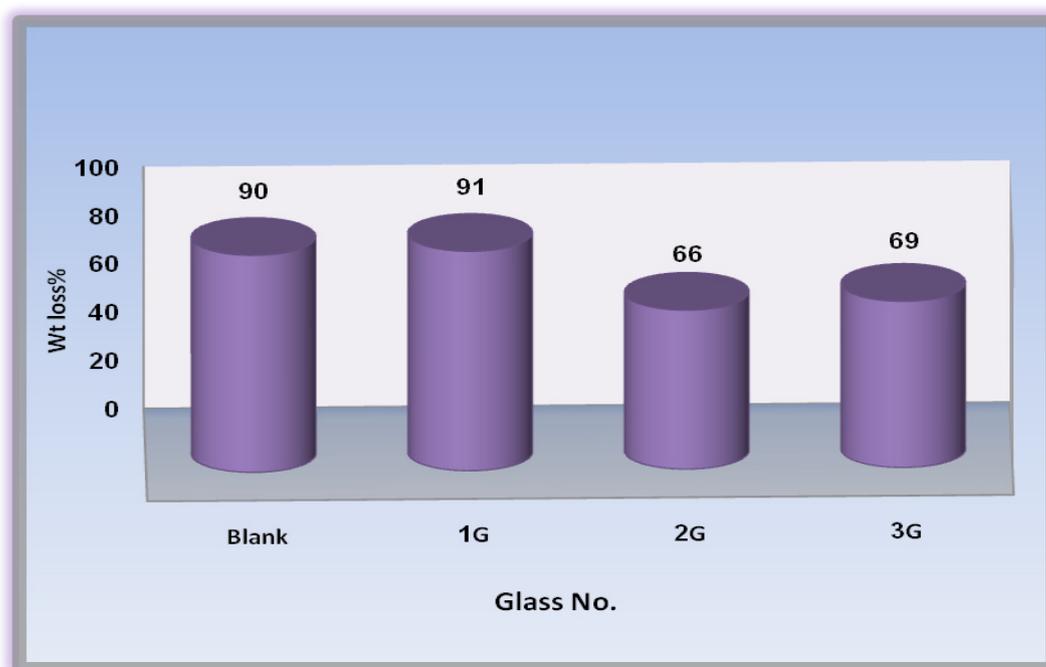


Fig.3. Wt loss% data of prepared glasses after immersion in SBF solution.

SEM

After samples immersion in SBF for 2 weeks, the SEM micrographs of the glass surface are presented in Fig. 4. Bioactive glasses convert to an amorphous calcium phosphate (ACP) or HA-like material [38, 39], which is in charge for their resilient bonding with the nearby tissue [40]. SEM micrographs exhibited a second phase material seemingly an amorphous calcium phosphate based material has entirely covered the surface of the samples (Fig. 4). As a function of doping element concentration, almost minor significant difference was observed on the shape and morphology of the borophosphate. While, in the case of glasses with cerium content a spherical second phase material of regular aggregates was observed on the surface, presumably an amorphous calcium phosphate or HA, mainly formed by cerium and phosphate [41]. The degradation of the borate bioactive glass in SBF occurs by the dissolution of components, such as Na_2O and B_2O_3 into the solution to form Na^+ , $(\text{BO}_3)^{3-}$, coupled with the reaction of Ca^{2+} ions from the glass with PO_4^{3-} from the solution to form the calcium phosphate layer on the glass [42]. Cerium content resulted in the formation of calcium rich HA layer over 14 days. This was attributed to the release of cerium ions which in turn competes with Ca ions to form phosphates [42]. In our glass system, all SEM images show a spherical shape which has better activity against bacterial infections in comparison to the needle-like form.

Antimicrobial experiment

The agar diffusion assay of the prepared glasses demonstrated different antimicrobial effects against tested micro-organisms depending on the sample composition. Table 2 & Fig.5&6 designate that, glass G1 exhibited an inhibitory effect against Gram positive bacteria *B. subtilis* and *S. aureus* with zones of the inhibition 17 and 16 mm, respectively. While glass G2 showed good wide range of antimicrobial activities against most of the micro-organisms tested with zones of inhibition range from 13 to 18 mm. Results also indicated that *S. aureus*, *C. albicans* and *F. solani* were found to be the most susceptible micro-organisms to glass G3 with zones of inhibition of 14, 16 and 25 mm. This result evidences that the G2 & G3 release Zn or Cu ions which react with proteins in the micro-organism causing the inactivation of proteins. The integration of Cu^{2+} in the borophosphate samples conveyed a development in the antibacterial effects [43]. The yield of antibacterial activity is well-defined conferring to numerous variables, including grain size, electron structure of the catalytic sample, speed of solubility, and nature of the microorganism considered for inactivation. For example, Cu^{2+} directed to a decrease in solubility but, due to the higher proportion of special surface to volume, the release of Cu^{2+} ions indicated a positive effect on antibacterial activity [43].

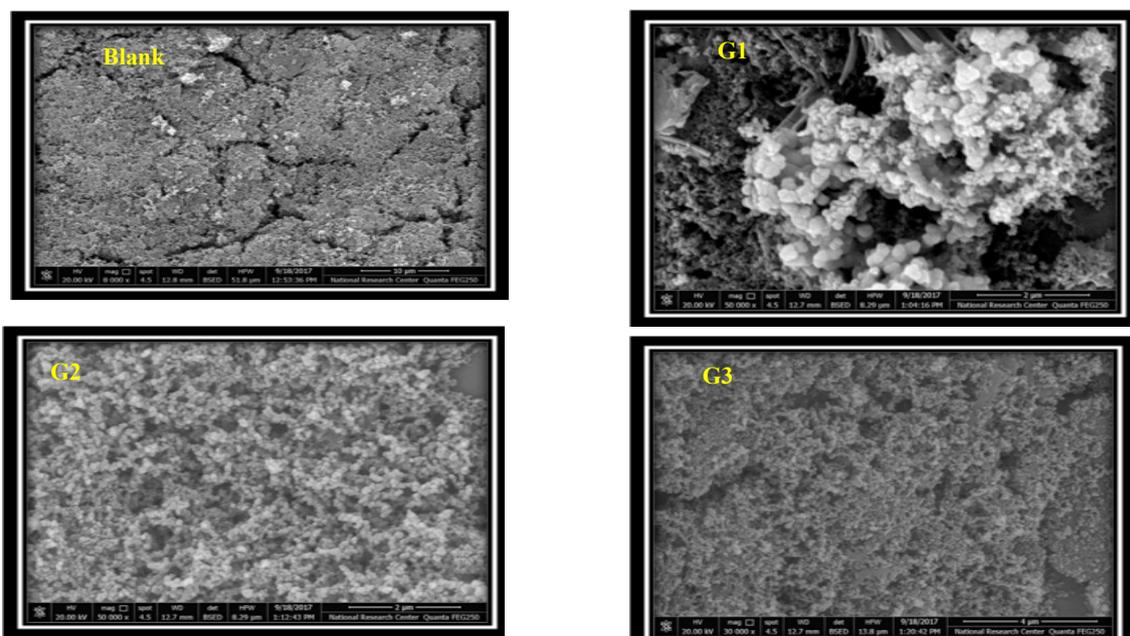


Fig . 4. SEM of prepared glasses after immersion in SBF.

TABLE 2. Antimicrobial activity of prepared glasses measured by the agar diffusion technique.

Glass Sample	Inhibition zone diameter (IZD) (mm)						
	Gram positive bacteria		Gram negative bacteria		Yeast	Fungi	
	B. subtilis ATCC 6633	S. aureus ATCC 29213	E. coli ATCC 25922	P.aeruginosa ATCC 27953	C.albicans ATCC 10321	F.solani NRC 18	A.niger NRC 53
Blank	20	18	N.A.	N.A.	13	13	N.A.
G1	17±1.2	16±0.3	N.A.	N.A.	N.A.	N.A.	N.A.
G2	13±0.5	15±0.2	N.A.	N.A.	18±0.4	15±1.6	N.A.
G3	N.A.	14±1.1	N.A.	N.A.	16±0.3	25±1.5	N.A.

N.A.No activity. The average values are reported as Mean ± SD calculated using MS Excel.

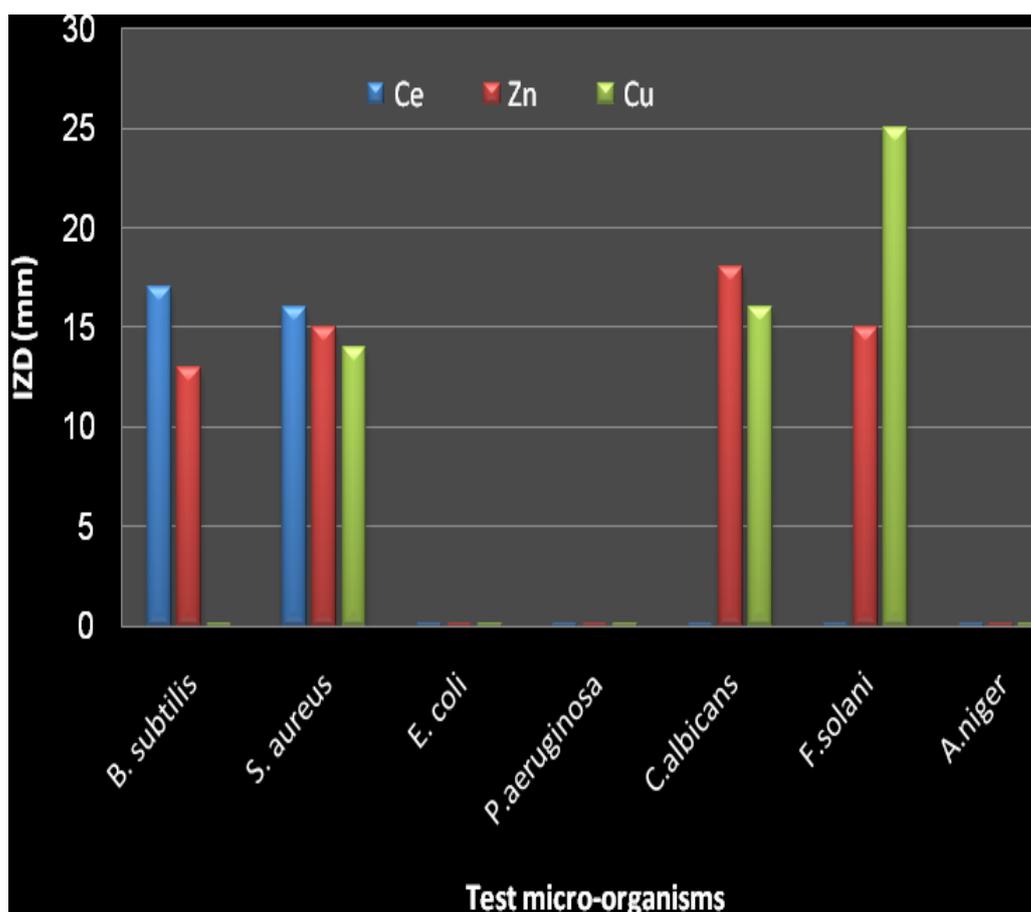


Fig .5. Antimicrobial activity of prepared glasses measured by the agar diffusion technique.

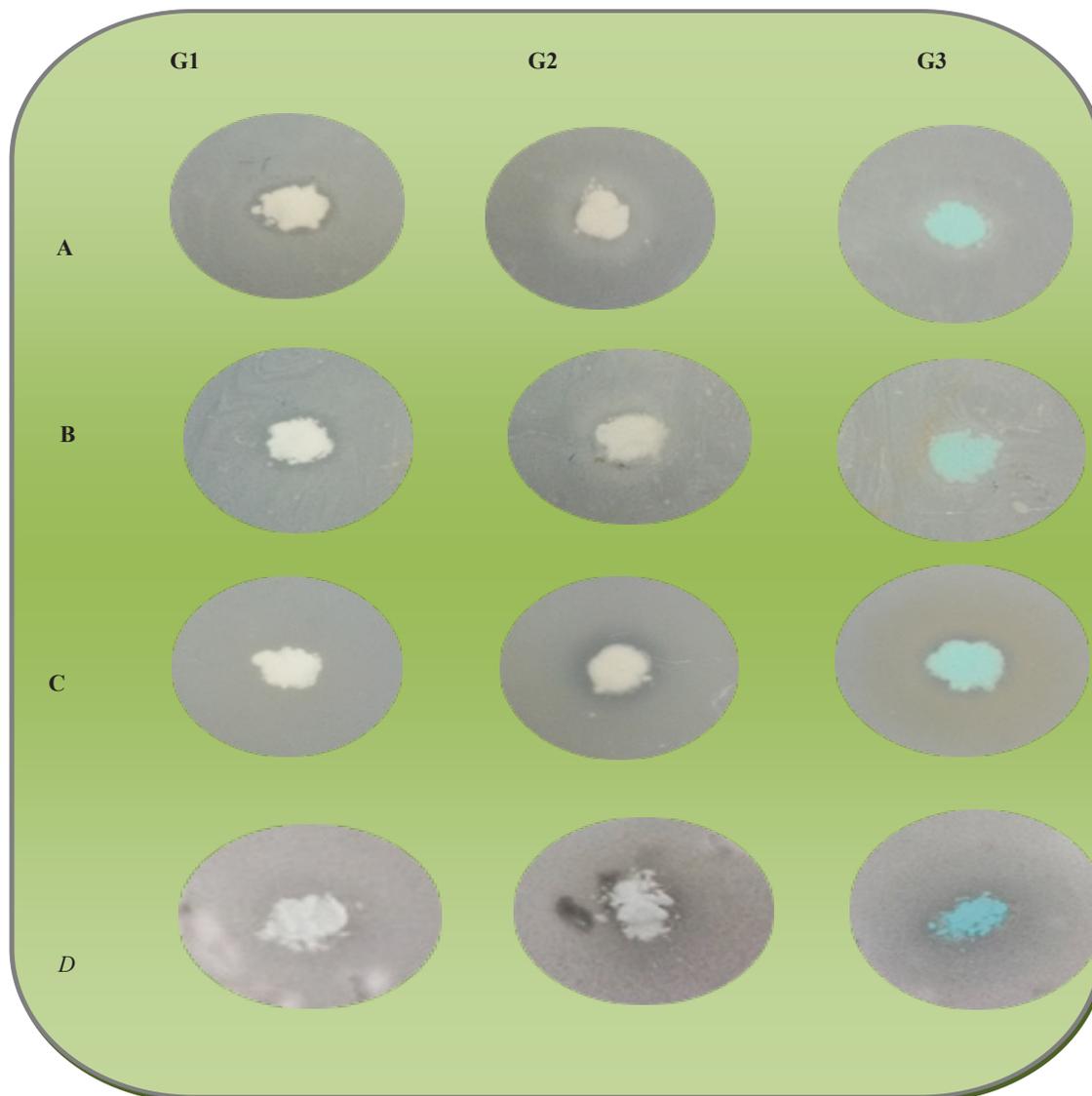


Fig .6. Photos of agar-diffusion assays of samples contained (Ce, Zn and Cu) with tested Micro-organisms. A, B, subtilis. B, S. aureus. C, C.albicans. D, F. solani.

Conclusions

Three different compositions in the system (B_2O_3 . P_2O_5 . CaF_2 . Na_2O . XO) where X is one of the antibacterial elements (Ce, Zn, Cu) were prepared. FTIR absorption spectra and weight loss% of the prepared glasses before and after immersion in SBF for 2 weeks were investigated. Samples showed a bioactive response in simulated body fluid (SBF). A Ca-P layer was indicated on the samples surface by the distinctive peaks of calcium phosphate group between 481 and 623 cm^{-1} . Also the antimicrobial application of the prepared glass was performed. All results assured

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the bioactivity of the prepared glasses. The dissolution of bioactive glass in SBF ensures the bioactivity especially in case of the CeO_2 sample. SEM similarly confirmed the bioactivity of the glass system by the development of a uniform spherical shape of particles and size which perform better against the bacterial infections.

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تجهيز و توصيف بعض زجاج البوروفوسفات المحتوى على أكاسيد مضاده للبكتريا والفطريات وتطبيقها فى الاحتياجات الحيويه

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^٤قسم الحراريات و السيراميك و مواد البناء، المركز القومى للبحوث، الدقى، الجيزه ، مصر

تم تحضير سلسله من التركيبات المختلفه من زجاج البوروفوسفات المحتوى على بعض الاكاسيد المقاومه للبكتريا مثل، أكسيد السيريوم و أكسيد الزنك و أكسيد النحاس، بطريقه الصهر. وتمت دراسه التركيب و الخصائص الحيويه لكل العينات المحضره عن طريق قياس طيف امتصاص الاشعه تحت الحمراء و النسبه المئويه للخساره فى الوزن قبل و بعد غمر الحبيبات الزجاجيه فى محلول مشابه لسوائل الجسم البشرى لمدة اسبوعين. وقد تم تأكيد الخصائص الحيويه للزجاج المحضر باستخدام المجهر الاليكترونى الماسح الذى اوضح تكون طبقه من فوسفات الكالسيوم و التى تتميز بشكلها الكروى على سطح العينات الزجاجيه بعد غمرها فى المحلول المشابه لسوائل الجسم البشرى. كما اوضحت نتيجته قياس طيف الاشعه تحت الحمراء تكون المجموعات البنائيه الخاصه بفوسفات الكالسيوم. و اوضحت قياسات النسبه المئويه للخساره فى الوزن تغير قيمة الخساره تبعاً لتغير الاكسيد فى الزجاج المحضر. و اظهر الزجاج مقاومه واضحه ضد انواع معينه من البكتريا و الفطريات. و خلصت النتائج الى ان اضافته بعض الاكاسيد المقاومه للبكتريا و الفطريات الى زجاج البوروفوسفات اعطى نتائج ايجابيه فى النشاط الحيوي للزجاج المحضر وربما يجد مجالاً فى التطبيقات الحيويه الطبيه.