CHAPTER VI

Formation of hydroxyapatite layer in Ag$_2$O added phosphate based glasses

6.1. Introduction

Bioactive glasses are having a specific biological phenomenon which elicits a tight bond between bone and glass interface. These special glass systems are mainly composed of SiO$_2$, CaO, P$_2$O$_5$ and Na$_2$O. In earlier days, the bioactive glasses and glass-ceramics were developed based on SiO$_4^{4-}$ as a network forming anion, used for the replacement of damaged body parts [1]. The insoluble nature of these glasses makes hard to compatible with biological tissues even though it has potential applications. Further, the role of magnesium in the bioactivity of the glasses is not completely clear [2] and the long-term reaction of silica is still unknown [3]. It is interesting to note that the silica free phosphate glasses show good bioactivity due to its chemical composition, which is closer to that of natural bone [4, 5]. The tailors made solubility, low melting, low glass transition and softening temperatures are the identity of phosphate based bioactive glasses [6]. The addition of iron phosphate increases the cross-linkages in the network and makes the glass as a less susceptible to water attack [7]. Recently, more focus has been made on development of new bioactive glasses for different applications based on the preparation methods, the change in chemical compositions, different heat treatment conditions etc [8-11].

Attempts have been made to improve both the bioactivity and the mechanical property of bioactive glasses by adding several metal oxides such as MgO, TiO$_2$, ZrO$_2$, Al$_2$O$_3$, Ta$_2$O$_5$, La$_2$O$_3$ [12], FeO [13] etc. The added metal oxides shall act either as a network modifier or intermediate [2, 14]. It is interesting to study the structural changes in the glass samples as a function of the content of the added
metal oxides. Several parameters have to be considered for the optimisation process during the addition of metal oxides such as toxicity, solubility, mechanical property, bioactivity etc.

The antimicrobial properties of the Ag\textsuperscript{+} ion have been exploited for a long time in the biomedical field [16]. The significant feature of the silver ion is its broad-spectrum antimicrobial property, which is particularly significant for the polymicrobial colonization associated with biomaterial related infections [17]. The general finding is that bacteria show a low propensity to develop resistance to silver-based products, and therefore both metallic and ionic silver have been incorporated into the several biomaterials such as polyurethane [18], hydroxyapatite (HAp) [19] and bioactive glasses [16,20-22]. Silver containing products are also interesting materials for wound repair applications [23-25]. When metallic silver reacts with moisture on the skin surface or with wound fluids, silver ions are released, damaging bacterial RNA and DNA, thus inhibiting replication.

Sustained silver release products have a bactericidal action and manage wound exudates and odour [23, 24]. Bioactive glasses in the system SiO\textsubscript{2}–CaO–P\textsubscript{2}O\textsubscript{5}–Na\textsubscript{2}O have been shown to form a mechanically strong bond to bone and to soft tissues; bonding occurs by the rapid formation of a thin layer of hydroxyapatite (similar to biological apatite) on the glass surface when implanted or in contact with biological fluids [26]. In recent studies, the introduction of Ag\textsubscript{2}O into bioactive glass compositions aiming at minimising the risk of microbial contamination through the potential antimicrobial activity of the leaching Ag\textsuperscript{+} ions has been reported [16, 20, 22]. It has been shown that the bioactive glass composition doped with Ag\textsubscript{2}O is bacteriostatic and elicits rapid bactericidal reaction [22]. It was also demonstrated that
the incorporation of 3wt% Ag₂O conferred the existence of antimicrobial properties in the glass without compromising its bioactivity [22].

The phosphate based bioactive glass is a novel bioactive glass system composed of P₂O₅, CaO, Na₂O and Ag₂O. The addition of Ag₂O content into the glass system is aimed to study the changes in structural, physico-chemical and bioactive properties of the prepared glass sample as a function of added metal oxide. The cytotoxicity and antibacterial property nature of the prepared sample as a function of Ag₂O content is our particular interest and it will be proposed in future. In order to explore the physico-chemical properties, five series of 45P₂O₅-30CaO-(25-x)Na₂O-xAg₂O glasses, the different compositions (x = 0, 0.25, 0.5, 0.75 and 1 mol%) of the glasses have been prepared. The studies such as FTIR, SEM, pH and ultrasonic velocities and attenuation measurements have been done in all the glass compositions.

In the present investigation, an attempt has been made to study bioactivity and mechanical property of the phosphate based glasses with different compositions of Ag₂O. The bioactive nature of the glasses has been determined by immersing the glasses in a simulated body fluid (SBF) for 21 days at 310 K. The studies such as SEM and FTIR help to reveal the biocompatibility of the glasses before and after immersion in the SBF solution. Further, it also helps to optimise the glass composition for particular applications. The observed results have been discussed in terms of the change in structure, stability, mechanical properties and bioactivity of the prepared bioactive glass.

6.2 Preparation of glass sample

The 45P₂O₅-30CaO-(25-x)Na₂O-xAg₂O glass for different compositions (x = 0, 0.25, 0.5, 0.75 and 1mol%) have been prepared using commercially available
chemicals employing the normal melting quench method [27]. The different \( \text{Ag}_2\text{O} \) contents \( x = 0, 0.25, 0.5, 0.75, 1\text{mol\%} \) (here after termed as PCNA0, PCNA0.25, PCNA0.5, PCNA0.75, \& PCNA1.0 respectively) of the glasses have been prepared. The chemical components include \( \text{NH}_4\text{H}_2\text{PO}_4\cdot2\text{H}_2\text{O} \ (99.999\%), \ \text{CaCO}_3 \ (99.995\%), \ \text{Na}_2\text{CO}_3 \ (99.9\%), \ \& \ \text{Ag}_2\text{O} \ (99\%) \) were of analytical grade (Aldrich) and used without any further purifications. The addition of \( \text{Ag}_2\text{O} \) is used to act as the network modifier, to act as the nucleating agents and to improve the bioactivity [28]. The mixture was melted in an alumina crucible for 3 h at 1400 K in an electric furnace.

The molten glass was cast into a pre-heated graphite mould giving a plate (90 mm\( \times \)60 mm\( \times \)20 mm) and annealed at 573 K for 1 h using subsequent furnace cooling. After that, the glass sample left to cool overnight at a gradually descending temperature to 303 K. The glass plate was crushed and remelted to improve homogeneity of the glass. Finally, the homogenised melt were recast in a mould of rectangular shape. For the ultrasonic velocities and attenuation measurements, six glasses (rectangle) have been cut using a diamond saw from the prepared glasses. Plane parallelism between the opposite faces of the glasses has been ensured before the actual measurements. The plane parallelism between the surfaces of the glass was checked employing a surface plate and dial gauge. The percentage of error in the measurement of glass thickness is \( \pm 0.01\% \). In the present investigation, glasses were shaped in the form of disc of 10 mm diameter and 6 to 7 mm thickness. The opposite faces of the disc shaped glasses were highly polished using lapping papers. The foreign particle residues were removed by rinsing with acetone, followed by rinsing with ethanol.
6.3 Density measurements

Archimedes principle was employed to measure the density of all bioactive glass using CCl₄ as buoyant. The density of glass was obtained using the relation,
\[
\rho = \frac{W_a}{W_a - W_b} \times \rho_b
\]  
(5.1)

where \(W_a\) is the weight in air, \(W_b\) the weight in buoyant and \(\rho_b\) the density of buoyant.

All the weight measurements have been made using a digital balance (Sartorius, Model-BP221S, USA) having an accuracy of ± 0.0001 g. The experiment was repeated for five times to get the accurate value of density. The overall accuracy in the density measurement is ± 0.5 kg m⁻³. The percentage error in the measurement of density is ± 0.05 %.

6.4 Ultrasonic velocity and attenuation measurements

The longitudinal and shear ultrasonic velocity measurements have been carried out in all glasses using the cross correlation technique employing the pulse echo method as discussed in chapter-II. Ultrasonic process control system with a 100 MHz digital storage oscilloscope and a computer were employed to record the ultrasonic (rf) signals. X and Y-cut transducers operated at a fundamental frequency of 5 MHz were used both for the generation and detection of the longitudinal and shear waves respectively. The ultrasonic velocity \(U(L \text{ and } S)\) in glass was obtained using the relation [32],
\[
U = \frac{2d}{t} \text{ ms}^{-1}
\]  
(5.2)

where \(d\) is the thickness of the glass and \(t\) the precise transit time. The percentage of error in the measurement of velocity is ± 0.1 %. 

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6.5 Elastic constants

From the measured values of density ($\rho$), longitudinal ($U_L$) and shear ($U_S$) velocity in all the glass samples, the longitudinal ($\nu$), shear ($\nu$), Young’s ($Y$) and bulk ($K$) modulus, and Poisson’s ratio ($\sigma$) have been determined employing the relations which are mentioned in the Chapter II. Table 6.1 lists the above parameters at room temperature along with the compositions of glass sample.

6.6 In vitro studies

The *in vitro* studies were made to explore the bioactivity of all the prepared glasses. The SBF has been prepared in the lab whose pH value is equivalent to the pH value of the human blood plasma as given by Kokubo et al. [29, 30]. The analytical grade chemicals (Aldrich, purity > 99.95%) have been added suitably with continuous stirring in a polyethylene container to prepare the SBF solution. The methodology of preparing SBF and soaking of Ag$_2$O doped phosphate glass samples has been explained in chapter II. Both the biocompatibility and the structural changes on the surface of the glass samples before and after soaking in SBF have been characterised by SEM and FTIR studies.

6.7 pH measurements

The variation in pH values of SBF were measured in all the 21 days employing a pH meter in all glasses under identical conditions. The pH electrode has been calibrated using the standard pH of 4.01, 7.01 and 10.1 before doing pH measurements. The percentage of error in the measurement of pH is $\pm 0.005\%$.

6.8 Scanning Electron Microscopy

The scanning electron microscope (Hitachi, Model-514A, Japan) has been used to obtain surface image of all the glass samples to explore the glassy nature and the surface morphology. However, in case of bioactive glasses, the apatite layer
formation has been found by SEM. Thus, from the micrographs obtained using SEM microscope, the silica-rich layer and Ca, P layers formed on the bioactive glasses have been identified.

6.9 Fourier Transform Infra Red analysis

Infrared absorption of the powdered glass samples have been analysed from the FTIR patterns. FTIR absorption spectra have been recorded at the room temperature from 4000 to 400 cm\(^{-1}\) using FTIR (Shimatzu, Model-8700, Japan) spectrometer. A sample each of 4.0 mg has been mixed with 200 mg of KBr in agate mortar and then, pressed into pellet of 13 mm diameter [31]. For each sample, the FTIR spectrum has been normalised with the blank KBr pellet. The above studies have been made in all glass compositions before and after completing \textit{in vitro} studies.

6.10 Results

The observed density shows a negligible variation due to the initial addition of 0.25 mol\% of Ag\(_2\)O to the base glass PCNA0. A further addition of silver leads to a decrease in the density value up to 0.5 mol\% of Ag\(_2\)O content. Beyond which, a negligible changes in the density have been noticed the further addition of Ag\(_2\)O content. The Table 1.1 lists the obtained values of density, longitudinal and shear velocities, and the attenuation of the prepared glass samples as a function of Ag\(_2\)O content. The composition dependent behaviour of obtained properties such as density (\(\rho\)), longitudinal modulus (L), shear modulus (G), Young’s modulus (Y), bulk modulus (K), Poisson’s ratio (\(\sigma\)) and micro hardness (H) are shown respectively in Figs. 5.1-5.5. The elastic moduli and Poisson’s ratio reveal similar trend of vibrations as that of the density and velocities. On the other hand, the micro hardness shows a reverse trend as that of density values. The attenuation (\(\alpha_L\) & \(\alpha_S\)) show an opposite trend to that of density and velocities in all the compositions.
The change in pH value in all the samples is shown in Fig. 11. The obtained pH value reveals the influence Ag₂O content on the base glass while immersed in SBF solution. During the in vitro studies, the pH value of the SBF solution in all glass samples exhibit a uniform variation and it reaches a maximum value at the end of the 3rd day. However, the observed variations in the pH value are quite different in each glass after the 3rd day. The final pH value of the SBF solution after the 21st day along with the initial and 3rd day are given in Table 6.2 for comparison. The glass with 0.25 mol% of Ag₂O (PCNA0.25) exhibits a least value of pH as 5.58, while the glass PCNA1 shows a high value close to neutral. The pH values of the other glasses remain in between the above two values.

Figs. 6-10 reveal the SEM images of all glasses which are used to explore the surface nature of the glasses after the in vitro studies. A partial precipitation over the surface of the glass PCNA0 after the in vitro studies has been noticed. The glass with least addition of Ag₂O has shown negligible changes after the in vitro studies. The surface morphology of the glass samples PCNA0.5 and PCNA0.75 reveal a gradual amount of precipitated layer at the period of immersion. On contrary, the glass PCNA1 shows a porous surface with wet precipitations after the in vitro studies.

The FTIR patterns of the prepared glasses obtained before and after in vitro studies are shown respectively in Figs. 12 & 13. Table 6.3 lists the observed characteristic absorptions band from the FTIR patterns before and after the in vitro studies. The corresponding band assignments have been made for the clear understanding. The symmetric bending of PO₃ absorption has been noticed in all glass samples at 494 cm⁻¹ before the in vitro studies [32]. The symmetric stretching mode of vibration of P-O-P bonds noticed at 755 cm⁻¹ after the in vitro studies has been shifted to 720 cm⁻¹ with the addition of Ag₂O content [33]. The above vibrations
presented at 720 cm\(^{-1}\) is only in the glass samples PCNA0 and PCNA1 after in vitro studies. The characteristics absorption of PO\(_3\) symmetric stretching of \(\gamma\)-Ca\(_2\)P\(_2\)O\(_7\) is exhibited at 1001 cm\(^{-1}\) in the glass samples PCNA0 and PCNA1. This absorption is narrow downed to 550 cm\(^{-1}\) for the glass samples PCNA0.25, PCNA0.5 and PCNA0.75 after in vitro studies. Particularly, the glass sample PCNA1 show the above peak at 530 cm\(^{-1}\). It is observed from the Fig. 12, that the relative absorption to the P-O-P asymmetric stretching vibration is presented in all the glass samples at 891 cm\(^{-1}\)[35]. The same has been shifted to 891 cm\(^{-1}\) after in vitro studies. The absorption band at 1116 cm\(^{-1}\) has been noticed both before and after the in vitro studies confirm the presence of P-O stretching mode of vibration [36]. The Ag\(_2\)O added glasses exhibit a PO\(_2\) asymmetric stretching vibrations at 1275 cm\(^{-1}\) both before and after immersion while, the same has been found to be absent in the base glass (PCNA0) [37]. During the immersion, the calcium phosphate layers can be grown over the surface of the glasses. The hydrogen bending mode of water (CaH\(_2\)PO\(_4\)).2H\(_2\)O has been recorded at 1630 cm\(^{-1}\) in all glasses after the in vitro studies. The H\(_2\)O element associated with HAp has been recorded at 3430 cm\(^{-1}\) [38] in all the glasses after the in vitro studies.
Fig. 1. Variation of density with change in Ag₂O content

Fig. 2. Variation of longitudinal (L) and shear modulus (G) with Ag₂O content
Fig. 3. Variation of Young’s (Y) and bulk (K) modulus with MgO content

Fig. 4. Variation of Poisson’s ratio (\(\sigma\)) with \(\text{Ag}_2\text{O}\) content
Fig. 5. Variation of microhardness with AgO$_2$ content

Fig. 6. SEM micrograph of the surface of the sample with 0 mol% of Ag$_2$O content
Fig. 7. SEM micrograph of the surface of the sample with 0.25 mol% of Ag₂O content

ig. 8. SEM micrograph of the surface of the sample with 0.5 mol% of Ag₂O content
Fig. 9. SEM micrograph of the surface of the sample with 0.75 mol% of Ag₂O content

Fig. 10. SEM micrograph of the surface of the sample with 1.0 mol% of Ag₂O content
Table 1  Ultrasonic longitudinal velocity ($U_L$), shear velocity ($U_S$), longitudinal attenuation ($\alpha_L$) and shear attenuation ($\alpha_S$) along with the composition of phosphate based glass at 303 K.

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Glass composition mol%</th>
<th>Density kg m⁻³</th>
<th>Velocity m s⁻¹</th>
<th>Attenuation dB cm⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P₂O₅ CaO Na₂O Ag₂O</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PCNA0</td>
<td>45 30 25.0 0</td>
<td>2707</td>
<td>5104</td>
<td>2801</td>
</tr>
<tr>
<td>PCNA0.25</td>
<td>45 30 24.75 0.25</td>
<td>2704</td>
<td>5083</td>
<td>2766</td>
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<tr>
<td>PCNA0.5</td>
<td>45 30 24.5 0.5</td>
<td>2668</td>
<td>4553</td>
<td>2753</td>
</tr>
<tr>
<td>PCNA0.75</td>
<td>45 30 24.25 0.75</td>
<td>2704</td>
<td>5219</td>
<td>2766</td>
</tr>
<tr>
<td>PCNA1.0</td>
<td>45 30 24.0 1.0</td>
<td>2706</td>
<td>5163</td>
<td>2748</td>
</tr>
</tbody>
</table>

Table 2  The predominant variations in pH values

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>pH variations</th>
<th>Nature of the SBF solution on 21st Day</th>
<th>Bioactivity level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0 Day 3</td>
<td>Day 21</td>
<td></td>
</tr>
<tr>
<td>PCNA0</td>
<td>7.4 8.04</td>
<td>Acid</td>
<td>Low</td>
</tr>
<tr>
<td>PCNA1</td>
<td>7.4 7.72</td>
<td>Acid</td>
<td>Low</td>
</tr>
<tr>
<td>PCNA2.5</td>
<td>7.4 7.68</td>
<td>Acid</td>
<td>Low</td>
</tr>
<tr>
<td>PCNA5</td>
<td>7.4 8.05</td>
<td>Weak Acid</td>
<td>Medium</td>
</tr>
<tr>
<td>PCNA10</td>
<td>7.4 8.08</td>
<td>Weak Acid</td>
<td>High</td>
</tr>
</tbody>
</table>
Table 3: FTIR absorption bands of Ag₂O added glass system

<table>
<thead>
<tr>
<th>Wave numbers (cm⁻¹)</th>
<th>Assignments</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Before in vitro</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC N A 0 0.25 0.5 0.75 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>After in vitro</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC N A 0 0.25 0.5 0.75 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>471</td>
<td>PO₃&lt;sup&gt;3−&lt;/sup&gt; O-P-O bending</td>
<td>32</td>
</tr>
<tr>
<td>494 494 494 494 494</td>
<td>Symmetric bending PO₃</td>
<td>32</td>
</tr>
<tr>
<td>530</td>
<td>Hydroxyapatite layer</td>
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</tr>
<tr>
<td>550 550 550</td>
<td>Symmetric stretching mode of P-O</td>
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<tr>
<td>720</td>
<td>\nu&lt;sub&gt;s&lt;/sub&gt; P-O-P stretching mode</td>
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<tr>
<td>774 774 774 774 774</td>
<td>P-O-P symmetric stretching</td>
<td>32</td>
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<tr>
<td>893 893 893 893 893</td>
<td>P-O-P asymmetric stretching</td>
<td>35</td>
</tr>
<tr>
<td>1001</td>
<td>\nu&lt;sub&gt;s&lt;/sub&gt; PO₄ ( ν-Ca₃P₂O₇)</td>
<td>34</td>
</tr>
<tr>
<td>1116 1116 1116 1116 1116</td>
<td>P-O stretching mode of vibration</td>
<td>36</td>
</tr>
<tr>
<td>- 1275 1275 1275 1275</td>
<td>PO₃ asymmetric stretching</td>
<td>37</td>
</tr>
<tr>
<td>- - - - -</td>
<td>Hydrogen bending modes of water of (CaH₂PO₄)·2H₂O</td>
<td>38</td>
</tr>
<tr>
<td>- - - - -</td>
<td>Water associated with HAp</td>
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</table>
Fig. 11. Variation of pH of SBF solutions with time
Fig. 12. FTIR transmittance spectra of bioglass samples before immersion in SBF solution
Fig. 13. FTIR transmittance spectra of bioglass samples after 21 days of immersion in SBF solution
6.11 Discussions

The phosphate glasses with different Ag$_2$O contents have been used to characterise the structural modifications due to the inclusion of silver in the glass network. The ultrasonic characterisation technique is one of the well known techniques employed to optimise the structural modifications and mechanical properties. Generally, the addition of metal oxides in the glass network depolymerise and hence, breaks the P-O-P linkages and thereby creates more number of non-bridging oxygens (NBOs) [39].

The addition of metal oxides which is replaced by alkali content has acted as the network intermediate results in a change in the glass network. The change in glass network due to the addition of metal oxide by replacing the sodium content has been reported elsewhere [14]. It reveals that the phosphate network was unaltered by exchanging sodium with silver for up to one quarter of its initial content [40]. The obtained modifications in density and ultrasonic velocity, and thereby results in elastic constants have been evidenced that there is no change due to the addition of 0.25 mol% of Ag$_2$O content. A further addition of metal oxide leads to the depolymerisation of the phosphate network results in an increase in the NBOs. As results a loose packing in the glass structure has been obtained. The density, velocity and elastic moduli reveal a shift to a lower value due to the addition of 0.5 mol% of Ag$_2$O (PCNA0.5) content (Figs. 1-5). When the content of Ag$_2$O has been increased as 0.75 %, it is incorporated into the glass network at the interstitial position. Thus, it gives a tight packing in the glass structure and helps to strengthen the structure to make them as compacted. In addition, the modified cations can also provide the ionic cross-linking between the non-bridging oxygen of two phosphate chains [27].
an increase in the bond strength of these ionic cross-links lead to an improvement in the density of the glass structure. This confirms that the structural re-modifications in the glass network due to the addition of 0.75 mol% of Ag$_2$O content and hence, results in the packing of the glass structure. The dense packing of the glass structure above 0.75 mol% of silver has not been altered even with the further addition of Ag$_2$O as 1 mol%. PCNA1 and PCNA0.75 show similar observations with negligible difference.

The present study show the possibility of obtaining the better solubility with the addition of metal oxides namely, Ag$_2$O. The rate of biodegradation of the bioactive material is a complex one and it plays an important role on stability. During the in vitro studies, the immediate release of Na$^+$ from the glass samples increases the pH value in all the SBF solutions soaked with glass samples (initial pH value is 7.4) up to 8.08. As a result, a sudden change in the pH value is observed in the SBF solution within 3 days of immersion. After the 3$^{rd}$ day, the phosphate ion has started to release and dominate the Na$^+$ concentrations as reported elsewhere [41]. Thus, a decreasing trend in the pH value has been observed there after. The observed linear and non-linear variations in the pH values in SBF solution have been extensively studied in P$_2$O$_5$-CaO-Na$_2$O glass systems [42]. The above studies support our observations i.e., the obtained high initial pH value followed by a non-linear variation with increase in time [Fig. 11]. However, P$_2$O$_5$ is the network forming component and hence, the dissolved phosphate is directly linked with dissolution of the glass sample. Thus, it also helps to reveal the other ions namely Na$^+$, Ca$^+$ etc. which is dissolved in the solutions. The solubility of the glasses strongly depends on the glass compositions.

It is evident from the above results that the glass sample PCNA1 has a
compact glass structure. A decrease in the solubility of the glasses due to the addition of metal oxide and hence, the structural compactness have been studied extensively [43]. As a result, the controlled dissolution leads to the reduced release of PO$_4$ ions and hence, contributes a less variation in pH value after the 3rd day onwards. It is concluded that the Ag$_2$O may be presented in the interstitial position in the glass network results in a decrease in the solubility against an increase in the Ag$_2$O content. The role of metal oxide on the solubility of the glass sample has been discussed elsewhere [14].

The characteristic absorption related to symmetric bending of PO$_3$ which is presented before in vitro is shifted to a new position after the immersion. The glass samples of PCNA0.25, PCNA0.5 and PCNA0.75 exhibit the P-O group of vibrations at 550 cm$^{-1}$. The glass PCNA1 shows a relative vibration of the above peak at 530 cm$^{-1}$ which is ascribed due to the presence of crystalline substance of HAp. During the immersion, the growth of hydroxyapatite layer shows the evidence for the presence of bioactivity. The recorded vibrations at 530 cm$^{-1}$ particularly in the glass sample PCNA1 after immersion is due to the presence of HAp layer on the surface of the glass. The P-O-P stretching mode of vibrations observed both at 774 and 720 cm$^{-1}$ have been recorded in all the glass samples. Particularly, the glass samples PCNA0 and PCNA1 have shown similar absorptions after the in vitro studies. Similarly, the glass samples PCNA0 and PCNA1 have recorded the characteristics absorption at 1001 cm$^{-1}$ which is due to the $\gamma$-Ca$_2$P$_2$O$_7$. The above studies confirm the growth of apatite crystalline layer particularly over the glass samples PCNA0 and PCNA1. The existence of hydrogen bending of (CaH$_2$PO$_4$)$_2$H$_2$O at 1630 cm$^{-1}$ has been noticed in all the samples after the in vitro studies. The above characteristic bands help to reveal the ability of the samples to produce the calcium phosphate layer on the surface of the
samples during the immersion in SBF. In the same way, the soaked glasses has produced a support for the development of HAp layer by exhibiting the absorption at 3430 cm\(^{-1}\) related to the vibrations of water associated with HAp. The obtained FTIR patterns reveal the presence of additional apatite layer over all the glass samples during \textit{in vitro} studies. Particularly, a significant magnitude in the absorption band at 3430 cm\(^{-1}\) has been presented in the samples PCNA0 and PCNA1.

The differences among the five SEM micrographs reveal that the influence of Ag\(_2\)O content in terms of rate of formation of hydroxyapatite layer on the surface. The SEM micrograph of the base glass PCNA0 shows the wet precipitation over its surface [44]. The glass sample PCNA1 exhibits a porous formation of dense hydroxyapatite layer after \textit{in vitro} studies. The observed SEM results (Figs 6-10) confirm that the formation of apatite layer on the glass surface is independent of the Ag\(_2\)O content beyond a certain percentage. It is evident from the SEM studies, among all the glass samples, a higher magnitude of the formation of the hydroxyapatite layer has been found in PCNA1. A similar observation has been made on CaO–SiO\(_2\) glass samples support the present observations on PCNA1 glass samples.

6.12 Conclusions

The physico-chemical properties and the bioactivity of phosphate based glass system with different Ag\(_2\)O contents from 0 to 1.0 mol\% in place of Na\(_2\)O have been prepared with a fixed content of P\(_2\)O\(_5\) (45 mol\%) and CaO (30 mol\%) by keeping the ratio of P/Ca as 1.5. The initial addition of Ag\(_2\)O content up to 0.5 mol\% decreases the density of the glass with results a breaking in the networks due to the formation of NBOs. Further, addition of Ag\(_2\)O leads to an increase in the packing density, controlled solubility and increased bioactivity. The ultrasonic velocities and attenuation measurements confirm the structural changes with the addition of Ag\(_2\)O.
content. The initial addition of Ag₂O up to 0.5 mol% in the well packed glass network helps to modify the structure leading to a loose packing as evidenced in by the elastic moduli. The observed sudden modification in the structure leads to an increases in the solubility during \textit{in vitro} studies. The pH value is increased for the first three days because of the instantaneous release of sodium ions. After the third day, the phosphate ion starts to release and dominate the acidity due to phosphate molecules. All the glasses have shown a similar trend of non-uniform pH variations during \textit{in vitro} studies. The PCNA1 glass shows a higher pH value at the end of \textit{in vitro} studies. The above studies have proved that the existence of higher bioactivity in the sample PCNA1. The observed absorption bands in all the glasses at 3430 and 1630 cm\(^{-1}\) band confirm respectively the presence of O-H groups and the calcium apatite crystal. Further, SEM studies also confirm the existence of hydroxyapatite layer in all the glass samples. However in PCNA1 glass sample, a better Ca-P rich layer than the other samples. It is inferred from the above studies that all the prepared glasses are bioactive in nature. However, a higher bioactivity has been recorded in glass sample PCNA1 which is well reflected in all the studies.
References


