



Sol-gel derived fluoride-doped bioactive glass powders: Structural and long-term fluoride release/pH analysis

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ABSTRACT

The study aimed to compare the fluoride release behavior of newly synthesized bioactive glass powders and its effect on the pH. The bioactive glass (BG) and three groups of fluoride-doped (2.5, 5 and 7.5 mol%) bioactive glasses (F-BG) powders were synthesized by a base-catalyzed sol-gel method. The prepared samples were heat-treated and were characterized by Fourier Transform Infrared Spectroscopy, X-ray Diffraction, Scanning Electron Microscopy, and Brunauer–Emmett–Teller (BET) surface area analyzer. The prepared heat-treated F-BG samples were amorphous in nature, except F-BAG with 2.5 mol% fluoride which was glass-ceramic with few characteristic peaks of fluorapatite and tetracalcium phosphate phases. High surface area of these prepared powders were observed, however, it was decreased with the increase in fluoride contents and particle size. A 6 month long fluoride release behavior was studied periodically in deionized water using an Ion Selective Electrode. Fluoride release was maximum with F-BG (7.5% mol%), however, the insignificant difference was observed among samples. Initially, high pH value was exhibited by all samples, where highest pH was observed with BG and least with F-BAG (5 mol%). The resulting F-BG (5% mol%) has potential to be used in dentifrices, restorative materials and for other dental applications.

1. Introduction

Currently, there is an emerging trend of developing “smart” ion-releasing bioactive dental restorative materials with therapeutic effects to re-mineralize the demineralized dentin [1, 2]. Bioceramics such as amorphous calcium phosphate (CaP), hydroxyapatite (HA) and bioactive glass (BG) initially explored in the field of biomedical materials as synthetic bone grafting materials [3, 4]. Later they were found to be beneficial in cases of hypersensitive dentin [3, 5–7] and as a reinforcing agent in dental restorative materials [8–10] to minimize the incidence of secondary decay. Among these, BG has gained special attention not only in research but it is also under focus in clinical dentistry. Since fluoride is popular in dentistry for controlling tooth decay due to its anti-bacterial and re-mineralizing nature. It was believed that marrying the two into one may synergize the re-mineralizing and anti-microbial effects in fluoride-containing bioactive glasses (F-BGs) [11–13].

It is established that upon exposure to physiological fluids, ion exchange processes start at the interface between the surface of BG particles and the surrounding fluid resulting in the development of silica

gel layer on the exterior of BG particles [14–16]. BG exhibits greater alkalinity (pH \geq 9) in aqueous media, which can prove hazardous to the oral soft tissues [14]. To decrease alkalinity of the BG, various attempts were made to modify its composition. One such successful attempt was the addition of fluoride, which displayed buffering action to some extent by fluoride release in physiological solutions [17, 18]. The fluoride release of up to 20 ppm exhibits direct bactericidal action, however, with aging, the amount drops, therefore the direct bactericidal effect of fluoride is irrelevant clinically [19]. Enamel remineralization is directly proportional to the amount of fluoride ions release from restorative materials, thus, a composite releasing 200–300 $\mu\text{g}/\text{cm}^2$ fluoride over a 1-month period would completely inhibit recurrent caries [20].

Prof. Hench in 1969 first developed “Bioglass®” by a melt-quench route which displayed greater loss of CaF by reaction with humidity [21, 22]. Later sol-gel systems were utilized extensively to produce BGs with less crystallinity, greater porosity and surface area with improved bioactivity [12, 22–24]. F-BGs form less soluble crystalline fluorapatite, therefore, is of greater interest in dentistry. By increasing the CaF

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content, improved network connectivity in BGs has been observed as fluorine is coordinated with calcium ions which may lower their chemical reactivity [12].

Previously, F-BGs were synthesized by a melt-quench derived method and reported seven day fluoride release in acetate-buffered MEM. It was found that low fluoride content bioactive glass formed fluorapatite well at low pH (i.e. < 6) [25]. In another week-long study, F-BG showed a linear proportionality between fluoride release and fluoride concentrations [18]. It is reported in fourteen days study that low fluoride content (i.e. 0.97 and 4.17 mol%) melt-derived BGs showed increased fluoride release upon aging, whereas, higher fluoride content BG showed less pronounced pH rise [21]. Still much work is needed to explore the potentials of fluoride-containing BGs specifically for the prolonged period of time.

The aim of the study was to measure and compare the amount of fluoride release from the experimental F-BG powders synthesized by co-precipitation method (i.e. sol-gel) and to evaluate the effect of various concentrations on pH for six months. The hypothesis was that the fluoride release rate of the experimental powders decreases upon aging; however, the pH of the conditioning bath remained constant and exhibit no relation to the amount of fluoride release.

2. Materials and methods

2.1. Materials

The analytical grade chemicals used in the synthesis of F-BGs included tetraethylorthosilicate [TEOS, $(\text{Si}(\text{OC}_2\text{H}_5)_4)$, Merck], calcium hydroxide [$\text{Ca}(\text{OH})_2$, Acros Organics], ammonium hydroxide (NH_4OH , Merck), diammonium hydrogen phosphate [$(\text{NH}_4)_2\text{HPO}_4$, Sigma Aldrich], nitric acid (HNO_3 , Analar), ethanol (99.08%, Analar) and sodium fluoride (NaF, Sigma Aldrich).

2.2. Synthesis of bioactive glasses

Three different types of F-BG samples with compositions (mol.%) 46SiO_2 ; $28.5-x\text{CaO}$; $23\text{Na}_2\text{O}$; $2.5\text{P}_2\text{O}_5$; $x\text{NaF}$ (where $x = 2.5/5/7.5$) were synthesized using various concentrations (2.5, 5 and 7.5 mol%) of fluoride through co-precipitation method. Initially, TEOS (23.0 mmol) was dissolved in 24 mL ethanol and pH of the solution was adjusted up to 1–2 by using 1.0 N HNO_3 . Then $\text{Ca}(\text{OH})_2$ (14.0 mmol) and NaOH (11.5 mmol) solution were mixed in TEOS solution. Later $(\text{NH}_4)_2\text{HPO}_4$ (1.25 mmol) was dissolved in 400 mL deionized water and pH was maintained at 11 using ammonia solution. The mixture of TEOS, $\text{Ca}(\text{OH})_2$, NaOH, and NaF were added dropwise into this solution raising the final volume up to 600 mL. The final solution was stirred for 48 h. The prepared solution was filtered and washed with deionized water to neutralize the product and dried at 75°C for 24 h and calcined at 600°C for 6 h at the heating rate of 2°C min^{-1} . The mole percentage of all components for the synthesis of BG and F-BGs is given in Table 1. The prepared BG samples with 2.5, 5, and 7.5 mol% of fluoride were labeled as F-BG 2.5, F-BG 5, and F-BG 7.5 respectively.

Table 1

Mole percentages of bioactive glasses and fluoride substituted bioactive glass nanoparticles.

	Mole (%)				
	SiO_2	CaO	Na_2O	P_2O_5	NaF
BG	46	28.5	23	2.5	Nil
F-BG 2.5	46	26	23	2.5	2.5
F-BG 5	46	23.5	23	2.5	5
F-BG 7.5	46	21	23	2.5	7.5

2.3. Characterizations

2.3.1. Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) was used to analyze the functional groups present in BG and F-BG and the observed structural changes using Thermo Nicolet 6700 USA having Attenuated Total Reflectance (ATR) mode. The spectral range was $4000\text{--}550\text{ cm}^{-1}$ at the resolution of 8 cm^{-1} with 256 number of scans.

2.3.2. X-ray diffraction (XRD)

X-ray diffractometer evaluated the crystalline phases of BG and F-BGs using PANalytical XPERT-PRO with $\text{Cu K}\alpha$ radiation operated at 40 mA and 40 kV. An angle range of $2\theta \approx 10^\circ\text{--}60^\circ$ and a step size of 0.02° was used to investigate the sample.

2.3.3. Scanning Electron microscopy (SEM)

Surface morphology of F-BG was done using SEM (TESCAN Vega3 LMU). Prior to scan, the particles were sonicated for 15 min in ethanol solution to separate the nanoparticles. Samples were coated with gold for 90 s using a sputter coater (Quorum Technologies). To get SEM images of samples, an acceleration voltage of 20 kV with a beam intensity of 4 pA was used.

2.3.4. Brunauer–Emmett–Teller (BET) analysis

Brunauer–Emmett–Teller (BET) method was used to analyze the surface properties of three samples from each group using Micromeritics; New TriStar II Surface Area and Porosity system. Before analysis, degassing conditions at 150°C with $10^\circ\text{C min}^{-1}$ heating ramp was used for 3 h under nitrogen environment.

2.3.5. Fluoride release and pH analysis

For fluoride release analysis, 0.3 g of each sample ($n = 7$) was immersed in 10 mL deionized water and were allowed to condition in an incubator (Fisher Isotemp, Model 413D) set at 37°C . During conditioning, elutes were extracted periodically i.e. 1, 3, 9, 16, 24, 40, 60, 90, 120, 150, and 180 days. For elute collection, the samples were centrifuged (Hettich EBA 20) at 6000 rpm for 10 min. The pH of elutes was measured with pH/Ion selective electrode (ISE) meter (Hanna HI3222 pH/ISE meter). For fluoride release analysis, 1 mL TISAB III agent was added to each 10 mL elute sample and the analysis was carried out by using pH/ISE meter and Fluoride electrode.

The mean and standard deviation values were calculated. The statistical analysis was done by One-way repeated measures ANOVA and Post Hoc Tukey's test using SPSS version 22.

3. Results

3.1. Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of non-calcined and calcined BG and F-BG sintered at 600°C is given in Fig. 1(a–d). The broad band around $3600\text{--}3300\text{ cm}^{-1}$ and the peak at 1645 cm^{-1} showed the presence of molecular water in the form of intermolecular hydrogen bonded to hydroxyl (OH^-) group in non-calcined samples. Stretching asymmetric peak of carbonate appeared at 1454 cm^{-1} and the stretching absorption peak specific to Si-O-Si was observed at 1028 cm^{-1} [26]. A symmetric stretching peak at 969 cm^{-1} showed the presence of phosphate functional group. A peak at 871 cm^{-1} was attributed to an asymmetric bending peak of carbonate [27]. After calcination, the broad $-\text{OH}$ band ($3600\text{--}3300\text{ cm}^{-1}$) disappeared and intensity of carbonate peaks reduced. Whereas, the stretching peak of Si-O-Si became broader and overlapped the P-O peak and converted into a band, where P-O appeared as shoulder peak. The intensity and area of Si-O-Si spectral band were increased after calcination. The area of Si-O-Si spectral band before/after calcination of F-BG 2.5, F-BG 5, and F-BG 7.5 was 7.5/13.41, 15.6/20.04, and 21.02/25.3 respectively.

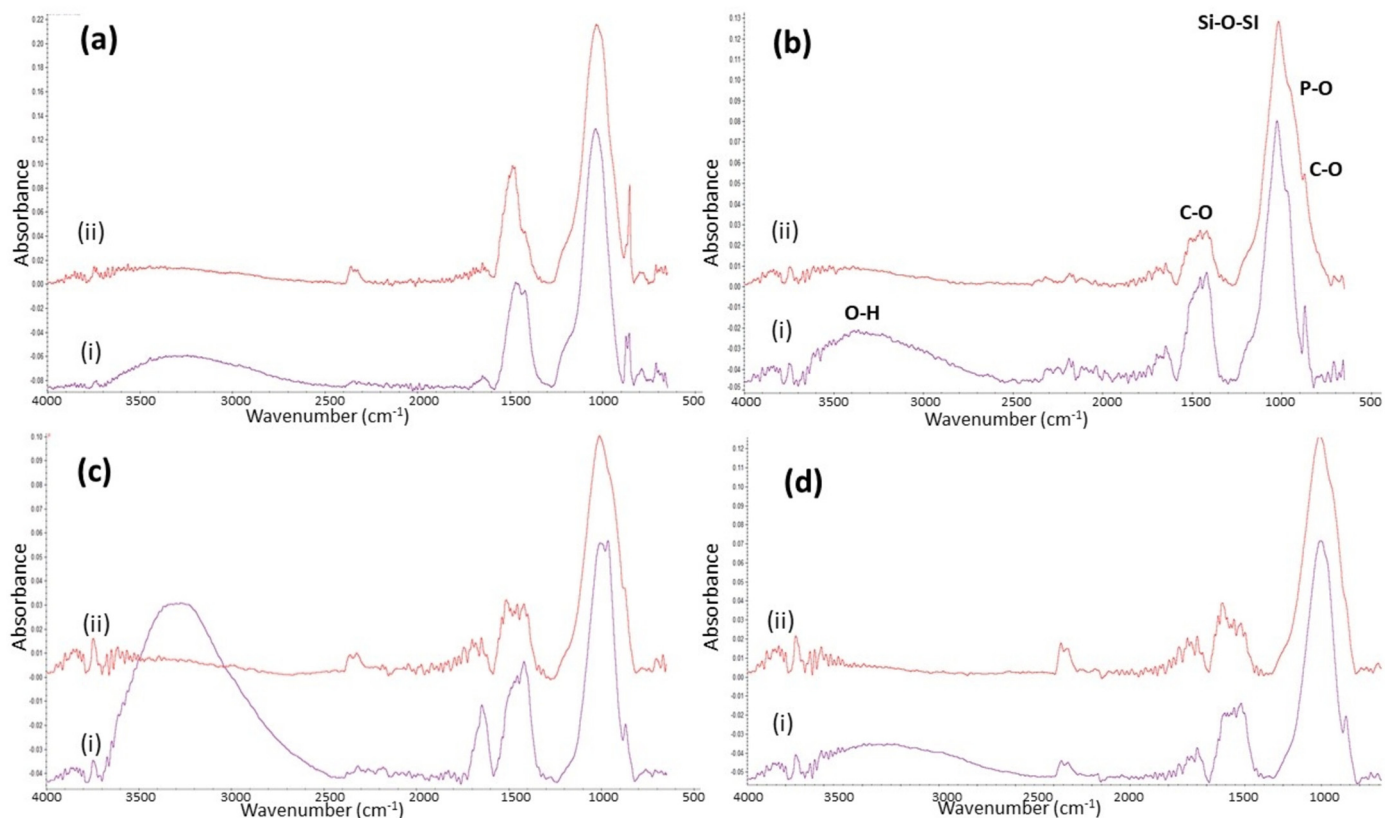


Fig. 1. Comparative FTIR spectra (a) BG, (b) F-BG 2.5, (c) F-BG 5, and (d) F-BG 7.5. The image showing the (i) uncalcined and (ii) calcined spectra.

3.2. X-ray diffraction (XRD)

The phase purity and structure of non-calcined and calcined samples were investigated through XRD analysis and diffraction pattern are shown in Fig. 2. Both non-calcined and calcined F-BG 2.5 were glass-ceramic in nature with characteristic peaks of tetracalcium phosphate phase (PDF# 70-1379) with 2θ values 25.34° (d-spacing 3.51 \AA), 29.79° (d-spacing 2.99 \AA), 32.82° (d-spacing 2.99 \AA), 36.2° (d-spacing 2.47 \AA) [26] and fluorapatite phase (PDF# 015-0876) with 2θ values 31.9° (d-spacing 2.8 \AA), 32.3° (d-spacing 2.7°) and 49.5° (d-spacing 1.8 \AA) [28]. All other samples were almost amorphous/glassy in nature

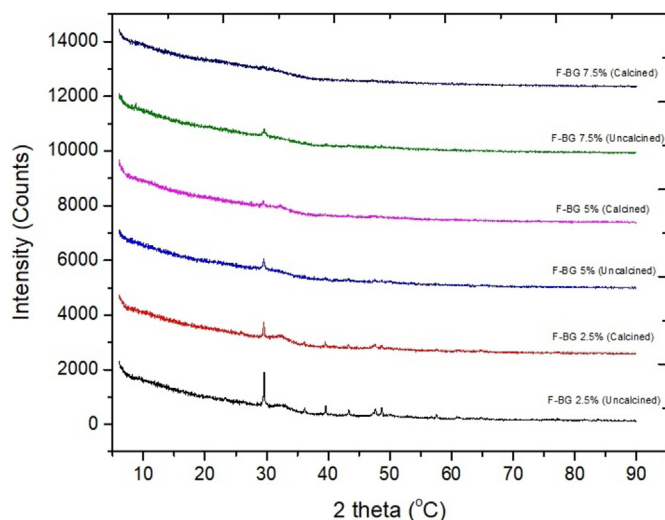


Fig. 2. Comparative X-ray Diffractogram of the calcined and uncalcined fluoride doped bioactive glasses (i.e. F-BG 2.5%, F-BG 5%, and F-BG 7.5%).

with a small peak at 2θ value 29.7 was appeared in uncalcined samples, which might be CaCO_3 (JCPDS data file No.001-0482), and disappeared after heat treatment [29].

3.3. Scanning electron microscope (SEM)

SEM images of non-calcined F-BG (Fig. 3) showed the presence of nano-particles ($30\text{--}50 \text{ nm}$) which were aggregated at some places. The size of particles was increased with the increase in mol.% of fluoride. The degree of agglomeration increased after calcination, however, no crystallization was observed, as also discussed in XRD results.

3.4. Brunauer–Emmett–Teller (BET) analysis

Surface properties of samples were examined using BET analysis and the surface area of F-BG 2.5, F-BG 5, and F-BG 7.5 was $76.0 \text{ m}^2 \text{ g}^{-1}$, $64.3 \text{ m}^2 \text{ g}^{-1}$, and $61.4 \text{ m}^2 \text{ g}^{-1}$ respectively. It was observed that surface area was decreased with the increase in fluoride contents and particle size. The average pore volume for all sample was $0.1 \text{ cm}^3/\text{g}$ and pore size for F-BG 2.5, F-BG 5, and F-BG 7.5 was 6.3 nm , 8.0 nm , and 8.2 nm respectively.

3.5. Fluoride Release Analysis

Maximum fluoride release was initially exhibited by F-BG 5 ($1.55 \pm 0.04 \text{ ppm}$) followed by F-BG 2.5 ($1.48 \pm 0.03 \text{ ppm}$) and F-BG 7.5 ($1.42 \pm 0.03 \text{ ppm}$). The mean daily fluoride release values of all groups of the experimental BG powders after periodic intervals are plotted against time in Fig. 4a. Similarly, the cumulative fluoride ions released upon aging in deionized water for all experimental powders were calculated and plotted against time as given in Fig. 4b. The highest cumulative fluoride release (ppm) during 180 days was: F-BG 7.5 ($17.07 \pm 0.4 \text{ ppm}$) > F-BG 5 ($16.99 \pm 0.17 \text{ ppm}$) > F-BG 2.5

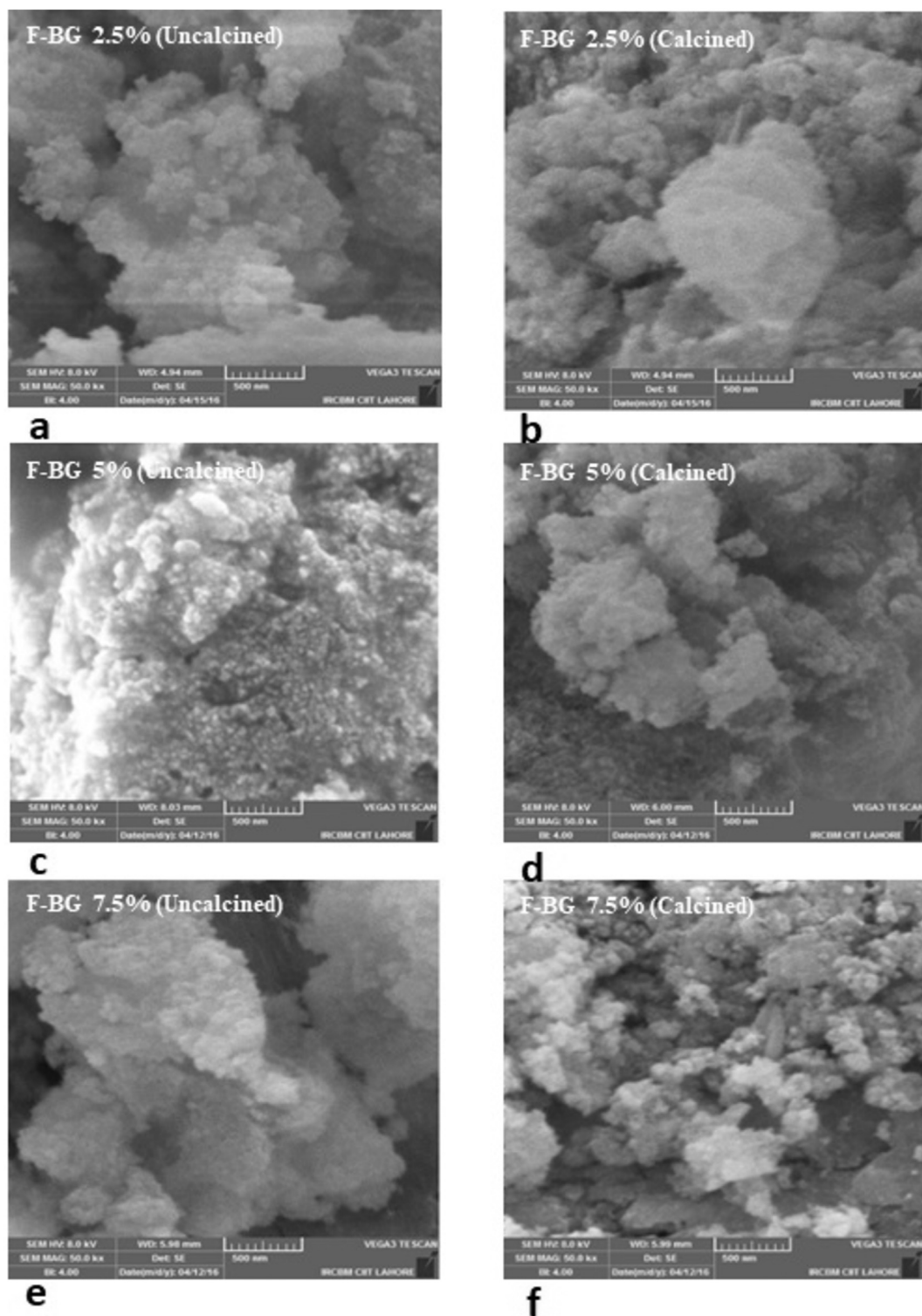


Fig. 3. SEM images showing surface morphology of (a) uncalcined F-BG 2.5%, (b) calcined F-BG 2.5%, (c) uncalcined F-BG 5%, (d) calcined F-BG 5%, (e) uncalcined F-BG 7.5%, and (f) calcined F-BG 7.5%.

(15.86 ± 0.63 ppm).

The ANOVA displayed a statistically significant difference in the cumulative fluoride release values over the period of observation within each study group ($p \leq 0.0001$). However, a statistically insignificant difference in cumulative fluoride release was detected between the three fluoride containing study groups ($p \leq 0.005$). The significant difference was observed due to the inclusion of the fluoride-free control group in the analysis, which had no fluoride release.

3.6. pH analysis

Initially, highest pH was exhibited by BG, the control group (9.42 ± 0.02) followed by F-BG 7.5 (8.28 ± 0.02), F-BG 2.5 (8.07 ± 0.03), and F-BG 5 (8.01 ± 0.08). For all the study groups, the mean daily pH values of the collected elutes were plotted against time in Fig. 5. In the present study, BG, the fluoride-free control group showed the highest pH throughout the study which ranged between

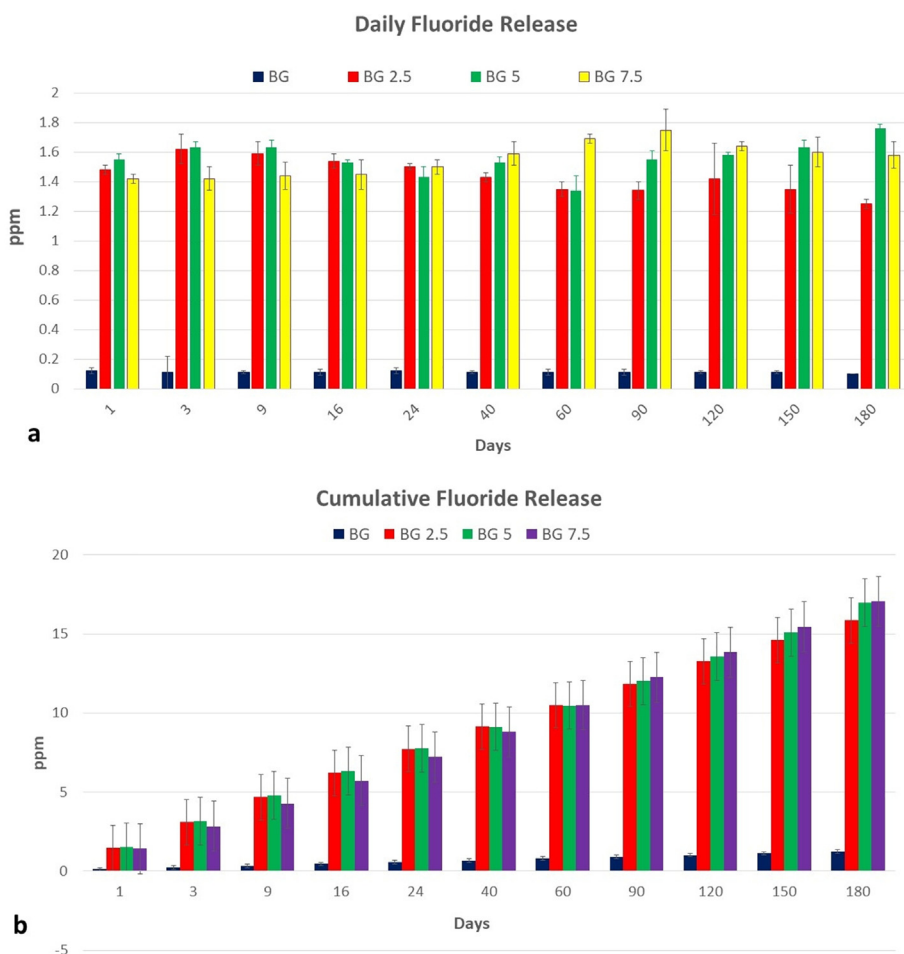


Fig. 4. (a) Pattern of daily fluoride release and (b) cumulative fluoride release of the experimental bioactive glass powders.

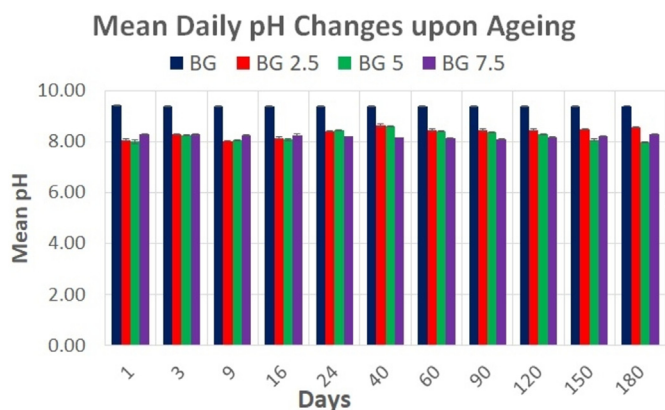


Fig. 5. Pattern of Daily pH of the Elutes of the Experimental Bioactive Glass Powders.

9.42 ± 0.02 to 9.38 ± 0.01. Tests within subject effects displayed a statistically significant difference in elute's pH values over observation period within each group (p ≤ 0.0001). The pair-wise comparison by Post hoc Tukey test showed a statistically significant difference in the daily pH values between F-BG 5 and F-BG 7.5 only. However, an insignificant pair-wise difference in the daily pH values was observed between all the other study groups.

4. Discussion

The study confirmed the synthesis of fluoride-containing bioactive glass. It is anticipated that fluoride ions may be fused into the glass as calcium fluoride either by keeping the persistent ratios of other constituents or by partial substitution of the oxides which are used as glass network modifiers [30]. In this study, various concentrations of fluoride were incorporated successfully in BG network. It was observed that varying the mol.% of fluoride from 2.5 to 7.5 evade the formation of ceramic phases due to decrease in the concentration of calcium ions. Therefore calcined F-BG 7.5 had pure amorphous structure.

The pattern of cumulative fluoride release by all the study F-BG groups exhibited similar linear region which was the representative of a diffusion-like procedure. In the surface controlled process, the solution adjacent to the crystal exterior is under-saturated with respect to BG [16]. Therefore, in this study, it can be assumed that diffusion was the dominant mechanism controlling the solubility of F-BG particles. The solubility of BG is either kinetic controlled or diffusion controlled or both. This diffusion controlled process followed Fickian laws of diffusion which depends mainly on the concentration gradient between the surrounding liquid and crystal surface along with the diffusion constant [31].

The highest amount of cumulative fluoride release in 180 days was exhibited by F-BG 7.5 showing a direct proportionality of fluoride release with the fluoride content. The steady fluoride ions elution rate indicating that F-BG powders exhibited a potential of releasing greater than one ppm fluoride daily in deionized water for 180 days. F-BG 2.5 and F-BG 5 showed similar cumulative fluoride release pattern from day 1 till day 60. All F-BG groups showed similar cumulative fluoride

release at day 60. Whereas, at day 180 F-BG 7.5 (17.07 ppm) and F-BG 5 (16.99 ppm) exhibited almost similar maximum cumulative fluoride release.

The fluoride release pattern by F-BG 2.5 exhibited a preliminary burst during the initial nine days trailed by a gradual drop as the material aged. The initial burst was associated with the presence of fluoride based crystalline phase. F-BG 5 exhibited spurt in the first three days, followed by a drop till day 60 after which the second burst of fluoride was observed which sustained till the end of the study. In contrast, BG 7.5 exhibited a gradual but steady increase in the daily fluoride elusion initially which peaked at day 90, followed by a gradual drop as the material aged. This steady release was due to the homogeneity of the bioactive glass.

In the present study, BG exhibited almost similar pH values of the collected elute samples at all-time intervals. It is expected that ionic exchange occurred at the surface of BG particles and sodium or calcium ions next to the glass surface were liberated into the adjacent solution in exchange for hydrogen ions formed by hydrolysis, which resulted in a pH rise. In F-BGs, fluoride ions eluted, and hydroxyl ions were taken up from the solution, thus buffering the alkali effect. F-BG displayed higher fluoride release is believed to show less marked pH rise, which could be less irritant to soft oral tissues [14, 15]. This relationship of fluoride release with pH is evident in the present study as well.

The drop in the pH upon aging could be attributed to a decrease in the solubility of BG particles due to the formation of apatite on its surface upon aging [17]. It is assumed that fluoride release in F-BG dropped soon afterward despite a higher fluoride content. Fluoride-containing groups, F-BG 2.5 and F-BG 5 showed almost similar pH values till day 60 which might be attributed to the similar fluoride release exhibited by these two groups during this period. After day 60, elute's pH values for F-BG 2.5 increased gradually whereas pH values of F-BG 5 elute dropped which continued till the end of the study. The pH values of F-BG 7.5 elute were stable till day nine after which a gradual but constant drop was observed till day 90, later it showed a gradual increase in the pH values till the end of the study.

A long study period made the present study innovative, which was focused not only on the amount and pattern of fluoride release upon aging but also on the effect of fluoride release on the pH. Though at high pH better apatite deposition had been reported reported but at the same time increased alkalinity led to compromised biocompatibility by negatively affecting the mucosa of the oral cavity [14]. Considering the growing preference of using composite resins in restorative dentistry, there is a necessity to explore the effect of loading F-BG fillers in resin-based dental restorative composites as no such research work is done yet.

5. Conclusions

The maximum cumulative fluoride release in the present study seemed directly proportional to the fluoride content of the F-BG powders. However, the pattern of release was independent of the fluoride content of the study groups. F-BG 7.5 showed the maximum total cumulative fluoride release, followed by F-BG 5 and F-BG 2.5. However, the pH of the conditioning baths was inversely proportional to the amount of daily fluoride elusion i.e. sample with highest daily fluoride elusion exhibited the lowest pH of the conditioning bath and vice versa.

Among the study groups, F-BG 5 exhibited not only the burst fluoride release initially but also showed nearly highest total cumulative fluoride release compounded with least alkalinity (pH = 8.01), therefore, was regarded as the best among the study groups. The daily fluoride release rate of the experimental powders remained nearly constant (i.e. > 1 ppm) upon aging; however, the pH of the conditioning bath fluctuated and was dependent on the amount of daily fluoride elusion, thus, the “null hypothesis” was rejected. It is recommended that F-BG 5 has potential to be used as bioactive fillers in dentifrices, dental adhesives and in restorative materials. However,

further evaluation with these materials is required.

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Declaration of interest

None.

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