INTRODUCTION

Phosphate glasses have been recently introduced in the field of medicine and dentistry for therapeutic purposes. They are bioactive degradable materials which are being used nowadays for replacing lost or diseased tissues, hence restoring their form and functions.\(^1\) They have become applicable in a variety of clinical applications such as dental, maxillofacial and orthopedic implants, bone regeneration procedures and as means of ion delivery for antimicrobial effects.\(^2\) This application is due to the fact that these bioactive materials have the ability to degrade or being soluble in an aqueous media, and releasing the respective ions required for the purpose. They degrade at a rate of \(10^{-4}\) to \(10^{-9}\) g cm\(^{-2}\) min\(^{-1}\).\(^3\)

They have very low melting points and glass transition temperatures. On the other hand they have a high coefficient of thermal expansion. The main component which forms the basis and backbone of the phosphate glass structure is PO\(_4\)\(^3-\), which is surrounded by metal cations which balance the whole structure.\(^4\) This forms a tetrahedral structure and with the help of adjacent ions it forms a three dimensional network.

Fluoride has been known to inhibit the formation of dental cavities by preventing enamel and dentine demineralization, enhancing the process of remineralization of enamel. For this study six bioactive Phosphate glasses of variable compositions containing Phosphate, Calcium, Sodium, Fluoride and Strontium ions were made for this study. Each powder mixture was melted in a platinum crucible at 1150°C and was quenched between stainless-steel plates to produce glasses. The glasses were milled into a powder. Demineralizing solution containing 0.1 M of acetic acid having a pH of 4.0 was prepared. 75 grams of each glass powder was immersed into 50 ml of the demineralization solution and kept in an incubator at 37°C shaking at a constant pace for different time points. After every time point, the solution was checked for precipitates and was filtered out. The filtered solution was sent for Inductively Coupled Plasma Optical Emission Spectroscopy for measurement of Strontium ion release, and Fluoride Ion Selective Electrode for the measurement of Fluoride release. 0.17 ppm of fluoride was released out of the maximum predicted release of 28 ppm which counts to 1.2% of the total release as measured by the fluoride electrode; along with 7.4 ppm of strontium out of the maximum predicted release of 32 ppm which counts to 23% of the total release as measured by ICP. Maximum release was from glasses incorporated with both strontium and fluoride in the same glass exhibiting synergism.

Key Words: Phosphate Glasses, Strontium, Fluoride, Synergism.
Transferases, hence the production of bacteria and biofilm layer.\textsuperscript{6}

Strontium is thought to replace Ca\textsuperscript{2+} in hydroxyapatite and the resultant compound formed is more resistant to caries attack.\textsuperscript{7} In this way strontium is thought to enhance the process of remineralization. Both when combined together have an enhanced effect, by improving apatite crystallinity and reducing acid reactivity of synthetic carbonated apatites. When fluoride is available simultaneously with calcium and phosphate, it has been suggested to accelerate the remineralizing process by adsorbing to the enamel surface and attracting calcium ions as it has a high affinity for calcium. When strontium is used in conjunction with fluoride, it results in the enhancement of remineralization.

Another study suggested that addition of 43ppm strontium with fluoride enhances the formation of fluoride substituted apatite, up to higher concentrations of fluoride. This also proved the synergistic effect of fluoride and strontium during remineralization.

Strontium and fluoride, both have been observed to play a synergistic role in caries reduction and remineralization, although both of them have their own mechanism of action respectively.\textsuperscript{8}

**METHODOLOGY**

Six compositions of bioactive phosphate glasses were prepared for this study. One composition was set as a control and was devoid of fluoride and strontium ions. The rest five glasses had variation in the compositions. One set had both fluoride and strontium incorporated together. Titanium was added to control the degradation of the glasses, prolong the ions release and result in amorphous glass formation with no or minimal crystallization.

Approximately 80 grams of each composition powder was measured on a weighing balance and then collected and mixed in a Platinum/Rhodium crucible as this crucible was used to melt these powders as it collected and mixed in a Platinum/Rhodium crucible. This crucible was used to measure fluoride release as a result of dissolution of the glass in the demineralization solution. An ORION model 720 meter and ORION 9609BNWP fluoride ion selective electrode were used.

<table>
<thead>
<tr>
<th></th>
<th>P\textsubscript{2}O\textsubscript{5}</th>
<th>TiO\textsubscript{2}</th>
<th>CaO</th>
<th>Na\textsubscript{2}O</th>
<th>CaF\textsubscript{2}</th>
<th>SrO</th>
</tr>
</thead>
<tbody>
<tr>
<td>COMPO1</td>
<td>50.00</td>
<td>3.00</td>
<td>37.00</td>
<td>10.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>COMPO2</td>
<td>48.75</td>
<td>2.93</td>
<td>33.58</td>
<td>9.75</td>
<td>2.50</td>
<td>2.50</td>
</tr>
<tr>
<td>COMPO3</td>
<td>48.75</td>
<td>2.93</td>
<td>36.08</td>
<td>9.75</td>
<td>2.50</td>
<td>0.00</td>
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<tr>
<td>COMPO4</td>
<td>47.50</td>
<td>2.85</td>
<td>35.15</td>
<td>9.50</td>
<td>5.00</td>
<td>0.00</td>
</tr>
<tr>
<td>COMPO5</td>
<td>50.00</td>
<td>3.00</td>
<td>32.00</td>
<td>10.00</td>
<td>0.00</td>
<td>5.00</td>
</tr>
<tr>
<td>COMPO6</td>
<td>50.00</td>
<td>3.00</td>
<td>34.50</td>
<td>10.00</td>
<td>0.00</td>
<td>2.50</td>
</tr>
</tbody>
</table>

1 was kept as a control and had no traces of fluoride and strontium in it.

The crucible was placed in the furnace at room temperature of around 21°C and it was ramped to 100°C at the rate of 5°C/min and dwelled for 1 hour. This process was repeated with different dwelling times and ramping temperatures till the temperature was increased to 1150°C. After one hour the crucible was removed from the furnace and the molten glass was carefully and immediately poured on a metal plate and left to cool for 10 minutes then collected in a new sample bag. This whole process was done for all of the six batches of glasses.

All 6 batches of glass were collected in separate labeled sample bags and then were milled in a Gyro mill for 7 minutes to produce powdered glass.

The demineralizing medium was used in the form of 0.1 M acetic acid at pH 4.0. For this purpose a 1000ml volumetric flask was used and was filled with 500 ml of deionized water. The pH measured by a calibrated pH meter was 2.5. Therefore it required neutralization and for this purpose small pellets of Sodium Hydroxide were used. Pellets of NaOH were added into the solution till the pH was risen up to 4.01.

**Immersion of Glass Powder in Demineralization Solution**

The next step was to immerse the glass powders into the demineralization solution. 75 mg of each composition of glass powder was measured on a weighing scale and was immersed in 50 ml of the demineralization solution and placed in an incubator at 37°C and at different time points of 1 hour, 3 hours, 6 hours, 24 hours, 3 days and 7 days respectively.

**Filtration of Glass Powders**

After each specific time point was complete, the demineralization solution was filtered out with the help of a filter paper. The drained solutions were placed back in the jars whereas the residues collected in the filter paper were kept for drying in heated air at 37°C. These were kept for drying overnight and then collected in small sample bags to be run for FTIR. The drained solutions were kept in the refrigerator so they could be used to measure fluoride and strontium release.

**Fluoride Electrode**

The next step was to calibrate the fluoride electrode which was to be used to measure fluoride release as a result of dissolution of the glass in the demineralization solution. An ORION model 720 meter and ORION 9609BNWP fluoride ion selective electrode were used.
were diluted in 100ml of acetic acid. The calibration was started as mentioned above with the immersion of the electrode into the solutions for three minutes and recording the values in mV after every 3 minutes till the difference between two consecutive values was 0.1. The same process was repeated for all the concentrations, ending up in a calibration curve.

As it was confirmed that the electrode was calibrated, fluoride release from the demineralization solution was to be measured. As mentioned in Table 2, it can be seen that there was only 3 sets of glasses which had fluoride incorporated in them, COMPO 2, COMPO 3 and COMPO 4. The measurements were made and recorded in the same pattern as electrode calibration was performed i.e recording values after every 3 minutes till the difference was 0.1. All readings were recorded in mV from which the concentration of fluoride released in ppm was calculated for each time point release.

**ICP Sample Preparation**

Strontium release was also to be measured and this was performed by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). Standard solutions were prepared for all ions ranging from 0.1 ppm to 100ppm. 10 ml of stock solutions of each respective ion was collected in a volumetric flask and diluted with deionized water till the 1 L mark. Then from this solution all 4 concentrations were prepared to act as standards.

The demineralization solution samples which were to be measured were prepared. 10 ml of sample was

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### TABLE 2: FLUORIDE RELEASE MEASUREMENTS RESULTS

<table>
<thead>
<tr>
<th>NaF/ ppm</th>
<th>F- mol/L</th>
<th>-log F</th>
<th>E, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>2.3809E-06</td>
<td>-5.623</td>
<td>214.5</td>
</tr>
<tr>
<td>0.5</td>
<td>1.1904E-05</td>
<td>-4.924</td>
<td>207.0</td>
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<tr>
<td>1</td>
<td>2.3809E-05</td>
<td>-4.263</td>
<td>161.1</td>
</tr>
<tr>
<td>2</td>
<td>4.7619E-05</td>
<td>-4.322</td>
<td>143.8</td>
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<tr>
<td>5</td>
<td>1.1904E-04</td>
<td>-3.922</td>
<td>118.9</td>
</tr>
<tr>
<td>10</td>
<td>2.38E-04</td>
<td>-3.623</td>
<td>105.0</td>
</tr>
<tr>
<td>50</td>
<td>1.1904E-03</td>
<td>-2.924</td>
<td>61.0</td>
</tr>
</tbody>
</table>

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Fig 1: Fluoride Calibration Curve

Fig 2: Showing XRD results of various glass composition
prepared which included 1 ml of demineralization solution, 1 ml of 69% Nitric Acid and 8 ml of deionized water. Nitric Acid was added in order to dissolve any residual precipitation retained in the solution to avoid false readings. 36 samples were prepared of each composition with its respective time points and submitted for ICP.

Fig 3 shows the percentage of strontium released in the demineralizing solution. The figure shows that 23% of the total predicted strontium was released meaning 7.4 ppm of 32 ppm was released from COMPO 2 on the 3rd day. Composition 6 however displayed a constant release with only a slight increase with time starting from the 6th hour.

Fig 4 shows the percentage of fluoride released in the demineralizing solution. The figure shows that 1.2% of the total predicted fluoride was released, meaning 0.17 ppm of the total 14.1 ppm was released from COMPO 2 on the 3rd day.

DISCUSSION

The study was based upon studying the potential of fluoride and strontium containing phosphate glasses to release fluoride and strontium ions in a demineralizing medium and measuring the release of the respective ions altogether to serve as a delivery medium to provide an anti-cariogenic effect. Fluoride and Strontium have been observed to have the capability of inhibiting and reducing the demineralization of enamel apatite.
during an acid attack. It has also been observed that both strontium and fluoride have a synergistic effect in reducing demineralization of enamel and both when added together, act to enhance the process of remineralization. However, both fluoride and strontium have their individual mechanism of action, of which the mechanism of strontium is still not understood. But, it has also been noted that when strontium and fluoride are incorporated together the mechanism of action is different from the individual performance of the two individual ionic species.

In order to check the synergistic effect of strontium and fluoride, these ions were incorporated in one glass. But there was a fear of the reaction between strontium and fluoride that could cause crystallization when they both were incorporated together in glass COMPO 2. But the X-Ray diffraction spectrum had no sharp peaks indicating that no crystallization had occurred and the glass was amorphous.

The study showed that not only fluoride and strontium ions were released from all compositions but calcium, phosphorus and titanium ions were also released.

The results of ion release measurements show three different patterns of the ion release among the six glass compositions. COMPO 2 had the highest amount of fluoride release on the 3rd day as compared to the other set of glasses followed by a decrease of the release at a later time point. COMPO 6 with low strontium content had a distinguished pattern of ion release. The ion release increases rapidly until 6 hours but does not change significantly after that and remains constant at any later point. The release pattern from the COMPO 3 increases up to 3 days but levels out for the longer time points.

The amount of fluoride that was expected to be released based on the amount of fluoride incorporated was calculated to be in the range of 14 to 28 ppm. However, according to the results, the maximum amount of fluoride released from the glasses was 0.17 ppm, which counts to around 1.25% which according to the British Association for the Study of Community Dentistry is still not efficient enough to prevent enamel demineralization. The minimum amount advised by the above mentioned group in order to prevent tooth decay is accounted to be 0.5 ppm. However, the highest release just above 2% from the original content in glass was observed for the COMPO 4 on third day (Fig 23). Lynch et. al has established through his study that fluoride in the range of 0.01 – 0.2 ppm is sufficient enough to inhibit enamel demineralization and induce hydroxyapatite growth provided the fluoride is incorporated inside the site of the new crystal growth instead of the outer enamel surface.

The amount of strontium release from the COMPO 2 and COMPO 6 was significantly different, though both compositions contain the same mol% of strontium oxide; COMPO 2 with both fluoride and strontium release is almost double compared to the COMPO 6. The strontium release from COMPO 2 was still higher than from the COMPO 5, though the latter contain double amount of SrO. Several epidemiological studies have proposed the amount of strontium required in water to prevent enamel demineralization to be 5-10 ppm.

With regards to the synergistic effect of strontium and fluoride, it was noticed that maximum release of ions occurred from those glasses which had both strontium and fluoride together. This can be thought as a reason for the enhanced effect of remineralization and inhibition of demineralization as both working together have proved to have an enhanced effect in previous studies.

Although no change in $Q_{1}/Q_{2}$ ratio of glass was observed from the FTIR spectra of the glasses with variation in strontium and fluoride content, a clear difference in ions release was identified. It is possible that small substitution of strontium and addition of fluoride can still affect the kinetics of the ions release, particular when both fluoride and strontium are present in small amount in the calcium phosphate glass. The reason for this synergy should be further researched and exploited for development of the delivery of these therapeutic species for dentistry.

REFERENCES


Cariostatic Potential of the Strontium Fluorophosphate

5 Berkovitz, BKB. Oral biology, Edinburgh, Churchill Livingstone 2011; 75-78.