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ORIGINAL ARTICLE

# Assessment of fluoride ions concentration in novel toothpastes containing fluoridated bioactive glass fillers.

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**ABSTRACT… Objective:** To measure and compare fluoride release in elutes and suspensions of fluoride doped bioglass (F-BG) enriched novel and commercial dentifrices and to assess effect of filler loading on fluoride release of novel toothpastes. **Study Design:** In vitro study carried out in triplicates (n=3). **Setting:** Army Medical College, National University of Medical Sciences, Rawalpindi, Pakistan. **Period**: 3/2020 to 04/2023. **Methods:** Novel dentifrices were prepared by adding F-BG nanoparticles and zinc oxide as active ingredients to basic dentifrice formulation. Sensodyne F toothpaste was taken as control group. Fluoride release analysis of both suspensions and elutes of dentifrices was carried out via ion selective electrode potentiometry. Collected data was statistically analyzed via SPSS version 22. **Results:** All novel dentifrices exhibited greater fluoride elusion from Sensodyne F in both elutes and suspensions. In elute and suspension, the observed fluoride release exhibited statistically significant difference within and between all groups where $p > 0.05$ was taken as significant. **Conclusion:** In toothpastes, type of fluoride source directs fluoride release behavior. Direct proportionality of fluoride release of novel dentifrices to filler loading was observed.

**Key words:** Bioactive Glass, Fluoride Ions, Toothpastes.

## INTRODUCTION

Dental caries is a dynamic pathological process.[1-3] Initial dental caries clinically appears as "white spot lesion" on enamel which is a small sub-surface demineralized lesion. If preventive measures are not taken timely, then demineralization may progress deeply, involving underlying dentine as well and ultimately lead to cavitation.[4,5] Protective factors including salivary proteins, salivary flow and ionic calcium, phosphate and fluoride can halt, avoid or reverse the caries development.[2,6] Minimal intervention is the latest dental caries management approach which involves "nonrestorative" techniques aiming to prevent mineral loss. Tooth surface demineralization can be reversed by deploying topical mineralizing agents on teeth.[7] In cariology, bioactive materials have the potential of forming hydroxyapatite crystals on tooth surfaces and are capable of disrupting biofilm formation.[7]

Popular caries preventive measure is brushing teeth with fluoridated dentifrice, as it causes mechanical elimination of biofilm, adding to fluoride's therapeutic effects.[8] Fluoride ions availability and stability in the dentifrice are the key requirements for its efficacy to counter tooth decay.[9,10] For control of dental decay, at least 1000 ppm fluoride should be present in formulation of a dentifrice, fluoride must be in soluble state to impart anti-caries effect.[11,12] Factors, which govern the amount of soluble fluoride content in a dentifrice, are mainly. fluoride source, abrasive type, storage temperature and ageing. Dentifrice's abrasivity play an important part in deactivating free fluoride ions, leading to the formation of a low soluble product with reduced anti-caries effect.[9,10] At present, no guidelines specify the exact amount of overall fluoride preserved in a dentifrice preparation.[11]

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Bioactive glass (BG) has the potential of bonding chemically to the dental hard tissues. Upon dissolution in aqueous media, BG transforms not only structurally but also chemically leading to hydroxycarbonated apatite formation.[13] BG being a smart material, upon topical application to teeth not only minimize prevalence of primary caries via enamel remineralization but is also effective in treating dentine hypersensitivity by inducing dentinal tubules sealing.[14-18] Different in vitro studies have indicated that BG added toothpastes exhibited immediate dentinal tubule occlusion via hydroxyapatite formation.[15,17,19] Orative, Novamin and BioMin are commercial denitrifies enriched with fine BG 45S5 particles and are recommended in the treatment of hypersensitive teeth.[14,15] Formation of apatite in the teeth should occur before the dilution of BG enriched toothpaste by the saliva. While sleeping at night salivary flow is minimum, so the time required for apatite creation should be below eight hours.[20]

Keeping in mind >75 years long history of topical fluoride usage in dentistry for caries control[2,5] and teeth remineralization potentials of BG, fluoride doped bioglass (F-BG) was synthesized in an attempt to synergize bioactivity.[20,21] Recently, F-BG nanoparticles were produced by our group, followed by evaluation of prolonged fluoride release and its effect on pH was carried out.[22]

In the present study, the free fluoride ions in elute and suspensions of the three novel toothpastes and a commercial toothpaste (Sensodyne F) were calculated. The objective of this study was assessment and comparison of fluoride release among novel and commercial dentifrices. The hypothesis was that dentifrices containing F-BG with more filler loading would exhibit greater fluoride release.

## METHODS

This invitro study was conducted at Army Medical College from March 2020 to April 2020 after approval from ethics committee (ERC/10/260 Dated: 02/3/23). In present study, the chemical used for the preparation of novel toothpastes were of analytical grade. Initially, optimized ratio of fundamental constituents was mixed homogeneously followed by incremental addition of titanium dioxide, 1.5% wt. (Sigma Aldrich, USA) and zinc oxide nanoparticles, 3% wt. nanoparticles. F-BG (7.5% mol. fluoride) was added to the experimental dentifrices in many different proportions i.e., 1.5%, 3%, and 4% wt/wt. Table-I presents the composition of the experimental and control toothpaste groups.

The research procedure used in the present study was the modified version of the one proposed by Pearce.[11,23] This study was carried out in quadruplicates. 100mg dentifrice sample against each group was dissolved in 10mL deionized in 15ml plastic centrifuge tubes. 5ml from this suspension was then centrifuged for ten minutes at 6000 rpm to eliminate insoluble fluoride in the filler particles. From the extracted elute, 2.5ml was shifted to two centrifuge tubes. 2.5ml of 2M hydrochloric acid (BDH, UK) was then added to each 2.5ml elute/suspension which was then conditioned at 45°C for an hour. After adding 5mL of 1M sodium hydroxide (Sigma Aldrich, USA) and one mL of TISAB III reagent (Hanna Instruments; Woonsocket, Rhode Island, United States), fluoride release analysis was done via pre-calibrated ion selective electrode (ISE) potentiometry (Hanna HI3222 pH/ISE meter and fluoride electrode; Hanna Instruments, Woonsocket, Rhode Island, United States).

SPSS version 22 was employed to estimate the means and standard deviations. Statistical difference within and between the study groups was calculated via one-way ANOVA and for pairwise comparison between groups Posthoc Tukey's test was employed.

## RESULTS

Fluoride release in parts per million (ppm) for both suspensions and elutes of all the toothpaste groups of this study are given in Figure-1. Amongst elutes of the toothpastes, highest mean fluoride elusion was noticed in E3 (6.9±0.45ppm), followed by E2 (6.1±0.04ppm), then E1 (5.24±0.03ppm) and least in CD (6.71±0.02ppm).

| S# | Name of TP Company | Abrasive | Other Ingredients (% wt.) | Fluoride Agent %age | | Group Name |
|---|---|---|---|---|---|---|
| 1. | Sensodyne F (Glaxo Smith Kline, Karachi, Pakistan) | Silica | **Basic Ingredients:** Pentasodium Triphosphate, Sodium Methyl Cocoyl Taurate, Cocamidopropyl Betaine, Sorbitol, PEG-6, Hydrated Silica, Xanthan Gum, Sodium Hydroxide, Potassium Nitrate, 5.00%w/w, Titanium Dioxide, Aqua/Water, Glycerin, Sodium Saccharin, Aroma/Flavor | Sodium fluoride (NaF) 0.315 w/w<br>Strontium Fluoride Acetate ($NaFC_2H_2O_2$) 8%<br>(1426ppm Fluoride) | | CD |
| 2. | Experimental toothpastes | Calcium carbonate ($CaCO_3$) 27%wt. | **Basic Ingredients:** Water ~ 36%<br>Methyelcellulose ~1%<br>Glycerol ~32%<br>Sodium Lauryl Sulphate ~ 2%<br>Sodium Benzoate ~1%<br>Zinc oxide (ZnO) nanoparticles 3%<br>Titanium dioxide ($TiO_2$) 1.5%<br>Peppermint (Flavoring Agent) ~ 1% | F-BG with 7.5% mol. Fluoride | F-BG 1.5% w/w | E1 |
| | | | | | F-BG 3% w/w | E2 |
| | | | | | F-BG 4% w/w | E3 |

**Table-I. Fluoride content of the experimental dentifrices**

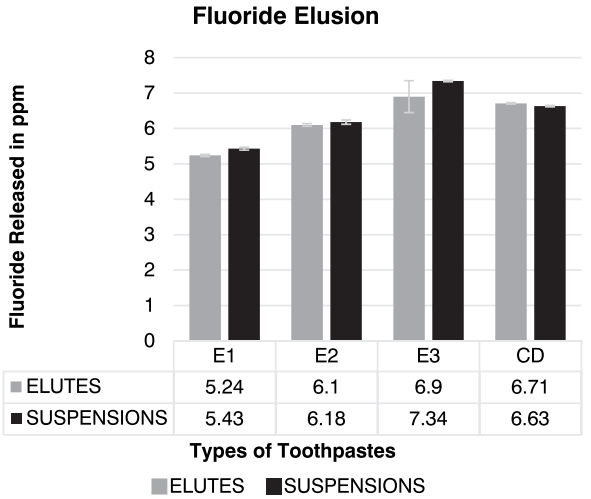


**Figure-1. Fluoride Elusion Means with Standard Deviations (Error Bars) in elutes and suspensions of the experimental toothpastes**

Among suspensions of the study toothpastes, highest mean fluoride elusion was shown by E3 (7.34±0.02ppm), succeeded by CD (6.63±0.02 ppm), then E2 (6.18±0.02 ppm) and least by E1 (5.43±0.04 ppm). In both suspensions and elutes of the study toothpastes, a rise in mean fluoride elusion was detected in novel dentifrices with increase in filler quantity.

A statistically significant difference in fluoride elusion was observed within and between toothpaste groups ($p<0.05$). Post hoc Tukey test disclosed that all the study toothpaste groups exhibited a statistically significant difference between mean fluoride release of the toothpaste suspensions and between the mean fluoride release of toothpaste elutes.

## DISCUSSION

Fluoride doped bioglass enriched dentifrices as topical fluoride agents are effective means of reversing white spot lesions. In fluoride doped bioglass (F-BG), fluoride is not within the glass structure which is beneficial and ensures the creation of strong crystals of fluorapatite which are more acid-resistant than carbonated HA.[24,25] Kanwal et al. discovered that fluoride addition in a BG dentifrice in Tris buffer solution yields stronger fluoridated apatite.[26] BioMinF© is a commercial F-BG enriched toothpaste when assessed in vitro showed the potential of effective dentinal tubule occlusion.[24]

In this study novel toothpastes enriched with F-BG with 7.5 wt% fluoride was synthesized with varying filler loading. These dentifrices were then assessed for their fluoride release in both elutes and suspensions. Among the novel dentifrices, upon rising the F-BG filler content, surge in

fluoride elusion rate in both suspensions and elutes of novel toothpastes was detected, thus, obeying the hypothesis of this study. Whereby, a direct proportionality of fluoride elusion rate with F-BG filler loading in novel toothpastes was seen.

During teeth brushing procedure, toothpaste is used in suspension form, therefore, fluoride released in toothpaste suspensions is of more clinical significance than the fluoride released in the toothpaste elutes. Though, no remarked difference was seen in fluoride release values of suspensions and elutes of same group. Among the novel toothpastes, group E3, loaded with F-BG filler with 7.5wt% fluoride with 4% wt/wt filler content, exhibited maximum mean fluoride release. A direct proportionality of fluoride release in all experimental toothpastes can be observed with filler loading.

The commercial dentifrice used in this study i.e., CD (Sensodyne), contained Sodium fluoride (NaF) which is a water soluble, inorganic fluoride salt. A study by Cury et al. revealed that all the commercial fluoridated toothpastes sold at Brazil showed lower concentration of total soluble fluoride than that of total fluoride content in dentifrices, which also hold true in this study for the control group, CD.[11] However, the control group (Sensodyne F) with total fluoride content of 1426 ppm, exhibited much inferior fluoride release in both elutes and suspensions as compared to the novel dentifrices, thus, classifying F-BG with 7.5wt% fluoride fillers as a better fluoride source than sodium fluoride in dentifrices.

Among the novel F-BG enriched dentifrices, group E3 exhibited the highest fluoride elusion and was the only novel toothpaste exhibiting greater fluoride release than commercial toothpaste group in both elutes and suspension form. The other two groups i.e., E1 and E2, were inferior in fluoride elusion than the control group. The difference in fluoride elusion exhibited by the experimental toothpastes groups in this study could be attributed to variations in fluoride content of the dentifrices.

Dentifrices containing calcium-based abrasives lose free available fluoride upon ageing at high temperatures. Calcium carbonate, react with ionic fluoride forming insoluble fluoride.[10,11,27] Tropical zones of earth experience long hot summers, therefore, greater chances are that these products lose more than a quarter of their free available fluoride content within a year under uncontrolled storage conditions before reaching their consumers as highlighted by the studies of Benzian et al. and Nascimento et al. Though the control group taken in the present study was within 03 years of expiry, still storage at high temperatures (before arriving to research laboratory) can be held responsible for the reduced amount of free available fluoride in the suspensions and elutes of commercial toothpaste.[27,12]

The existing literature does not bring substantial evidence about the active amount of free existing fluoride in toothpastes to produce top anti-carious efficiency. Cosmetic toothpaste's guidelines primarily emphasize only on amount of total fluoride and affirm that total fluoride in a toothpaste should not surpass 1500 ppm. Similarly, ISO Standard 11609 comments only about the amount of total fluoride in a toothpaste and exclude the necessity to state about the amount of free fluoride in a toothpaste, though this is an indispensable condition for anti-caries effectiveness. Only the US Food and Drug Administration, express that sodium fluoride or sodium monofluorophosphate enriched toothpastes containing total fluoride of 850– 1150 ppm should carry ≥ 650ppm and ≥ 800ppm free fluoride ions respectively. Though validation for these values are still debatable.[27]

Commercial BG 45S5 particles enriched dentifrices i.e., Orative, Novamin and BioMin, do not contain fluoride-based BG fillers. Only a single similar study that too conducted by our group was covered fluoride elusion of novel toothpastes enriched with fluoride doped BG fillers with 5% mol. Fluoride.[28] In the current study, the novel toothpastes containing fluoride doped BG fillers with 7.5% mol had higher fluoride content than toothpastes of the previous study reported by Gul et al. but instead of improved fluoride elusion due to higher fluoride content,

inferior fluoride elusion was reported by the novel toothpastes in this study. Thus, showing that further increasing the fluoride content of fluoride doped BG fluoride fillers decrease its fluoride elusion potentials in toothpastes. Further in vitro studies need to be done to evaluate antibacterial and teeth remineralization capacities of these novel toothpastes to measure their usefulness in reducing white spot lesions of enamel and hypersensitivity of dentine. Lastly, there is a need to carry out the cytotoxicity testing and in vivo usage tests to develop an appropriate toothpaste formulation before introducing it commercially.

## CONCLUSION

In toothpastes, principally the source of fluoride directs its fluoride elusion. Fluoride elusion in suspensions and elutes of novel toothpastes showed direct proportionality to filler loading, obeying the hypothesis. Among the novel toothpastes, only group E3 with maximum filler loading i.e., 4%wt. fluoride doped bioactive glass particles, showed superior fluoride release in both suspensions and elutes than the commercial toothpaste i.e. Sensodyne F.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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