CHAPTER III

In vitro bioactivity of zirconium doped phosphate glass system

3.1 Introduction

In recent years, more attentions have been made by researchers on biocompatible materials for implant applications. Bioactive glasses and glass-ceramics play an important role in the replacement of bone. Recent studies [1-7] indicate the successful implantations of bioactive glasses such as the replacement of damaged or diseased body parts. Bioactive glasses have been used as a filler material in dental prosthetics and orthopedics implants. However, the microstructure, physico-chemical and mechanical properties of bioactive glasses widen the range of the options for clinical applications of this material [8, 9]. Attempts have been made to explain the bioactivity on thermal treatment conditions [10, 11]. The mechanical interactions between the implant and the surrounding tissues, the knowledge of the bone stiffness and elastic properties of bioactive glasses [12] etc., are essentially required while developing a new bioactive glass for different clinical applications.

Initially, the bioactive glasses and glass-ceramics were developed based on SiO₄⁴⁻ as a network forming anion, used for surgical implants [13]. These glasses have potential applications but hard to compatible with biological tissues due to its insoluble properties. Further, the long-term reaction of silica, both locally and systematically, is still unknown [14]. 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 [15, 16]. The role of phosphate based bioactive glasses have shown their own identity in the field of biomaterials due to its tailor made solubility with low melting, low glass transition and low softening temperatures [17]. The addition of calcium phosphate increases the cross-linkages in the network and
makes the glass as a less susceptible to water attack [18]. Recently, more focus have been made on development of new bioactive glasses for different applications based on the preparation methods, change in chemical compositions, different heat treatment conditions etc. [10, 11, 19, 20].

In order to explore the mechanical properties of the glasses, the ultrasonic studies have been made. Ultrasonic non-destructive testing (NDT) technique has been widely accepted as a unique tool for materials characterisation [21, 22]. This may be possible due to the interaction of ultrasonic waves with macro, micro and sub-microscopic particles during the propagation of ultrasonic waves into the glass, and also the availability of multimode vibrations and a wide range of frequency selection.

In the present investigation, an attempt has been made to study bioactivity and mechanical property of the phosphate based glasses added with different compositions of ZrO2. 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 bioactivity of the glasses before and after immersion in the SBF solution. Further, it also helps to optimise the suitable 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 glasses.

### 3.2 Preparation of glass sample

The 45P2O5-30CaO-(25-\(x\))Na2O-\(x\)ZrO2 glass for different compositions (\(x = 0, 0.25, 0.5, 0.75\) and 1.0 mol%) have been prepared using commercially available chemicals employing the normal melting quench method [19]. The glasses with ZrO2 content \(x = 0, 0.25, 0.5, 0.75\) & 1.0 mol% (here after termed as PCNZ0, PCNZ0.25, PCNZ0.5, PCNZ0.75 & PCNZ1.0 respectively) have been prepared. The
components include NH$_4$H$_2$PO$_4$.2H$_2$O [99.999%], CaCO$_3$ [99.995%], Na$_2$CO$_3$ [99.9%], and ZrO$_2$ [99%] were of analytical grade (Aldrich) have been used without any further purification. The addition of ZrO$_2$ helps to lower the melting temperature of the mixture, to act as the nucleating agents and to improve the bioactivity [23]. The mixture was ground well in agate mortar for 3 h and then melted in an alumina crucible for 3 h at 1300 K in an electric furnace. The molten glass was cast into a pre-heated graphite mould giving a plate (90 mm×60 mm×20 mm) and kept at 573 K for 1 h. After that, the glass sample left to cool in a furnace 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.

From the prepared glasses, six pieces (rectangle) have been cut using a diamond saw for ultrasonic velocities and attenuation measurements. Plane parallelism between the opposite faces of the glasses has been ensured before the actual measurements and checked employing a surface plate and dial gauge. The percentage of error in the measurement of glass thickness is ±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 on the surface of glass sample were removed by rinsing with acetone, followed by rinsing with ethanol.

### 3.3 Density measurements

Archimedes principle was employed to measure the density of all bioactive glass using CCl$_4$ as buoyant. The density of glass was obtained using the relation,
\[ \rho = \frac{W_a}{W_a - W_b} \times \rho_b \]  

(3.1)

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 \( \pm \ 0.0001 \) g. The experiment was repeated for five times to get the accurate value of density. The overall accuracy in the density measurement is \( \pm \ 0.5 \) kg m\(^{-3}\). The percentage error in the measurement of density is \( \pm \ 0.05 \) %.

**3.4 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 \( (U_L \) and \( U_S \)) in glass was obtained using the relation [24],

\[ U = \frac{2d}{t} \ \text{ms}^{-1} \]  

(3.2)

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

**3.5 Elastic constants**

From the measured values of density \( (\rho) \), longitudinal \( (U_L) \) and shear \( (U_S) \) velocity in all the glass samples, the longitudinal \( (L) \), shear \( (G) \), 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 3.1 lists the above parameters at room
temperature along with the compositions of glass sample.

3.6 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. [25, 26]. 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 ZrO₂ 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.

3.7 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 ±0.005%.

3.8 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.
3.9 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 [27]. 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.

3.10 Results

The experimental values of ultrasonic velocities (\(U_L\)) & (\(U_S\)) and attenuation (\(\alpha_L\)) & (\(\alpha_S\)) at 5MHz have been given in the Table 1 along with different compositions of the glass samples. The table also lists the experimental density, velocity and attenuation data for different compositions. It is inferred from the Table 3.1, that there is an initial decrease in velocity and an increase in attenuation up to 0.75 mol\% of ZrO\(_2\), beyond which both velocity and attenuation exhibit a reverse trend i.e., an increase in velocity and decrease in attenuation with further addition of ZrO\(_2\) content has been noticed. The composition dependent density shows an initial decrease up to 0.75 mol\% of ZrO\(_2\) followed by an increase with further addition of ZrO\(_2\) as that of velocity as shown in Fig. 3.1.

All the modulii exhibit the same trend of variations for entire range of compositions with addition of ZrO\(_2\) content as shown in Figs. 3.1 to 3.5. An initial decrease in longitudinal (L), shear (G), Young’s (Y) and bulk (K) modulus up to 0.25 mol\% of ZrO\(_2\) has been observed. A gradual increase in the same up to 0.75 mol\% followed by a decrease in trend for further addition of ZrO\(_2\) is observed (Figs. 3.2 & 3.3). On the other hand, the Poisson’s ratio (\(\alpha\)) shows an initial decrease up to
Table 3.1 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>( \text{P}_2\text{O}_5 )</td>
<td>( \text{CaO} )</td>
<td>( \text{Na}_2\text{O} )</td>
<td>( \text{ZrO}_2 )</td>
</tr>
<tr>
<td>PCNZ0</td>
<td>45</td>
<td>30</td>
<td>25.0</td>
<td>0</td>
</tr>
<tr>
<td>PCNZ0.25</td>
<td>45</td>
<td>30</td>
<td>24.75</td>
<td>0.25</td>
</tr>
<tr>
<td>PCNZ0.5</td>
<td>45</td>
<td>30</td>
<td>24.5</td>
<td>0.5</td>
</tr>
<tr>
<td>PCNZ0.75</td>
<td>45</td>
<td>30</td>
<td>24.25</td>
<td>0.75</td>
</tr>
<tr>
<td>PCNZ1.0</td>
<td>45</td>
<td>30</td>
<td>24.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Table 3.2 The predominant variations in pH values

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>pH variations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0</td>
</tr>
<tr>
<td>PCNZ0</td>
<td>7.4</td>
</tr>
<tr>
<td>PCNZ0.25</td>
<td>7.4</td>
</tr>
<tr>
<td>PCNZ0.5</td>
<td>7.4</td>
</tr>
<tr>
<td>PCNZ0.75</td>
<td>7.4</td>
</tr>
<tr>
<td>PCNZ1.0</td>
<td>7.4</td>
</tr>
</tbody>
</table>
Table 3.3 FTIR absorption bands of ZrO$_2$ 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>P CNZ 0</td>
<td>480 480 480</td>
<td></td>
</tr>
<tr>
<td>P CNZ 0.25</td>
<td>560 560 560</td>
<td></td>
</tr>
<tr>
<td>P CNZ 0.5</td>
<td>720 720 720</td>
<td></td>
</tr>
<tr>
<td>P CNZ 0.75</td>
<td>986 986 986</td>
<td></td>
</tr>
<tr>
<td>P CNZ 1</td>
<td>- - - - -</td>
<td></td>
</tr>
<tr>
<td></td>
<td>560 560 560</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1100 1100 1100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1630 1630 1630</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3430 3430 3430</td>
<td></td>
</tr>
<tr>
<td><strong>After in vitro</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P CNZ 0</td>
<td>480 480 480</td>
<td></td>
</tr>
<tr>
<td>P CNZ 0.25</td>
<td>560 560 560</td>
<td></td>
</tr>
<tr>
<td>P CNZ 0.5</td>
<td>720 720 720</td>
<td></td>
</tr>
<tr>
<td>P CNZ 0.75</td>
<td>986 986 986</td>
<td></td>
</tr>
<tr>
<td>P CNZ 1</td>
<td>- - - - -</td>
<td></td>
</tr>
<tr>
<td></td>
<td>560 560 560</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1100 1100 1100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1630 1630 1630</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3430 3430 3430</td>
<td></td>
</tr>
</tbody>
</table>

- PO$_4^{3-}$ O-P-O bending
- $v_4$ (P-O bending mode)
- P-O-P symmetric stretching mode
- $v_1$ (P-O-P Stretching mode)
- C-O vibrations in CO$_3^{2-}$
- $v_{as}$ PO$_4^{3-}$($\alpha$-Ca$_2$P$_2$O$_7$)
- OH$^-$
- CO$_3^{2-}$ & HCO$_3^-$

31, 32, 33, 34, 28, 38, 35
of ZrO\textsubscript{2}. The Poisson’s ratio remains almost constant with a small change in ZrO\textsubscript{2} content from 0.272 to 0.274 (Fig. 3.4). The micro hardness is increased as ZrO\textsubscript{2} increases and attains the maximum at 0.75 mol\% of ZrO\textsubscript{2} as shown in Fig. 3.5.

The SEM analysis for the glass samples with 0, 0.25, 0.5, 0.75 and 1 mol\% of added ZrO\textsubscript{2} are shown respectively in the Figs. 3.6 to 3.10. It is evident from the above micrographs that more concentration of hydroxyl apatite was found in the sample with 0.75 mol\% of ZrO\textsubscript{2}. A continuous change in pH value as a function of time has been noticed in all the glass samples. There was an increase in pH value on the 4\textsuperscript{th} day while a decrease in the same was noticed from 5\textsuperscript{th} day onwards (Fig. 3.11). The variation of pH shown in Fig. 3.11 reveals the influence of pH on different glasses immersed in SBF solution for different time periods. All the glasses exhibit a similar trend of variation from initial pH value of 7.4 to 8.0 within 3 days. The influence in pH value has been observed throughout 21 days in all glasses. However, the magnitude of the variation and the change in values are quite different in each glass after 3 days. The observed change in pH of the SBF solution immersed with PCNZ0.75 glass is very less at the end of 21 days while the all other glasses (PCNZ0, PCNZ0.25, PCNZ0.5 and PCNZ1.0) show a considerable decrease in pH value.

The FTIR spectra of all the glass samples (PCNZ0, PCNZ0.25, PCNZ0.5, PCNZ0.75 and PCNZ1.0) before and after \textit{in vitro} studies are shown in Figs. 3.12 and 3.13 respectively. The FTIR patterns before and after \textit{in vitro} studies (Fig. 3.12 & 3.13) of all glasses exhibit the evidence for the presence of phosphate group. Among the various absorptions of phosphate group of bonds the peaks at 986 and 720 cm\textsuperscript{-1} are the evidence for symmetric stretching vibrations which is attributed to the PO\textsubscript{3} and P-O-P band group respectively. The trough observed at 1100 to 1090 cm\textsuperscript{-1} can be
Fig. 3.1 Variation of density with change in ZrO₂ content

Fig. 3.2 Variation of longitudinal (L) and shear modulus (G) with ZrO₂ content
Fig. 3.3 Variation of Young’s modulus (Y) and bulk modulus (K) with ZrO₂ content

Fig. 3.4 Variation of Poisson’s ratio (σ) with ZrO₂ content
Fig. 3.5 Variation of micro hardness with ZrO₂ content

Fig. 3.6 SEM image of the surface of the sample with 0 mol% of ZrO₂ content
Fig. 3.7 SEM image of the surface of the sample with 0.25 mol% of ZrO₂ content

Fig. 3.8 SEM image of the surface of the sample with 0.5 mol% of ZrO₂ content
Fig. 3.9 SEM image of the surface of the sample with 0.75 mol\% of ZrO$_2$ content

Fig. 3.10 SEM image of the surface of the sample with 1.0 mol\% of ZrO$_2$ content
assigned to the absorption due to the vibrations of phosphate group of bands [28]. The increase in intensity observed at 1100 cm⁻¹ is possibly due to the \( \text{PO}_4^{3-} \) absorption corresponding to asymmetric stretching mode of \( \text{Ca(H}_2\text{PO}_4)_2\cdot\text{H}_2\text{O} \) [29] vibrations which is formed during the \textit{in vitro} studies. The observed band at 1630 cm⁻¹ is attributed to the hydrogen bending modes of water of \( \text{Ca(H}_2\text{PO}_4)_2\cdot\text{H}_2\text{O} \). The observed less intensity absorption at 720 cm⁻¹ corresponds to the stretching mode of the P-O-P bands. The broad absorption peak present from 560 to 480 cm⁻¹ is evident for the presence of phosphate group before and after \textit{in vitro} studies. The intensity of absorption band increases and exhibit a similar trend of fashion in all the samples confirm the existence of characteristics of apatite crystals [30]. The above results support the possibility of the formation of apatite crystalline layer over the amorphous glass samples after \textit{in vitro} studies.

The observed FTIR patterns after \textit{in vitro} studies confirm the presence of \( \text{CO}_3^{2-} \) around 1460 to 1420 cm⁻¹, and the existence of \( \text{CaCO}_3 \) group of carbonated apatite along with O–H stretching peaks of \( \text{Ca(OH)}_2 \) with a strong absorption around 3630 cm⁻¹ after immersion in SBF solution which coincide with the earlier studies [36]. It is evident from Fig. 11, the above band are totally absent before \textit{in vitro}. The observed absorption peaks after \textit{in vitro} studies corresponding to OH⁻ group of bonds around at 3430 cm⁻¹ and 1630 cm⁻¹ proves the existence of hydroxyl components orginated from \( \text{H}_2\text{O} \).
Fig. 3.11 Variation of pH of SBF with time
Fig. 3.12 FTIR transmittance spectra of glass samples before immersed in SBF
Fig. 3.13 FTIR transmittance spectra of glass samples after immersed 21 days in SBF
3.11 Discussion

Generally, when metal oxides are added into the glass network either a decrease or an increase in density will be noticed without any abnormal behaviour. In the present investigation, the observed anomalous behaviour i.e., minimum in density at 0.5 mol% of ZrO\(_2\) is interesting. The initial decrease in density with addition of ZrO\(_2\) may affect the structure resulting in the loose packing of the atoms. As a result, a decrease in density with addition of ZrO\(_2\) up to 0.5 mol% is observed as shown in Fig. 3.1. A further addition of ZrO\(_2\) leads to an increase in density from 0.5 mol% of ZrO\(_2\) content. The increase in density with addition of ZrO\(_2\) content leads to a decrease in volume due to the charge polarity of the atoms resulting in compactness of the glass network. The observed results beyond 0.5 mol% can be explained as follows: The initial introduction of the network intermediate namely ZrO\(_2\) \cite{37} leads to the breaking of P–O–P bonds phosphate network and formation of terminal oxygens. Thus, the Zr\(^{4+}\) ions are connected to PO\(_4\) tetrahedra through non-bridging oxygens (NBOs). The above results indicate that the existence of the elongation of P=O and P–O–P bonds with the addition of ZrO\(_2\). As a result, an initial decrease in density with addition of ZrO\(_2\) (<0.5 mol%) was observed. A further addition of ZrO\(_2\) (>0.5 mol%) in the glass network results in the creation of ionic cross-linking between non-bridging oxygens of two different chains, thereby reinforces the glass structure \cite{31, 38}.

The decrease in longitudinal (L), shear (G), Young’s (Y) and bulk (K) modulus up to 0.25 mol% of ZrO\(_2\) beyond which an increase in the same with further addition of ZrO\(_2\) was observed. The observed peak at 0.75 mol% shows a decreasing trend beyond 0.75 mol% of ZrO\(_2\) as shown in Figs. 2 and 3. On the other hand, the
Poisson’s ratio ($\sigma$) almost remains constant with a small change from 0.278 to 0.275 with an increase in ZrO$_2$ content from 0 to 2.5 mol\% (Fig. 4).

The size of the foreign ions plays a dominant role in regulating the chemical stability of glasses. Thus, the metallic ions with small ionic radii and high electrical charge contribute to the formation of stronger P–O–M bonds in the P$_2$O$_5$–Na$_2$O–CaO–MO glass system. Several modifying ions such as (M) Fe$^{3+}$, Al$^{3+}$, Zn$^{2+}$, Ti$^{4+}$ and Zr$^{4+}$ have been used for reinforcement in glass system [33]. Among them, Ti$^{4+}$ has been found to be very effective to improve the stability and mechanical properties of the glasses. The observed increase in density of glasses beyond 0.5 mol\% of ZrO$_2$ content confirms the strengthening of the glass structure as indicated by the increase in Young’s modulus (Figs. 2 and 3). In the present glasses, the ratio of P/Ca was kept constant as 3.00. The composition dependence of velocity shows similar observation as that of density and also exhibits a minimum at 0.5 mol\% of TiO$_2$ beyond which a continuous increase in velocity with addition of TiO$_2$ content (Table 1). The longitudinal and shear attenuation show a reverse trend to that of velocity and also exhibit a maximum at 0.5 mol\% of ZrO$_2$ content (Table 3.1).

Generally, the immersed glasses exhibit the property of solubility in SBF solution. The initial increase in pH values in the glasses to the base region may because of the release of sodium ion during the initiation of solubility. The increase of pH of SBF solution due to the release of Na$^+$ will accelerate the formation of apatite as reported elsewhere [39]. Later, the decrease in pH value increasing time is mainly because of the increase of solubility of the glasses and decrease in the sodium ion [39]. The increase in dissolution rate of the sample leads to a decrease in the pH value of the solution. The variations in the pH values ensure the exchange of ion between
the sample and SBF solution. Thus, the exchange of ions is the only essential reaction during in vitro studies to ensure the formation of hydroxyapatite layer.

The observed absorption bands in the FTIR pattern at 560 cm\(^{-1}\) (Fig. 3.13) for the apatite crystals clearly supports the possibility of the formation of apatite crystalline layer over the amorphous glass samples during the immersion in SBF. A similar observation has been noticed in MgO doped phosphate based bioactive glass [34]. The CaO and P\(_2\)O\(_5\) elements form glass with OH\(^-\) and CO\(_3^{2-}\) anions of the solution is a mixed hydroxyl carbonate apatite layer in all the immersed glasses in SBF. The presence of absorption group of CO\(_3^{2-}\) ions after in vitro reveals the formation of hydroxyl carbonate apatite layer in all the glasses which are added with ZrO\(_2\). The SEM micrograph studies also support the same observations made in FTIR i.e., the existence of hydroxyl carbonate apatite layer in all the glass samples after in vitro studies. The above studies indicate the bioactive nature of the prepared glass sample even after adding with ZrO\(_2\). Further, it is interesting to note from the SEM micrograph that the formation of hydroxyl carbonate apatite layer is in rich PCNZ0.5 and PCNZ0.75 glasses than the other glasses.

3.12 Conclusions

The P\(_2\)O\(_5\)-Na\(_2\)O-CaO-ZrO\(_2\) glass system with different ZrO\(_2\) 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 observed minima in density, velocities, modulus and a maxima in attenuation with change in ZrO\(_2\) content confirms the softening of glass network up to 0.75 mol% of ZrO\(_2\) content beyond which an increase in the compactness of glass network with further addition of ZrO\(_2\) content has been noticed. The observed results confirm the breaking of P-O-P network leads to the formation of NBO network. The results beyond 0.5 mol% of
ZrO$_2$ confirms the reinforce of the glass structure due to ionic cross linking between NBOs. 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. Therefore, a random change from 3$^{\text{rd}}$ day onwards has been noticed. All the glasses have similar trend of non uniform pH variations while PCNT0.75 glass shows higher pH value which proves the existence of higher bioactivity. The observed absorption bands in all the in vitro glass at 3430 and 1630 cm$^{-1}$ band confirms respectively the presence of OH groups and the calcium apatite crystal. Further, SEM studies also confirm the existence of hydroxyapatite layer in all the glass samples. It is inferred from the above studies that all the prepared glasses are bioactive in nature. However, the higher bioactivity has been recorded in 45P$_2$O$_5$-30CaO-24.25Na$_2$O-0.75ZrO$_2$ glass than the other compositions.
References


