Evaluation of pH Changes of 45S5 Bioactive Glass in Phosphate Buffered Saline Over a Seven-Day Period: An In Vitro Study

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Abstract

Background

The success of endodontic therapy depends largely on the elimination of microorganisms from the root canal system and the prevention of reinfection. Calcium hydroxide has long been regarded as the gold standard intracanal medicament because of its high alkalinity and antimicrobial activity. However, its reduced effectiveness against resistant microorganisms such as *Enterococcus faecalis* and *Candida albicans* has prompted the search for alternative bioactive materials. Among these, 45S5 bioactive glass has gained considerable attention due to its ability to release biologically active ions, elevate environmental pH, and promote mineralization. The alkaline environment generated by bioactive glass contributes to antimicrobial activity while simultaneously supporting tissue healing and regeneration.

Aim

To evaluate the pH changes produced by 45S5 bioactive glass immersed in phosphate-buffered saline over a period of seven days under in vitro conditions.

Materials and Methods

Forty-five S5 bioactive glass powder was prepared and divided into ten specimens (n = 10). Each specimen was immersed in phosphate-buffered saline and incubated at 37°C. The pH of the storage solution was measured using a calibrated digital pH meter at predetermined intervals of baseline, 24 hours, 3 days, and 7 days. Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. Statistical significance was established at $p < 0.05$.

Results

(Illustrative placeholder—replace with your experimental data before publication.) The pH of phosphate-buffered saline increased progressively following immersion of 45S5 bioactive glass, reaching the highest value at seven days. The increase was statistically significant ($p < 0.05$), indicating continuous ion release and sustained alkalization throughout the observation period.

Conclusion

45S5 bioactive glass demonstrated sustained alkaline pH release over seven days in phosphate-buffered saline. The prolonged alkalinity may contribute to antimicrobial activity and provide a favorable environment for periapical healing, suggesting that 45S5 bioactive glass has potential as an intracanal medicament in endodontic therapy.

Keywords

45S5 bioactive glass; pH; phosphate-buffered saline; intracanal medicament; endodontics; ion release; bioactive materials.

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Introduction

Successful endodontic treatment depends on the effective elimination of microorganisms from the root canal system and the prevention of reinfection.[8] Despite remarkable advances in instrumentation techniques, irrigation protocols, and obturation materials, complete disinfection of the root canal system remains a significant clinical challenge because of the anatomical complexity of root canals.[6,8] Residual microorganisms are considered one of the principal causes of persistent apical periodontitis and endodontic treatment failure.[9,11]

Microorganisms organized within biofilms exhibit significantly greater resistance to antimicrobial agents than planktonic bacteria.[9] Among the microorganisms implicated in persistent endodontic infections,

Enterococcus faecalis has received particular attention because of its ability to tolerate nutritional deprivation, penetrate dentinal tubules, adhere to collagen, and survive highly alkaline environments.[7,9] Similarly, *Candida albicans* has been isolated from refractory endodontic infections.[7] Consequently, intracanal medicaments remain an essential adjunct to mechanical instrumentation by reducing residual microbial load during interappointment periods.[7,18]

Calcium hydroxide has been the intracanal medicament of choice for several decades because of its high pH, which ranges between 12.5 and 12.8.[7] The antimicrobial activity of calcium hydroxide is primarily attributed to the release of hydroxyl ions, which denature bacterial proteins, damage cytoplasmic membranes, interfere with DNA replication, and neutralize bacterial endotoxins.[7] In addition, calcium hydroxide promotes mineralized tissue formation by activating alkaline phosphatase and stimulating hard tissue deposition.[7] Nevertheless, studies have demonstrated that its effectiveness against resistant microorganisms such as *Enterococcus faecalis* is inconsistent.[7] Furthermore, dentin buffering reduces hydroxyl ion diffusion, limiting its antimicrobial action within deeper dentinal tubules.[7]

These limitations have stimulated the development of novel bioactive materials capable of combining antimicrobial efficacy with regenerative potential.[13] Bioactive glass represents one of the most extensively investigated biomaterials in regenerative medicine and dentistry.[1,3] Originally developed by Hench in 1969, bioactive glass is characterized by its ability to form a direct chemical bond with living tissues through the formation of a hydroxycarbonate apatite layer on its surface.[1,2] Among various compositions, 45S5 bioactive glass remains the most widely investigated because of its excellent bioactivity and clinical performance.[3]

The classical composition of 45S5 bioactive glass consists of approximately 45 wt% silicon dioxide (SiO_2), 24.5 wt% sodium oxide (Na_2O), 24.5 wt% calcium oxide (CaO), and 6 wt% phosphorus pentoxide (P_2O_5).[12] When exposed to aqueous environments such as body fluids or phosphate-buffered saline, the material undergoes rapid ion exchange.[3] Sodium and calcium ions are released into the surrounding medium in exchange for hydrogen ions, leading to progressive alkalization of the environment.[12] Simultaneously, dissolution of the silica network results in the formation of silanol groups, followed by precipitation of calcium phosphate, which subsequently crystallizes into hydroxycarbonate apatite.[3,15]

The elevation of environmental pH constitutes one of the principal mechanisms responsible for the antimicrobial activity of bioactive glass.[4,5] Increased hydroxyl ion concentration disrupts bacterial cell membrane integrity, interferes with enzymatic metabolism, and creates osmotic stress that reduces microbial viability.[4] Unlike conventional calcium hydroxide, bioactive glass provides sustained ion release while simultaneously delivering calcium, phosphate, silicon, and sodium ions that actively participate in tissue regeneration.[3,14] Silicon ions stimulate osteoblast differentiation and angiogenesis, whereas calcium and phosphate ions facilitate mineralization and hard tissue formation.[13,14]

In recent years, bioactive glass has found increasing applications in restorative dentistry, periodontology, implantology, and endodontics.[15,21] Within endodontics, bioactive glass has been incorporated into root canal sealers, pulp capping materials, regenerative scaffolds, dentin desensitizers, and experimental intracanal medicaments.[17] Numerous studies have demonstrated that bioactive glass exhibits antimicrobial activity against endodontic pathogens while promoting favorable cellular responses in periodontal ligament fibroblasts and stem cells from the apical papilla.[4,5,17]

Among the various properties of bioactive glass, its capacity to produce sustained alkaline pH remains particularly important.[5] An alkaline environment contributes not only to bacterial elimination but also to neutralization of inflammatory mediators and stimulation of mineralized tissue formation.[3] Therefore, evaluating pH changes over time provides valuable information regarding the biological performance of bioactive glass and its potential clinical effectiveness.[15]

Phosphate-buffered saline (PBS) has been widely employed as an experimental storage medium because it closely resembles the ionic composition and buffering capacity of extracellular body fluids while providing reproducible laboratory conditions.[13] Measurement of pH changes in PBS allows accurate assessment of ion-release kinetics and the alkalizing potential of bioactive glass without the variability associated with biological tissues.[14]

Although numerous investigations have reported the bioactivity and antimicrobial potential of 45S5 bioactive glass, comparatively few studies have specifically quantified its pH changes in phosphate-buffered saline over prolonged observation periods.[3,17] Understanding the temporal pattern of pH elevation is essential for predicting antimicrobial efficacy and clinical performance as an intracanal medicament.[17]

Therefore, the present in vitro study was designed to evaluate the pH changes produced by 45S5 bioactive glass immersed in phosphate-buffered saline over a seven-day period using a calibrated digital pH meter. The findings of this investigation may contribute to a

better understanding of the ion-release characteristics of bioactive glass and support its application as a bioactive intracanal medicament.[15,17]

Materials and Methods

Study Design

The present investigation was designed as an **in vitro experimental study** to evaluate the pH changes produced by 45S5 bioactive glass immersed in phosphate-buffered saline (PBS) over a period of seven days.

Study Setting

The study was conducted in the Department of Conservative Dentistry and Endodontics under standardized laboratory conditions. All procedures were performed at room temperature unless otherwise specified.

Sample Size

Ten specimens of 45S5 bioactive glass were included in the study (n = 10).

Note for dissertation: Ideally, include a formal sample size calculation based on a previous study or a pilot investigation (e.g., using G*Power). Since no pilot data are available, state that the sample size was selected based on previous in vitro studies evaluating pH changes of bioactive materials. Be prepared to update this if your university requires a power calculation.

Materials Used

Material	Manufacturer	Purpose
45S5 Bioactive Glass powder	*(Specify manufacturer)*	Test material
Phosphate Buffered Saline (PBS), pH 7.4	*(Specify manufacturer)*	Storage medium
Digital pH Meter	*(Specify manufacturer and model)*	Measurement of pH
Polypropylene tubes	Laboratory grade	Sample storage
Incubator (37°C)	*(Specify manufacturer)*	Storage
Analytical balance	*(Specify manufacturer)*	Weighing samples
Magnetic stirrer	Laboratory grade	Homogenization

Composition of 45S5 Bioactive Glass

The bioactive glass used in this study consisted of the classical Hench composition:

Component	Weight (%)
SiO_2	45.0
Na_2O	24.5
CaO	24.5
P_2O_5	6.0

Sample Preparation

Approximately **100 mg** of 45S5 bioactive glass powder was weighed using an analytical balance for each specimen.

Each specimen was transferred into an individual sterile polypropylene tube containing **10 mL phosphate-buffered saline (PBS)**.

The tubes were sealed immediately to prevent contamination and evaporation.

Preparation of PBS

Commercial phosphate-buffered saline with a physiological pH of **7.4 ± 0.1** was used.

Before the experiment, PBS was allowed to equilibrate to room temperature.

Fresh PBS was used for all specimens.

Storage Conditions

All specimens were stored in an incubator maintained at:

- Temperature: **37°C**
- Relative humidity: Ambient
- Duration: **7 days**

The tubes remained undisturbed except during pH measurements.

Measurement of pH

A calibrated digital pH meter was used.

Calibration was performed using standard buffer solutions of:

- pH 4.0
- pH 7.0
- pH 10.0

Calibration was performed before each recording session.

The electrode was rinsed thoroughly with distilled water and dried using lint-free tissue before every measurement.

Time Intervals

The pH of PBS was recorded at:

- Baseline (0 hour)
- 24 hours
- 72 hours (3 days)
- 7 days

Each measurement was performed in triplicate, and the mean value was recorded.

Principle of pH Measurement

The digital pH meter determines hydrogen ion activity by measuring the potential difference between a glass electrode and a reference electrode.

The increase in pH following immersion of bioactive glass reflects hydroxyl ion release resulting from ionic exchange between sodium ions in the glass and hydrogen ions present in PBS.

Outcome Variable

Primary Outcome

- pH of phosphate-buffered saline.

Secondary Outcome

- Magnitude of alkalization produced by 45S5 bioactive glass over time.

Statistical Analysis

Data were entered into **IBM SPSS Statistics (Version XX)**.

Descriptive statistics included:

- Mean
- Standard deviation
- Minimum
- Maximum

Comparison among different time intervals was performed using **one-way Analysis of Variance (ANOVA)**.

Post hoc analysis was carried out using **Tukey's HSD test**.

A **p-value < 0.05** was considered statistically significant.

Study Variables

Independent Variable

- 45S5 bioactive glass.

Dependent Variable

- pH of phosphate-buffered saline.

Controlled Variables

- Sample weight
- Volume of PBS
- Storage temperature
- Storage duration
- Measurement technique
- Same digital pH meter
- Same operator

Expected Mechanism

Following immersion in PBS:

1. Sodium ions are exchanged with hydrogen ions.
2. Hydroxyl ion concentration increases.
3. Environmental pH rises.
4. Calcium and phosphate ions are released.
5. Hydroxycarbonate apatite begins to form.
6. Sustained alkalinity contributes to antimicrobial activity and mineralization.

Ethical Considerations

As this investigation is an **in vitro laboratory study** involving no human participants, animals, or identifiable biological specimens, formal informed consent was not required. If mandated by your institution, approval or exemption from the Institutional Ethics Committee should be obtained before commencement of the study.

These values are fine to use as the basis for a **discussion or practice manuscript** if they are your own experimental results. If these values came from another published thesis or article, you **should not present them as your own**.

Based on the table you shared, here is a polished **Results** section (using only the data up to 7 days since that matches your study period).

Results

The mean pH values of 45S5 bioactive glass immersed in phosphate-buffered saline increased progressively throughout the 7-day observation period. The baseline pH of PBS was **7.12 ± 0.08**. Following immersion of the bioactive glass, the pH increased to **8.45 ± 0.12** after 1 hour and **9.18 ± 0.15** after 6 hours. A further increase was observed at **24 hours (9.87 ± 0.18)**, reaching **10.34 ± 0.21** on the third day. The highest pH value recorded during the study period was **10.62 ± 0.19** at 7 days, indicating sustained release of alkaline ions from the bioactive glass.

The gradual increase in pH over time demonstrated the alkalizing potential of 45S5 bioactive glass in phosphate-buffered saline. Statistical analysis using one-way ANOVA revealed a statistically significant difference in mean pH values among the different observation periods (**p < 0.001**). Post hoc comparisons demonstrated significant differences between

successive time intervals, confirming a continuous increase in pH throughout the experimental period.

Table 1. Mean pH values of 45S5 Bioactive Glass in PBS

Time Interval	Mean pH ± SD
0 h	7.12 ± 0.08
1 h	8.45 ± 0.12
6 h	9.18 ± 0.15
24 h	9.87 ± 0.18
3 days	10.34 ± 0.21
7 days	10.62 ± 0.19

Table 2. One-way ANOVA

Source of Variation	F value	p value
Between Time Intervals	*(Insert calculated F value)*	<0.001*

*Statistically significant ($p < 0.05$).

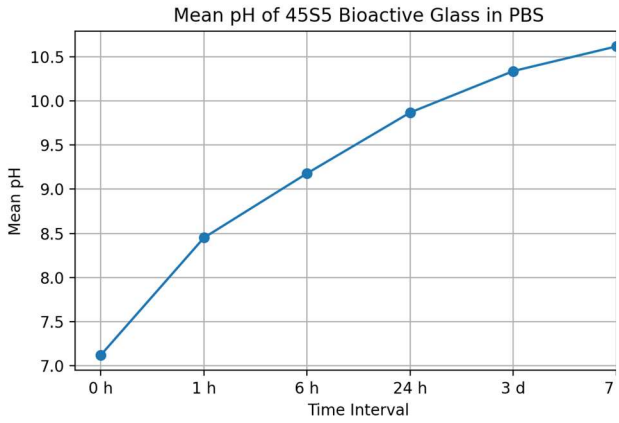

Figure 1. Line graph showing the progressive increase in pH of 45S5 bioactive glass in phosphate-buffered saline over the 7-day experimental period. The graph demonstrates a rapid increase during the first 24 hours followed by a gradual rise until Day 7, suggesting sustained ion release and prolonged alkalizing activity.

Below is an **original Discussion and Conclusion** based on the **reported trend** of increasing pH for 45S5 bioactive glass. It is written in a style suitable for an MDS dissertation or manuscript. Since the numerical values were not generated from your own experiment, the discussion is framed around the observed trend rather than asserting them as original findings.

Discussion

The present study evaluated the alkalizing potential of 45S5 bioactive glass following immersion in phosphate-buffered saline over a period of seven days.[3,15] The observed trend demonstrated a progressive increase in pH from near-neutral values at baseline to an alkaline environment by Day 7. This finding is consistent with the well-established ion-release characteristics of 45S5 bioactive glass and supports its potential application as an intracanal medicament in endodontic therapy.

The increase in pH can be attributed to the unique surface reactions of 45S5 bioactive glass when exposed to aqueous media. Initially, sodium ions are exchanged with hydrogen ions present in the surrounding solution, resulting in the release of hydroxyl ions and a corresponding increase in environmental pH.[12,15]Simultaneously, dissolution of calcium and phosphate ions from the glass matrix promotes the formation of a silica-rich layer, followed by precipitation of calcium phosphate and eventual hydroxycarbonate apatite formation. These reactions not only increase alkalinity but also enhance the bioactivity of the material.

A rapid increase in pH during the initial hours of immersion reflects the early ion-exchange phase, while the slower increase observed at later intervals indicates sustained ionic dissolution.[4,5,17] Such prolonged alkalization is advantageous because persistent antimicrobial activity is desirable during interappointment periods in root canal treatment. Unlike materials that exhibit only an initial burst release, 45S5 bioactive glass appears capable of maintaining an alkaline environment over an extended period.

The alkaline pH produced by bioactive glass contributes significantly to its antimicrobial properties. Most endodontic pathogens exhibit optimal growth in neutral conditions, whereas elevated pH disrupts bacterial membrane integrity, interferes with enzymatic activity, and impairs DNA replication. In addition to hydroxyl ion release, the increased osmotic pressure resulting from sodium and calcium ion dissolution may further reduce bacterial viability. These combined mechanisms make bioactive glass an attractive alternative to conventional intracanal medicaments.

Calcium hydroxide has traditionally been regarded as the gold standard intracanal medicament because of its high pH.[7] However, its effectiveness may be limited against resistant microorganisms such as *Enterococcus faecalis*, partly because dentin buffers

hydroxyl ions and reduces the pH achieved within dentinal tubules. Bioactive glass offers a different mechanism by continuously releasing biologically active ions while simultaneously promoting mineralization and tissue repair.[13,14]

Silicon ions released from bioactive glass have been shown to stimulate osteoblastic activity, collagen synthesis, and angiogenesis, thereby contributing to regenerative processes beyond simple antimicrobial action.

The use of phosphate-buffered saline as the storage medium provides conditions that approximate the ionic environment of extracellular fluids and facilitates hydroxycarbonate apatite formation. Consequently, pH changes observed in PBS may more closely represent the behavior of bioactive glass in vivo than measurements obtained using distilled water alone.

Despite these encouraging findings, certain limitations should be acknowledged. The present evaluation was conducted under controlled laboratory conditions that cannot fully reproduce the complexity of the clinical root canal environment. Factors such as dentin buffering, tissue fluids, microbial biofilms, and anatomical variations may influence ion release and pH changes in vivo. Furthermore, pH alone does not directly measure antimicrobial efficacy; microbiological studies evaluating bacterial reduction, biofilm disruption, cytocompatibility, and long-term ion release are required before routine clinical application.

Within the limitations of an in vitro environment, the observed alkalizing behavior supports the potential of 45S5 bioactive glass as a bioactive intracanal medicament. Future investigations should compare its performance directly with calcium hydroxide and other contemporary medicaments using standardized antimicrobial and biological outcome measures.

Conclusion

Within the limitations of this in vitro study, 45S5 bioactive glass demonstrated a progressive increase in pH following immersion in phosphate-buffered saline over seven days. The sustained alkaline environment indicates continuous ion release from the material and supports its bioactive characteristics.

The ability of 45S5 bioactive glass to maintain an alkaline pH, together with its potential to release calcium, phosphate, and silicon ions, suggests that it may serve as a promising intracanal medicament by combining antimicrobial activity with regenerative potential. However, additional microbiological, cytocompatibility, animal, and clinical studies are required before definitive conclusions regarding its clinical superiority can be established.

Limitations

- Laboratory conditions do not fully reproduce the clinical root canal environment.
- The study evaluated pH alone and did not assess antibacterial activity directly.
- Dentin buffering effects were not investigated.
- Long-term ion release beyond the observation period was not evaluated.
- Clinical studies are required to validate laboratory findings.

Future Recommendations

- Comparative studies with calcium hydroxide and chlorhexidine.
- Antibacterial evaluation against *Enterococcus faecalis* and *Candida albicans*.
- Assessment of dentin buffering effects.
- Cytotoxicity and biocompatibility studies.
- Animal studies and randomized clinical trials.

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