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RESEARCH

Preparation and Characterization of Dental Pit and Fissure Sealant Based on Calcium Sodium Silicate Bioactive Glasses

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Abstract

This study aimed to prepare a resin based dental sealant loaded with novel bioactive glass formulated from (50 wt% calcium silicate and 50 wt% sodium silicate) with different percentages of fluorapatite. Four glass batches were formulated then characterized using Fourier Transform Infrared Spectroscopy analysis, X-ray powder diffraction analysis and Transmission electron microscopy. Density, microhardness and bioactivity testing after insertion in artificial saliva were done. Four bisphenol A-glycidyl methacrylate (Bis-GMA) based sealants loaded with the glass batch that showed the preeminent properties and silica fillers were prepared. The prepared sealants were compared to commercial sealants regarding flow, curing depth, compressive strength and microhardness. Sealant composition that showed comparable properties to that of the commercial sealant was selected for pH changes and ion release testing after immersion in artificial saliva for different time intervals. Results indicated amorphous nature, bioactive behavior and apatite forming ability of the tested glass batches. Experimental sealant revealed comparable tested properties with lower compressive strength compared to the commercial sealants at $P < 0.001$. The mean pH values of the tested sealants ranged from 6.75 to 6.35 with extended calcium and phosphorus ion release up to 90 days. It was concluded that the 85 wt% calcium silicate and sodium silicate with 15 wt% fluorapatite had the best trend regarding ion release and apatite forming ability. Sealant loaded with 65 wt% bioactive glass, 10 wt% sintered nanosilica and 10 wt% nanosilica had the best acceptable mechanical properties. The novel pit and fissure sealant is a promising bioactive and ion releasing material.

Keywords Pit and fissure sealant · Bioactive glass · Ion release · Micro hardness · Compressive strength

1 Introduction

Dental caries in children and adults is a public health problem in different countries all over the world. Caries prevention is one of the main goals in the dental field, due to the medical, social, and economic consequences that may result from dental caries [1]. Most of this decay was detected at the molar teeth due to their nature of presence of narrow and deep pits and fissures on their occlusal surfaces [1].

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Therefore, sealing occlusal pits and fissures is one of the most important caries preventive measures, acts through creating a physical barrier that blocks the biofilm's nutrition; hence inhibiting the biofilm growth [2]. A clinical study with 360 children over an observation period of 15 years showed a reduction of 36% in dental caries when all first molars were sealed and a reduction of 54% when all posterior teeth were sealed [3].

A number of materials and techniques have been tried to seal posterior teeth pits and fissures. However, the durability of resin-based sealants and the fluoride-releasing properties of glass ionomer sealants made them the most commonly used as sealing materials [4]. Additionally, the capability of sealing materials to perfectly adhere to the tooth structure is the most important property for caries prevention [5].

The efficacy of resin-based sealing materials, have been proven in many studies [6, 7] as well in systematic review of the Cochrane library [8]. Resin-based sealants based on light-cured bisphenol A-glycidyl methacrylate (Bis-GMA)

monomers with their known proper physical properties; flow ability and wettability are the most commonly used type of resin materials [9]. However, they are more prone to accumulate biofilm and dental plaque on their surfaces and also suffer from micro leakage caused by polymerization shrinkage. Such drawbacks may cause bacterial invasion followed by marginal caries and are the main causes for their failure.

To prevent marginal caries at the teeth sealant interface, attempts were made to promote remineralization through the use of bioactive glass containing dental materials with phosphate and calcium ions release. Bioactive glasses can form a strong chemical bond with the tissues [10, 11] and the remineralizing effect of products containing those particles on artificially induced carious enamel lesion were confirmed through several studies [12, 13].

Bioactive glasses have well proven dental advantages. In investigation made by Yang at al. [14], when examining the enamel surface adjacent to sealants containing 45S5 bioactive glass under simulated microleakage in a cariogenic environment, using optical 3-dimensional surface profilometer, microhardness tester, and scanning electron microscopy, it was revealed that increasing the glass filler contents had a significant impact in preventing the demineralization of the enamel surface within microgaps between the material and the tooth [14]. Another study examined the effects of 45S5 bioactive glass on the acid neutralization, mechanical and physical properties of pit and fissure sealants and found that these novel sealants neutralized the acid solution (pH 4.0) and exhibited appropriate mechanical and physical properties. Therefore, these compounds proved to be suitable candidates as caries inhibiting dental materials [15].

Additionally, there are many attempts to retain many dental materials bioactive ones through adding bioactive glasses to their original composition. In a study conducted by Raszewski et al. [16], they prepared a bioactive acrylic material through adding S53P4, Biomin F, 45S5 and Biomin C glasses to poly methyl methacrylate resin and they found that Biomin C and S53P4 showed greater solubility values than those stated by the ISO 20795-1:2013 standard and that acrylic resins modified with 10% of these two glasses produce a promising bioactive denture base material.

Another study tried to create a material for 3D printing that possesses bioactive properties, where 3D-printed Foto Dent splint resin was modified by the addition of two types of bioactive glasses (Kavitan and Fritex), results showed that this modified polymethacrylate resin was capable of releasing fluoride ions in the oral cavity environment with small reduction in physical properties and minor cytotoxic effect on human fibroblasts [17].

Calcium silicate ($CaSiO_3$) is a simple chain silicate mineral proved to have proper bioactivity and biocompatibility when studied as biomaterials for artificial bones, tissue regeneration and dental implants [18]. Studies proved the rapid formation of apatite on $CaSiO_3$ based materials more than that on other bioactive glasses when tested in simulated body fluid [18].

This study aimed to prepare a resin based pit and fissure sealant loaded with a novel bioactive glass formulated from equal weight percent of calcium silicate and sodium silicate combined with different percentages of fluorapatite. The effects on pH change, ion release, mechanical strength properties of the glass loaded resin sealer, were investigated. It was hypothesized that the prepared pit and fissure sealant would have acceptable physical and mechanical properties comparable to a commercial control sealants; and would have greater and more sustained multiple ion release required for enamel remineralization than the control commercial sealant.

2 Materials and Methods

2.1 Glass Composition and Batches Preparation

Four glass batch compositions were formulated based on a mother sample enclosing mixture of equal weight percentages of calcium silicate ($CaSiO_3$) and sodium silicate (Na_2SiO_3), with different weight percentages of fluorapatite ($Ca_5(PO_4)_3F$) (Table 1).

The starting materials were quartz (< 100 μ, 99.97% - Elnasr chemical company - Egypt), calcium carbonate ($CaCO_3$, 98% - Sigma Aldrich - USA), sodium carbonate

Table 1 Chemical composition of the glass batches and percentage of raw materials used

Glass sample No.	Nominal composition (wt%)		Chemical composition of the glass samples (wt%)					Raw materials (wt%)				
	Calcium silicate + sodium silicate	Fluorapatite	SiO_2	CaO	Na_2O	CaF_2	P_2O_5	Quartz	$CaCO_3$	Na_2CO_3	CaF_2	$NH_4H_2PO_4$
G0	100	–	50.45	24.14	25.44	–	–	36.38	32.57	31.09	–	–
G5	95	5	47.93	25.43	24.17	0.38	2.12	34.05	33.81	29.09	0.27	2.77
G15	85	15	42.88	28.02	21.62	1.14	6.36	29.62	36.22	25.29	0.78	8.09
G25	75	25	37.84	30.61	19.08	1.93	10.56	25.87	39.16	22.08	1.31	11.59

(Na_2CO_3 99.0% - Sigma Aldrich - USA), calcium fluoride anhydrous (CaF_2, 99.99% - Sigma Aldrich - USA) and ammonium phosphate monobasic ($NH_4H_2PO_4$, 98% - Sigma Aldrich - USA) as shown in Table 1. The raw materials were weighed (Electronic balance, Fuzhou Huake Electronics Instrument Co. Ltd., China), then mixed in a laboratory size ball mill at 120 rpm for 2 hours (Rapid Laboratory Mills, Ceramic - Model Series SD, Ceramic Instruments s.r.l, Italy), and melted in a platinum crucible using electric furnace (Carbolite, UK) in the temperatures range of 1300 - 1350 °C for 50-75 minutes. After a homogenous melt free from air bubbles was assured; the glass melt was cast in air to obtain the cast samples. To prepare the samples in the powder shape, parts of the cast samples were ground in ceramic mills by alumina balls then sieved for powder size ˂45 µm.

2.2 Characterization of the Prepared Glass Batches

2.2.1 Fourier Transform Infrared Spectroscopy (FTIR) Analysis of the Prepared Glass Batches

The prepared glass batches were characterized using FTIR analysis to study the functional groups in glass batches and to observe structural changes using Thermo Nicolet 6700, USA, having Attenuated Total Reflectance (ATR) mode. The spectral range was 4000–550 cm^{-1} at the resolution of 8 cm^{-1} with 256 number of scans. FTIR analysis was done before and after 6 months immersion of 0.3 g glass powder in 10 mL distilled water at 37 °C.

2.2.2 XRD Analysis of the Glass Batches

To ensure the amorphous characteristics of the prepared glass and absence of uncontrolled spontaneous crystallization (devitrification); X-ray powder diffraction analysis (XRD) was done (XRD, Mini Flex of Rigaku-Japan).

2.2.3 Density and Microhardness Characterization of the Prepared Glass Batches

The densities of the prepared glass samples have been calculated using the Archimedes' method based on the following formula:

$$Density = \frac{ð1}{ð1 - ð2} \times density\ of\ liquid$$

where ð1 is the weight in air, ð2 is the weight in water. And the density of distilled water is assumed to be 1.

Indentation microhardness of the prepared glass samples was also measured using Vicker's microhardness tester (Innovatest, Impressions XT, Europe BV). Testing was done at 1 Kg load, with 20 sec dwell time (ASTM E92).

2.2.4 Transmission electron Microscopy (TEM) Examination of the Glass Batches

TEM examination with acceleration voltage 120 kV attached to CCD camera, Japan was done to detect changes in the microstructure of glass batches initially and after 9 months immersion of 0.3 g powder of each glass batch in 30 mL artificial saliva.

2.2.5 In Vitro Bioactivity Testing of Glass Batches

pH and Ion Release Measurement of the Prepared Glass Batches The prepared glass samples (0.3 g) were immersed in 30 mL artificial saliva fluid. The artificial saliva was prepared using 2.38 g sodium phosphate dibasic anhydrous (Na_2HPO_4, Loba Chemie Pvt. Ltd., India), 0.19 g potassium dihydrogen orthophosphate (KH_2PO_4, SDFCL, India) and 8.00 g Sodium chloride (NaCl, ADWIC, El Nasr Pharmaceutical Co., Egypt) per liter of distilled water adjusted with orthophosphoric acid (H_2PO_4, ADWIC, El Nasr Pharmaceutical Co., Egypt) to pH value of 6.75, and placed in polyethylene bottles [19]. The artificial saliva fluid (10 mL) was collected after the following time intervals (1 hour, 2 hours, 4 hours, 8 hours, 24 hours, 48 hours, 5 days, 7 days, 14 days, 28 days, 56 and 90 days) and was replenished by 10 mL fresh artificial saliva. The pH of the collected solutions was inspected using pH meter (Jenway 3505, Bibby Scientific Limited, UK). The concentrations of the released calcium (Ca), phosphorous (P), and silicon (Si) ions from the glass batches were measured using inductively coupled plasma atomic emission spectroscopy (ICP-AES) using Agilent 5100 Synchronous Vertical Dual View (SVDV).

Apatite Forming Ability of Glass Batches The apatite forming ability of the tested glass batches was done using scanning electron microscope imaging (SEM) (S.E.M.- model XL30, Phillips Holland) at 40000 × magnification before and after immersion of 0.3 g powder of each glass batch in 30 mL artificial saliva.

2.3 Preparation and Characterization of Resin Based Pit and Fissure Sealant

2.3.1 Preparation of the Resin Matrix

The resins matrix was formulated through mixing 50 wt% Bisphenol-A glycidyl methacrylate (Bis-GMA) (Sigma Aldrich, USA), and 50 wt% triethylene glycol dimethacrylate monomers (TEGDMA) (Sigma Aldrich, USA); with 0.5 wt% camphorquinone powder (Alpha Aesar, USA) as photo-initiator and 0.5 wt% ethyl 4-dimethyl-amino benzoate (EDAB) powder (Alpha Aesar, USA) as co-initiator at room temperature. The mixture was then stirred for 1 hour at room temperature in a dark glass container using magnetic stirrer (IKA C-MAG HS10 digital, IKA-Werke GmbH & Co. KG, Germany) [20, 21].

2.3.2 Preparation of Fillers for Loading in Sealant Resin Matrix

The glass batch that showed the preeminent properties was selected as filler for the preparation of the experimental sealer . In order to reinforce the final sealant product, clusters of silica nanoparticles were utilized. Clusters were obtained through sintering of silica nanoparticles (nano powder, 10-20 nm particle size (BET), 99.5% trace metals basis- sigma Aldrich- USA) at 1300 °C for 15 min, at heating rate of 20 °C/min in an electric furnace [21].

For silanization of glass and sintered nanosilica; silane coupling agent was prepared by proportioning 70 wt% of ethanol in glass beaker, then few drops of acetic acid were added gradually to decrease the pH to 3- 4. Trimethoxysilane (3 wt%) was then added and stirring was done using a magnetic stirrer (IKA C-MAG HS10 digital, IKA-Werke GmbH & Co. KG, Germany) for one hour at room temperature [21]. Silica nanoparticles clusters and the selected glass powder were immersed in 3 wt% silane coupling agent, centrifuged, and then dried in hot oven (Witeg vacuum oven WOV, Germany). Silanization was done for 2 hours then centrifuged (Megafuge 8R, Thermo Fisher, Germany) for 30 minutes. Silanization was achieved by trimethoxy sialne, upon hydrolysis, (Si-OCH_3) groups were hydrolyzed to silanol groups by the water present in the ethanol. Then the silanol groups were condensed with hydroxyl groups on the silica surface to form the covalent bonds. The silanol groups on adjacent silanes were also condensed with each other to form a polymer film on the silica surface. Moreover, hydrogen bonds were formed between the hydroxyl groups of silica particles surfaces and the carbonyl groups of silane coupling agent by Vander Waal interactions. Finally, the precipitate was dried for 1 hour in hot oven at 110 °C [22].

2.3.3 Loading of Fillers in Resin Matrix

Different bioactive glass and silica filler weight percentages were tried for the preparation of the experimental sealant with total filler loading of 85 wt% (Table 2). The silanized fillers were incrementally added, at ambient temperature, to the resin matrix and mixed thoroughly with plastic spatula to form a homogenous mix. The mix was stored in dark glass container at 15 °C for two hours before use [22–24].

2.3.4 Characterization of the Prepared Pit and Fissure Sealant Samples

The prepared pit and fissure sealant samples were characterized regarding flow, depth of cure, compressive strength and microhardness and results were compared to that of commercial sealants. The brand names, composition, and manufacturers of the commercial sealants are listed in Table 3.

Table 2 Weight percentages of bioactive glass and silica fillers loadings used for the preparation of the experimental pit and fissure sealant samples

Sample code	Bioactive Glass	Sintered nano-silica	Nanosilica
Sample A	85%	–	–
Sample B	75%	10%	–
Sample C	65%	20%	–
Sample D	65%	10%	10%

Specimens of the commercial pit and fissure sealants were prepared according to their manufacturers' instruction.

Flow Testing of Pit and Fissure Sealants Flow test for the prepared samples and the commercial sealant was measured following ISO 6876/2012 specification [25]. Two square shaped glass slabs 40 mm × 40 mm in dimensions and 5 mm in thickness and approximately 20 g in weight were used. Uncured sealant paste (0.05 ml) was placed in between the two glass slabs and a 100 g load was placed on top of the two slabs forming a total load of 120 g. After 10 minutes, the weight was removed and the maximum and minimum diameters of the formed discs were measured (mm). The average diameter was taken to indicate the material flow. The test was repeated 6 times for all tested material.

Depth of Cure Testing The depth of cure was tested following ISO 6874/2015 specification [26]. A cylindrical mold of 6 mm height and 4 mm diameter was used. Sealant was injected into the mold and cured from the top side for 20 seconds. The sample was then removed from the mold and the uncured resin was scraped from the bottom part till set material was reached. The remaining height (mm) was measured using a digital caliper. The curing depth value is 50% of the obtained height. The test was repeated 3 times for each of the tested materials.

Compressive Strength Testing A total of 66 rods; 6 mm in height and 4 mm in diameter were prepared following ANSI/ADA Specification No. 27 [27], ($n = 11$) for each tested group. Sealant was added in 2 mm increments and cured for 60 seconds each. The top increment was covered with a clear celluloid strip during curing. Compressive strength was measured using universal testing machine (SHIMADZU AG-5KNX plus, SHIMADZU CORPORATION, Kyoto 604-8511, Japan) with cross head speed of 1 mm/min after being stored in deionized water for 24 hours in an incubator at 37 °C.

Vickers Microhardness Testing A total of 55 sealant discs were prepared for each of the experimental samples and the commercial sealant, ($n = 11$) for each tested group. Discs were prepared using a Teflon mold 5 mm diameter and 2 mm thickness. Samples were cured for 60 seconds, and then were stored in

Table 3 Brand names, composition, and manufacturers of the commercial sealant

Brand name	Composition	Manufacturers
i-SEAL LC pit and fissure sealant	Methacrylate matrix with 30-50% grinded glass filler by weight.	i-dental, Siauliai, Lithuania
Composan glass sealant A3	Filler content: 78%by weight microfillers. Fluoride and glass fillers.	Promedica, Neumünster, German
Compobond NE	Bis-GMA, HEMA, BHT, acetone and organic acids	Promedica, Neumünster, German

deionized water for 24 hours in an incubator at 37 °C. Vickers microhardness testing was measured using digital Vickers hardness tester (NEXUS 4000 TM, INNOVATEST, model no. 4503, Netherlands) at 200 g load and dwell time 10 seconds [9].

The experimental pit and fissure sealant that showed acceptable mechanical properties comparable to that of the commercial sealant was selected for further evaluations and testing.

2.4 pH Changes and Ion Release Testing after Immersion of the Tested Sealant in Artificial Saliva

A total of six discs; 5 mm in diameter and 2 mm in thickness, of the selected experimental, as well as, the commercial sealants were immersed in 30 mL artificial saliva in a polyethylene

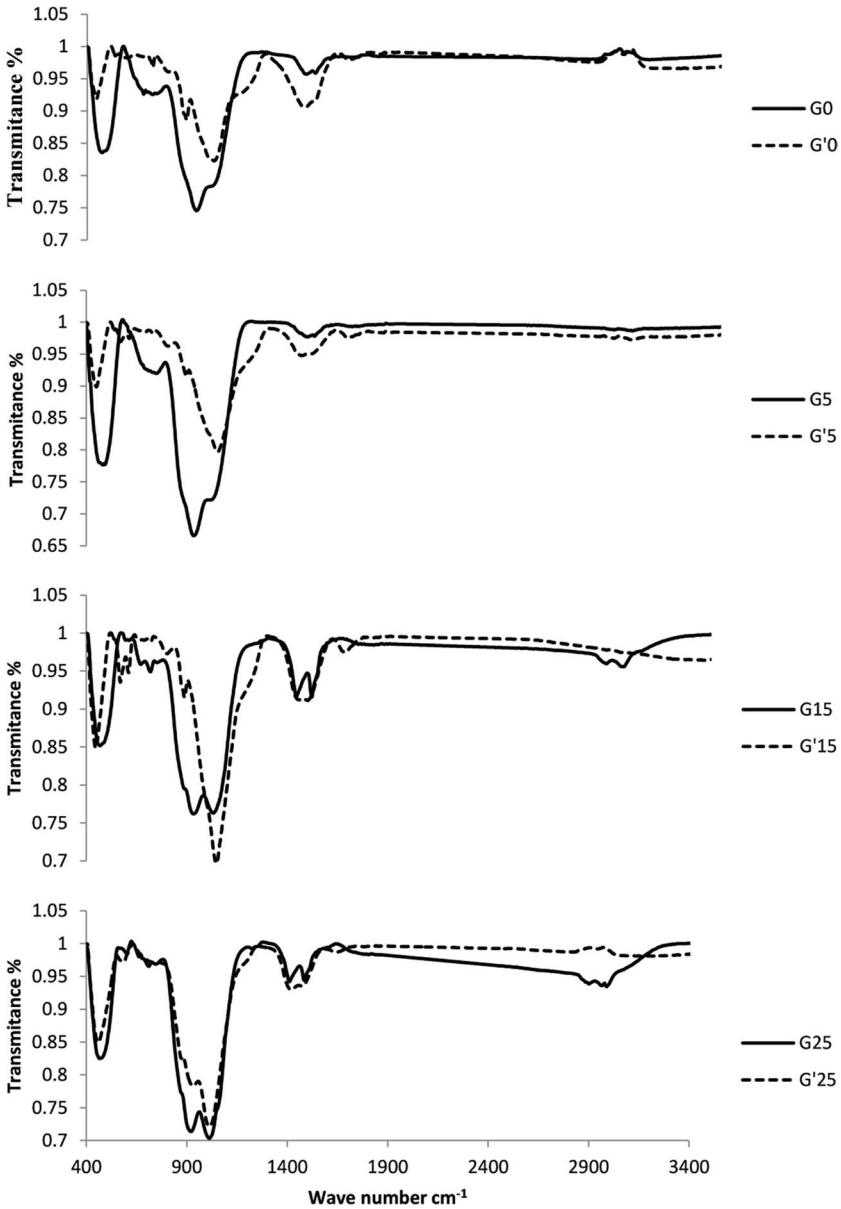

Fig. 1 FTIR transmittance spectra of the prepared glass batches before (G0, G5, G15 and G25) and after immersion in distilled water for 6 months (G'0, G'5, G'15 and G'25)

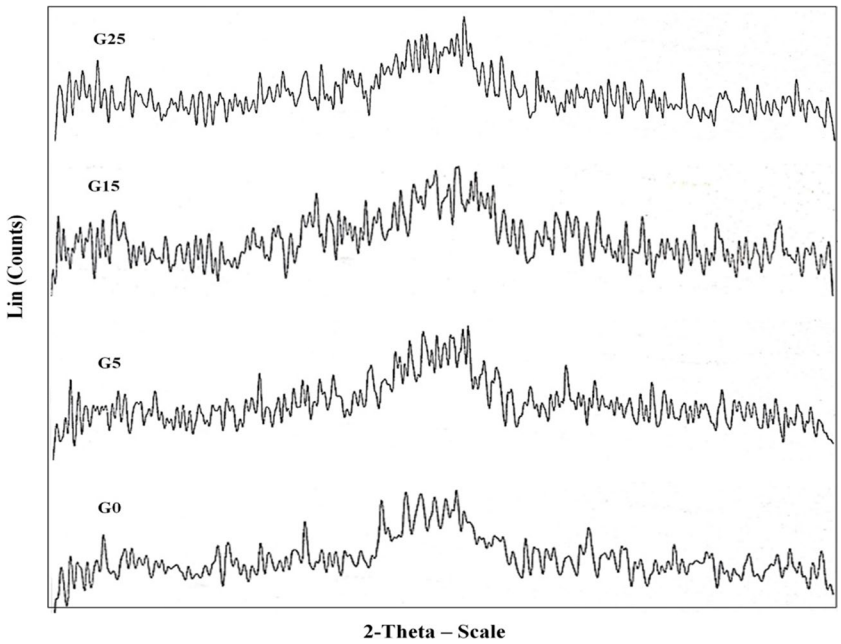

Fig. 2 XRD results of the prepared glass batches (G0, G5, G15 and G25)

bottles for (1 hour, 2 hours, 4 hours, 8 hours, 24 hours, 48 hours, 5 days, 7 days, 14 days, 28 days, 56 and 90 days). Artificial saliva was collected (10 mL) and replenished by fresh saliva at the end of each immersion time. The pH of the collected solutions was inspected using pH meter (Jenway 3505, Bibby Scientific Limited, UK), and the concentrations Ca, and P ions released were measured using inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Agilent 5100 Synchronous Vertical Dual View (SVDV).

2.5 Statistical Analysis

The data was presented as mean and standard deviation (SD). At first, the data was explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests where the significance level was set at $P \leq 0.05$. Afterwards, Kruskal Wallis test was used to compare between groups followed by Mann Whitney U Post hoc test for pairwise comparison. Statistical analysis was performed with IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp.

3 Results and Discussion

The purpose of this study was to prepare a new light curable pit and fissure sealant through loading it with a novel bioactive glass with remineralizing ability and proper mechanical and physical properties.

3.1 FTIR Results Analysis of the Prepared Glass Samples

For each glass batch, FTIR transmission spectra of the as prepared and soaked glass powder for 6 months in distilled water are shown in Fig. 1. In the FTIR spectra, the main transmittance band are in the 400–1500 cm^{-1} range, centered at ≈ 900 cm^{-1}. The distinct sharp peaks within the mid region for glass samples before soaking are at 470-478, 710-723, 908-918 and 1439-1443 cm^{-1} for G0 and G5 respectively. New peaks started to appear at 1010 and 1483 for G15 and G25 samples. After soaking in distilled water

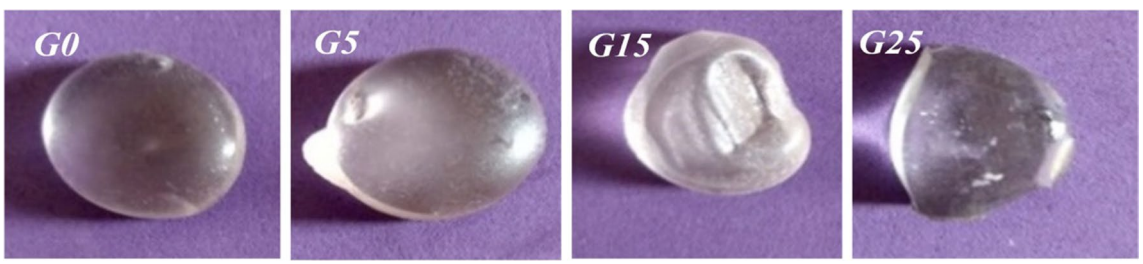

Fig. 3 Photographs for the prepared cast glass samples (G0, G5, G15 and G25)

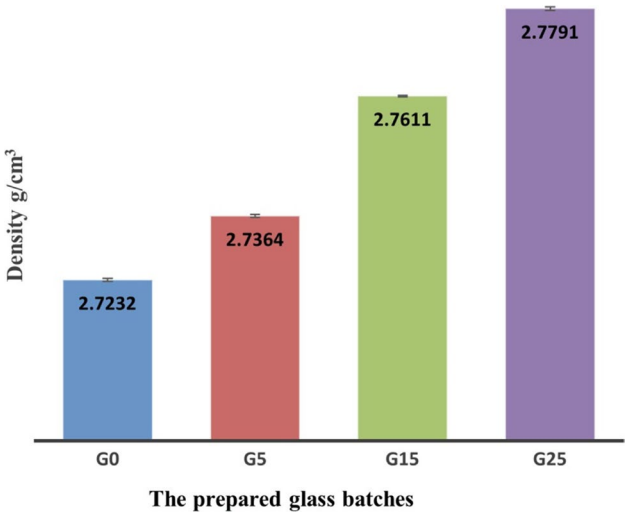

Fig. 4 Bar chart showing the mean density measurements of the four tested glass batches (G0, G5, G15 and G25)

for 6 months, there were slight shift in the peak position and intensity at bands from 470 to 1000 cm^{-1}, indicating hydrolysis and dissolution of the existing structural building units of all the bioactive glass samples.

3.2 XRD Analysis Results

The XRD results of the prepared glass batches are shown in Fig. 2. XRD results of all the tested groups didn't show the characteristic peaks of crystallization, indicating the amorphous nature of all the prepared glass batches.

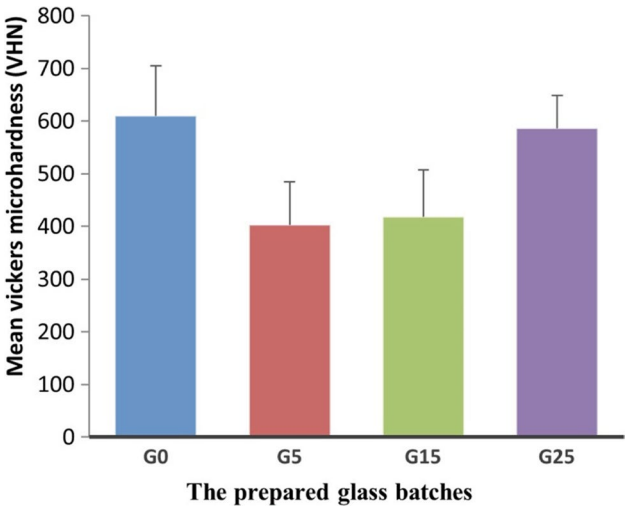

Fig. 5 Bar chart showing the mean microhardness measurement of the four prepared glass batches

3.3 Density Results of the Prepared Glass Batches

The cast glass samples used to inspect density and hardness of the prepared samples are shown in Fig. 3. Density results shown in Fig. 4 revealed statistical significant differences in density values between the tested glass batches (p value <0.001). A uniform increase in the density values was detected by the increase in fluorapatite content, from G0 to G25 glass.

3.4 Microhardness Results of the Prepared Glass Batches

Results of micro hardness testing shown in Fig. 5 revealed statistical insignificant differences in Vickers hardness values between G0 and G25 and between G5 and G15 (p value <0.001). On the other hand a statistical significant difference was found between G0, G25 and G5, G15 (p value <0.001).

3.5 In Vitro Bioactivity Results of Glass Batches

Silica-based bioactive glasses characterized by their ability to undergo a partial dissolution, initiating the formation of a silica rich layer, followed by the precipitation of amorphous calcium phosphate which then crystallizes in hydroxycarbonate apatite layer (HCA). The bioactive behavior of glasses and their apatite forming ability on their surfaces are usually done by soaking in simulated body fluids solutions since it contains a similar ionic concentration to human blood plasma. However in this study the bioactivity of the tested glass was evaluated by soaking the glass particles in artificial saliva fluid (pH =6.75), mimicking the conditions surrounding the pit and fissure sealant in the oral cavity. Different time intervals was tested for pH changes and ion

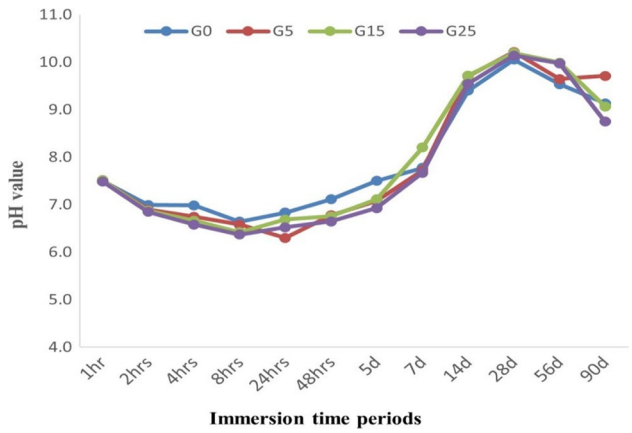

Fig. 6 Line chart showing the mean pH changes after immersion of four glass batches at different time intervals

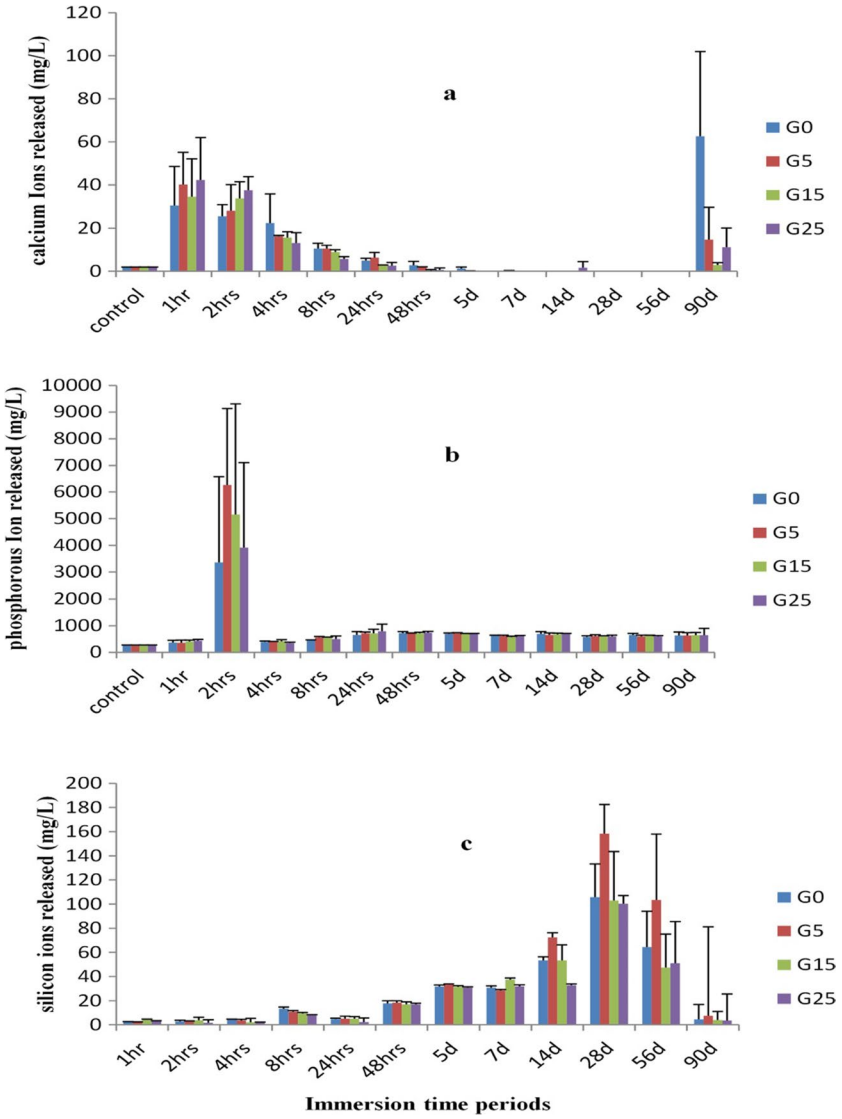

Fig. 7 Bar chart showing; **a** mean calcium ion, **b** mean phosphorus ion and (**c**) mean silicon ion, released from the four glass batches at different immersion time periods

release testing to detect initial and extended bioactive behavior of the tested glass.

3.5.1 pH Changes Result of the Artificial Saliva after Immersion of the Four Glass Batches at Different Time Intervals

Periodic pH inspection of the artificial saliva after immersion of the four glass batches at the predetermined time intervals are shown in Fig. 6. Kruskal Wallis test was used to compare between groups followed by Mann Whitney U Post hoc test for pairwise comparison. For all the tested glass batches; the higher pH values were observed after 14, 28 and 56 days immersion time (≈ 9.4-10.2) indicating higher glass dissolution rate and denoting the ion exchange between the cations from the glass and the protons of the solution, which is significantly affected by the dissolution rate of the glass. A decrease in pH values (≈ 8.7-9.7) was detected after 90 days immersion time indicating a decrease in glass dissolution rate by time.

3.5.2 Ion Release Results of Glass Batches

The amount of Ca, P and Si ions leached into the artificial saliva at the predetermined time intervals are shown in Fig. 7a, b and c respectively. Kruskal Wallis test was used to compare between groups followed by Mann Whitney U Post hoc test for pairwise comparison. For all glass batches a statistical significant differences was revealed regarding Ca ion release between each immersion time period (P value = 0.001). Ca ions showed increased release at the first two hours, and then it gradually decreased until almost disappeared after two days of immersion. An increase in Ca ion release was detected after 90 days of immersion time. As regards P ion release, a sudden increase was detected after 2 hours immersion

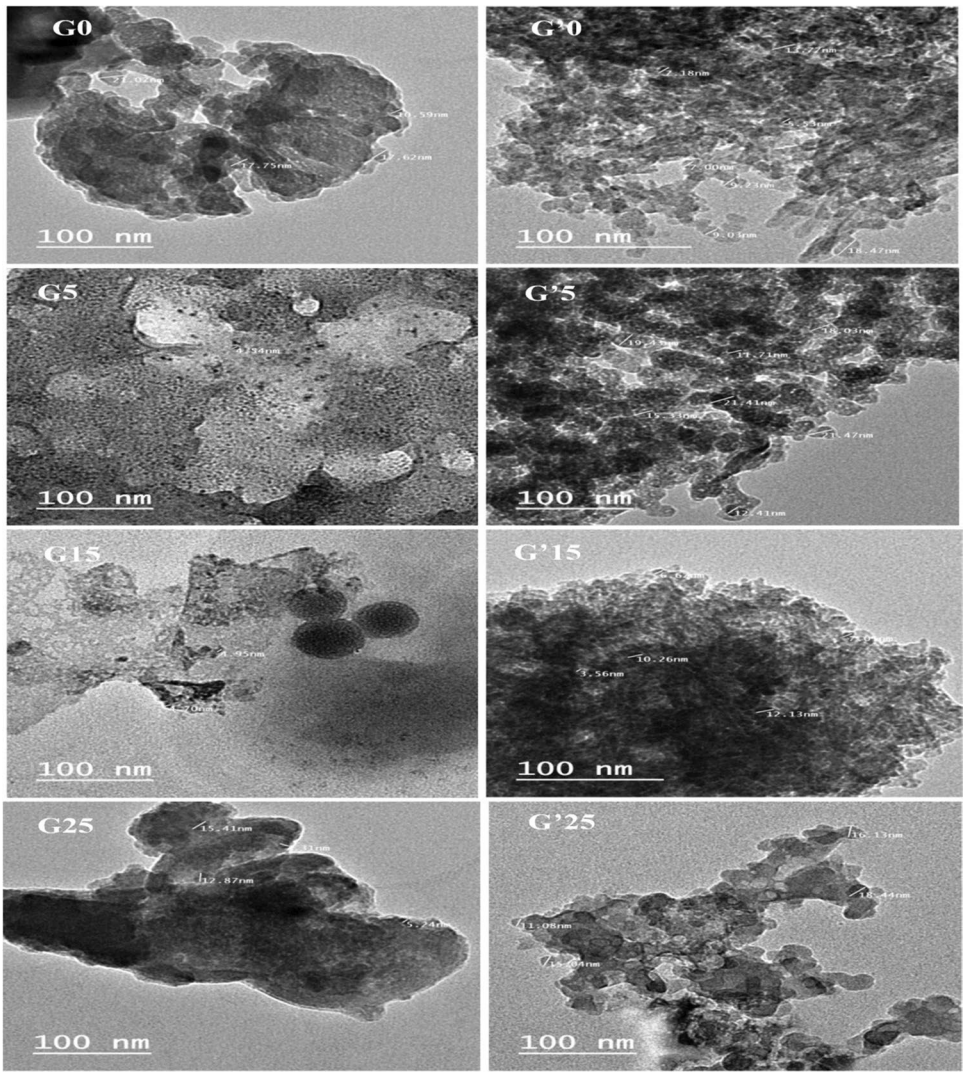

Fig. 8 TEM images of glass batches (G0, G5, G15, G25 and G'0, G'5, G'15, G'25) before and after 3 months immersion in artificial saliva respectively

time followed by a decrease steady release through all the investigated immersion time periods.

However, Si ions showed a delayed release during the first 24 hours, then a gradual increase started reaching the peak after 28 days where a decrease in release was detected again till the end of the 90 days immersion periods.

3.5.3 TEM Results of Glass Batches before and after 3 Months Immersion in Artificial Saliva

TEM images of the tested glass batches before and after immersion in artificial saliva are shown in Fig. 8. Images revealed that the glass particles were in the range of ≈ 9 nm to 65 nm before immersion and became obviously smaller and more distributed (≈ 3-20 nm), after immersion in artificial saliva for 3 months confirming the dissolution behavior of the glass particles.

3.5.4 SEM and EDAX Analysis Results of Glass Batches

SEM images of the inspected glass powders at 40000× magnification before and after immersion in artificial saliva are shown in Fig. 9. SEM images of the glass powders after immersion showed the characteristic flower shape of the apatite needle crystals in groups (G'0 and G'15). G'5 group showed spherical apatite crystals while G'25 revealed a scattered less dense precipitates indicating the bioactive nature of all the prepared glass groups.

Though the utilized bioactive glasses in the prepared groups do not contain phosphorus ions in their composition, yet during their contact with saliva (which contains phosphorus ions) hydrolysis of silicates occurs resulting in the formation of a layer of hydroxyapatite on their surfaces.

These findings were confirmed by the results of the EDAX analysis as shown in Fig. 12 and Table 4. As shown in Fig. 10, the EDAX graphs of the glass batches

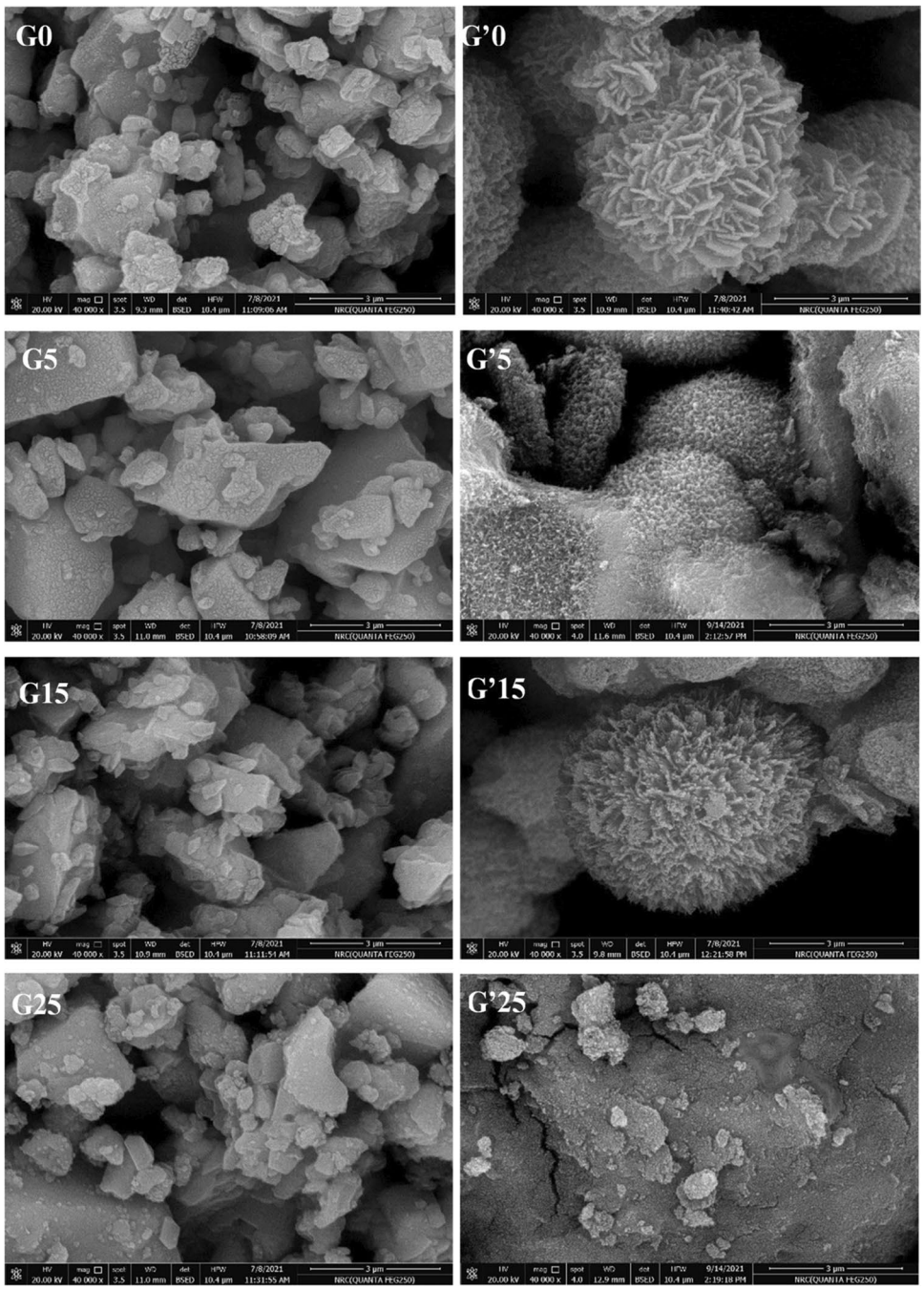

Fig. 9 SEM images of glass batches before (G0, G5, G15, G25) and after immersion for 9 months in artificial saliva (G'0, G'5, G'15, G'25) magnified at 40000×

before immersion in artificial saliva (G0,G5,G15,G25) revealed lower calcium and phosphorous content and higher silicon content than their consecutive immersed samples (G'0, G'5, G15, G'25). Additionally the calcium phosphate ratio for precipitates on G'0, G'15 and G'25 as presented in the EDAX results in Table 2 are optimal for hydroxyl apatite (1.67), confirming the formation of apatite on their surfaces.

Among the four glass batches, G15 showed the best trend regarding calcium, phosphorous and silicon ion release and appetite forming ability; therefore G15 was selected to be the bioactive filler for the preparation of the experimental pit and fissure sealant samples. To improve strength properties of the prepared sealant; clusters of silica nanoparticles were also added. Different weight percentages of bioactive glass and silica fillers were tested with total filler loading of 85 wt%. Since loading of properly bonded filler to resin matrix affects positively the material properties; hence, glass and silica particles were salinized in the present investigation to ensure bonding to resin matrix.

Table 4 Weight% of calcium and phosphorous and Ca:P ratio as presented in the EDAX results before and after immersion in artificial saliva

Sample name	Ca wt%	P wt%	Ca: P ratio
G0	19.34	–	–
G'0	19.63	12.51	1.569
G5	21.5	2.34	9.188
G'5	31.54	10.98	2.872
G15	22.37	3.64	6.145
G'15	22.06	14.01	1.574
G25	22.24	5.02	4.430
G'25	14.46	9.38	1.541

3.6 Results of Characterization of the Pit and Fissure Sealants

The survival of sealant is directly related to their ability to withstand the harsh oral environment including fluctuation of temperature and pH together with occlusal forces, water sorption, and polymerization shrinkage stresses etc. All these factors affect their capability to be retained in the occlusal pits and fissures.

In the present study two different types of pit and fissure sealant were selected as commercial control sealants; a resin based, light curing, highly flowable type (i-SEAL LC pit and fissure sealant) and a light-curing Compomer type (Composan glass). Composan glass commercial sealant combines the advantages of glass ionomer cements and composites with fluoride and glass filler content, while i-SEAL LC pit and fissure sealant is a resin based sealer containing 30-50% non-bioactive glass fillers. These commercial sealants were used to assess the physical properties and ion release characteristics of the prepared sealants.

The mean values and standard deviations of flow, depth of cure, Vickers hardness number and compressive strength results of all tested samples and the commercial sealant are listed in Table 5.

3.6.1 Flow Test Results

The ability of the sealant material to flow by itself in small cavities and fissures; helps in better application of the material clinically. According to Sébastien Beun et al. [28] the type of filler and surface treatment used affect the rheological behavior of the flowable resin materials than the amount of the filler loading. Such finding is in agreement with the results obtained in the present investigation where the flow results were significantly affected by the type of filler used. Decreasing the amount of glass filler significantly increased the mean flow values where sample (A) with 85 wt% glass filler revealed the lowest mean flow value when compared with other experimental samples. Results also revealed that the addition of nano silica particles in sample (D) significantly decreased the flow values when compared with sample (B and C). Statistical significant differences were found between all the tested materials. i-SEAL LC pit and fissure sealant type showed the highest mean disc diameter value indicating high flow properties, while the Composan glass type showed the lowest disc diameter value.

3.6.2 Depth of Cure Results

Depth of cure describes the extent of cure of a light polymerized sealant which probably will affect material properties, both in the bulk and at the interface. It also will affect their retention to etched enamel, hence affecting the success and longevity of the sealant. According to ANSI/ADA specification 39 for pit-and-fissure sealants the depth of cure of 0.75-mm is required [29]. While according to ISO 6874:2005, depth of cure requirements of dental sealant should be not less than 1.5 mm [26]. Clinically the thickness of dental sealant usually lay within this range; however, anatomical variation may result in thicker layer of sealant more than 0.75 or 1.5 mm.

The tested sealant in this study showed a statistical insignificant difference between all the tested groups. All the tested sealants met the ANSI/ADA specification 39 [29] and ISO 6874:2005 [26], depth of cure requirements of dental sealant.

3.6.3 Microhardness Results

Surface microhardness together with other strength properties provides insight into the ability of the sealant to resist scratching or penetration by external stresses. Concerning the VHN results; Composan glass revealed a significantly higher VHN values when compared to other tested groups. A statistical significant difference was found between all the experimental groups with higher significant VHN mean value was found for sample D (filled with 65 wt% bioactive glass, 10 wt% sintered silica nanoparticles and 10 wt% nanosilica). Sample D revealed higher significant mean VHN value than the commercial i-SEAL LC pit and fissure sealant. Results also revealed that the addition of sintered silica nanoparticles together with silica nanoparticles significantly improved the hardness value of the tested materials. Sample A filled with 85 wt% bioactive glass only; revealed the significantly lower VHN mean values when compared with other experimental samples. The results of this study are in consistent with another study which also reported that a decrease in strength properties was detected with increasing proportion of the added bioactive glass [30].

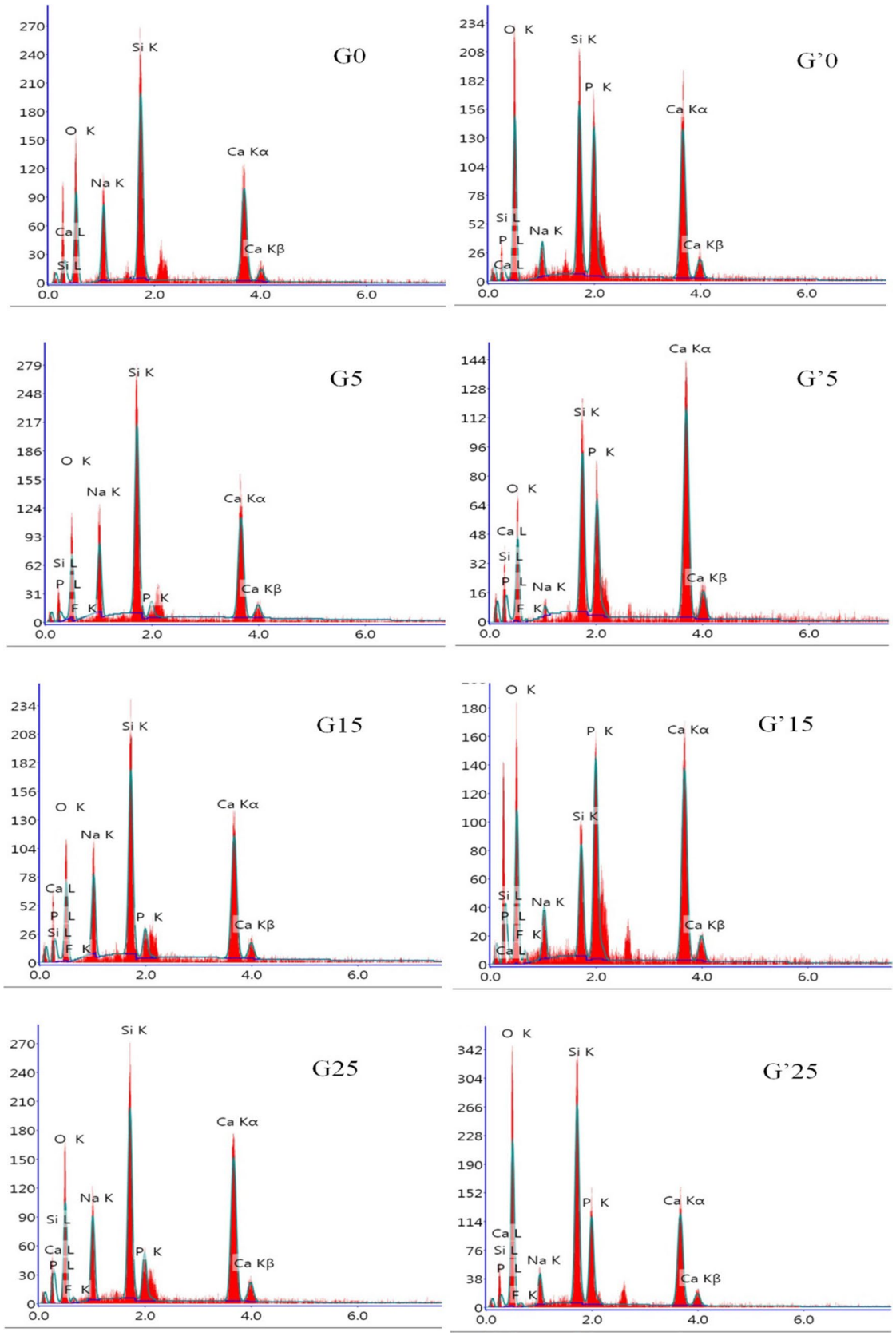

Fig. 10 EDAX graph of the prepared glass powder before (G0, G5, G15, G25) and after (G'0, G'5, G15, G'25) immersion in artificial saliva

Table 5 Mean values and standard deviations of flow, Depth of cure, Vickers microhardness and compressive strength of the tested sealant groups

Testing parameters Material type	Flow (mm)	Depth of cure (mm)	Vickers micro-hardness (VHN)	Compressive strength (MPa)
Composan glass	9.4 ^{a} ± 0.07906	2.98^{a} ± 0.02	64.05 ^{e} ± 3.41	255.85^{a} ± 27.84
i-SEAL LC pit and fissure sealant	19.3 ^{f} ± 0.03536	2.98^{a} ± 0.02	13.93 ^{b} ± 1.45	206.42^{b} ± 29.64
Sample A	14.5 ^{b} ± 0.0790569	2.97^{a} ± 0.01	11.40 ^{a} ± 0.39	145.11^{cd} ± 40.99
Sample B	16.2 ^{c} ± 0.07071	2.96^{a} ± 0.02	18.81 ^{c} ± 1.39	94.51^{e} ± 11.03
Sample C	18.4 ^{e} ± 0.05	2.98^{a} ± 0.02	16.01 ^{b} ± 1.39	114.11^{de} ± 21.12
Sample D	17.6 ^{d} ± 0.03536	2.97^{a} ± 0.02	22.88 ^{d} ± 1.15	157.02^{c} ± 30.99
p value	<0.001*	0.854 NS	<0.001*	<0.001*

NS Non-significant, Different letters within each column indicates significant difference

3.6.4 Compressive Strength Results

The mechanical properties of pit and fissure sealant is probably affected not only by the filler loading, particle size, distribution and filler type, but also on coupling between particles and matrix. Studies postulated that the stronger effect on the mechanical properties is due to the filler type and its fraction than the percentage of the filler. Such finding is in agreement with the results obtained in the present study, where Composan glass sealant had the lowest filler content 78 wt% compared to the experimental samples with total filler loadings of 85 wt%, however, Composan glass showed the highest mean compressive strength value. This may be attributed to the aggregation of filler particles at higher concentrations leading to defects that deteriorates the mechanical properties of the material.

Statistical significant differences in compressive strength mean values were found between all the tested groups. The two commercial sealants showed the highest mean values, while sample D revealed the highest compressive strength value when compared to that of the other experimental samples. According to Khvostenko et al. [31] hand mixing of bioactive glass impregnated resin based composite may result in incorporation of air voids causing a 30% decrease in strength properties.

In terms of the micro hardness, and compressive strength, the experimental samples performs less than the Composan glass sealant, which may be attributed to the development of not visually observed tiny bubbles produced during mixing of the filler and polymer matrix. Such bubbles act as stress concentrations area, reducing the mechanical properties of the experimental samples when compared with a professionally mixed and prepared commercial sealant [32].

3.7 pH Changes and Ion Release Results of the Tested Pit and Fissure Sealants

Due to the lack of bioactivity of i-SEAL LC pit and fissure sealant; only Composan glass sealant was used as a commercial control sealant regarding pH changes and ion release testing. Sample D showed the highest mean compressive and compressive strength properties compared with other experimental samples; therefore it was selected for further evaluations and testing.

3.7.1 pH Changes Results

Dental caries develop when bacteria in the oral cavity metabolize acid from fermentable carbohydrate, producing acids with significant drop in the pH value, causing demineralization of the hard tooth structure. Secondary caries around pit and fissure sealant produced due to either material loss or microleakage at tooth restoration interface [15].

Acid neutralizing composite is a strategy used to stop the development of dental caries. Regarding caries development a pH 6.0–5.5 is known as the theoretically cariogenic pH,

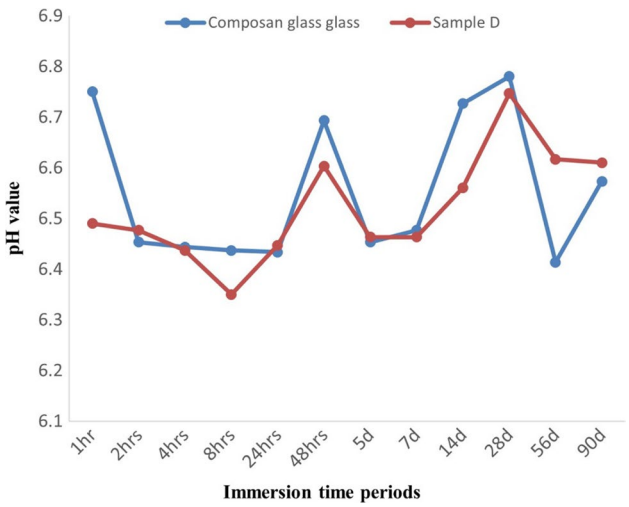

Fig. 11 A line chart showing the mean pH fluctuation for Composan glass and sample D at different immersion time periods

above 6.0 is the safe zone, and 5.5–4 is the danger cariogenic zone. For the pit and fissure sealant to inhibit caries it should be able to raise pH from 4.0 to pH of 5.5 or above.

A Line chart showing the mean pH fluctuation pattern for the Composan glass and sample D is shown in Fig. 11. No statistical significant difference was found in mean pH values between the two tested materials at all immersion time periods except for early pH change after 1 hour, where Composan glass showed a higher significant pH value of 6.75 (p value =0.0463) than that of sample D (6.49) (p value =0.0463). The mean pH values recorded for the two tested materials and during the tested immersion time periods is in the range of 6.75 to 6.35 with no statistical significant differences for Composan glass type results (p value =0.0081). Regarding pH changes in sample D a statistical significant decrease in pH value (6.35) was detected after 8 hours and a significant increase (6.75) (p value = 0.0049) after 28 days immersion time. This rise in pH values are probably due to the release of cations that originated from the alkaline glass filler.

3.7.2 Calcium and Phosphorous Ion Release Results from the Tested Sealant

The ability of resin based pit and fissure sealants to release active remineralizing ions in the surrounding enamel is highly recommended due to their known low retention to tooth structure. The released ions help to seal microgaps between restoration and tooth structure produced as a result of polymerization shrinkage stresses that may exceed the bond strength leading to debonding. Studies on bioactive resin based sealant confirmed that the release of multiple ions from restoration have an important role on increasing the self-repair ability and the long term durability against demineralization [33]. Hydroxyapatite, fluoride, tricalcium phosphate and β- tricalcium phosphate loaded composite resins capable of releasing fluoride, calcium and phosphate were investigated in previous studies [33].

In a systematic review done in 2022 on remineralizing effects of resin-based dental sealants it was found that most of the included studies (23 out of 39 studies) investigated

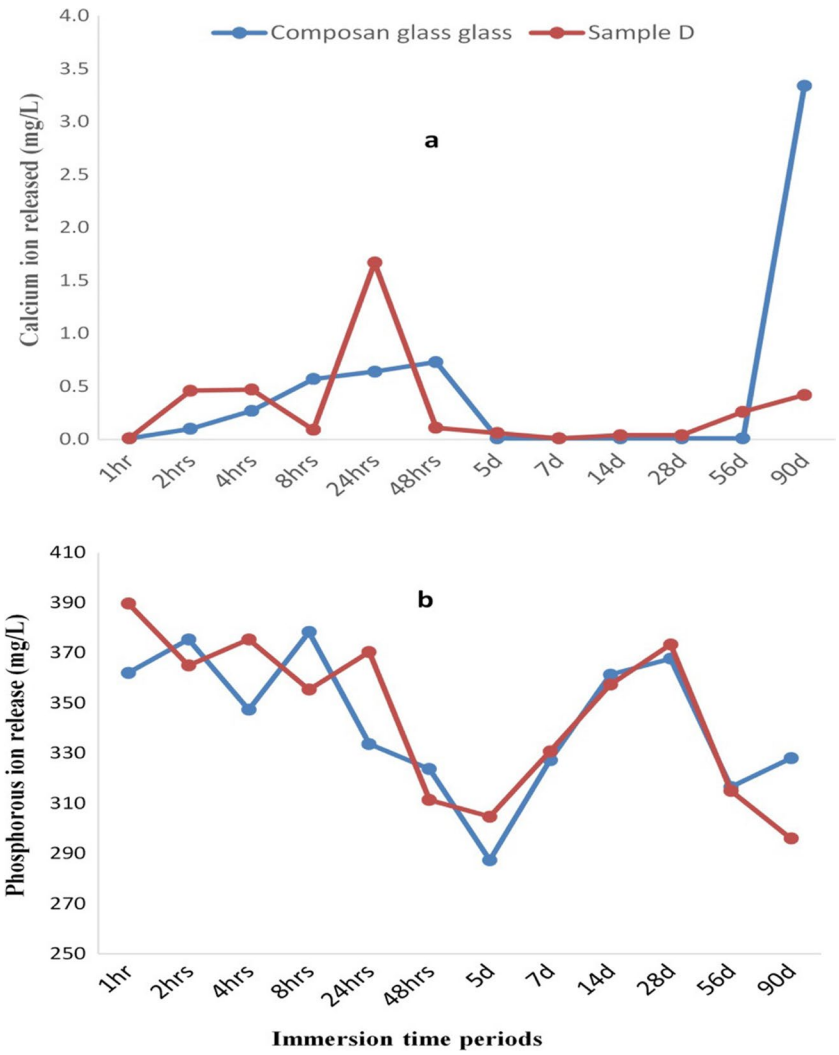

Fig. 12 Line chart showing; **a**: the mean calcium ions, **b**: mean phosphorus ions, released from sample D and Composan glass sealants after different immersion time periods

the release of F ion where a short term release of F ions (7–9 days) was detected [34]. Additionally few studies was done to assess Ca and P ion release where the release of these ions showed extended release up to more than 21–70 days. Therefore the aim of this investigation was to study the release of Ca and P ion release. The fluoride content in glass composition in the present investigation aimed to decrease the glass melting temperature.

Ca ion release results (mg/L) from sample D and Composan glass sealant during the tested periods are presented in Fig. 12a. Ca ion release pattern revealed a comparable statistical insignificant difference between Composan glass and sample D sealant during the tested time periods. Although after 24 hours, sample D showed a remarkably higher value (1.67 ± 1.81), than Composan glass (0.64 ± 1.09), yet, after 90 days, Composan glass showed a sudden increase in Ca ion release (3.34 ± 2.36) compared to Sample D (0.42 ± 0.08), which may indicate partial solubility of Composan glass.

The P ion release results (mg/L) from sample D and Composan glass sealants during the tested periods are presented in Fig. 12b. At each time interval, the tested materials released statistical insignificant amounts of P except after 90 days immersion time; where a statistical significant increase in P ions was released from Composan glass (328.00 ± 4.00) compared to that of sample D (296.00 ± 9.00) (P value $= 0.0495$).

4 Conclusions

In this study, the 85 wt% calcium silicate and sodium silicate with 15 wt% fluoroapatite had the best trend regarding calcium, phosphorous and silicon ion release and appetite forming ability. Pit and fissure sealant loaded with 65 wt% prepared bioactive glass, 10% sintered nanosilica had the best acceptable mechanical properties. The novel sealant showed a favorable pH values and extended Ca and P ion release. Therefore, it was concluded that the novel pit and fissure sealant is a promising bioactive and ion releasing material. Further attempts to improve compressive strength are required.

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Data Availability The data and materials that have been used in this work is not available to be shared, they are confidential data.

Declarations

Ethics Approval and Consent to Participate Not applicable.

Consent for Publication Not applicable.

Competing Interests The authors declare that they have no conflict of interest.

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