In-vitro Reactivity and Antibacterial Activity of Agro-waste Derived Silicate and Phosphate Glasses

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Research Article

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**In-vitro reactivity and antibacterial activity of agro-waste derived Silicate and Phosphate glasses**

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**Abstract:** Two different categories of bioactive glasses prepared from biomass using SiO\(_2\) and P\(_2\)O\(_5\) as glass formers are reported in this study. These glasses are prepared by melt-quench technique. The glasses are evaluated in-vitro for their bioactivity assessment with the help of simulated body fluid (SBF). The formation of hydroxyapatite (HAp) above the glass surface is taken as an indicator for the glasses to be bioactive. Hence, various experimental techniques like X-Ray diffraction, Scanning electron microscopy, Energy dispersive X-ray spectroscopy, Fourier transform infrared spectroscopy, and Microwave plasma atomic emission spectroscopy are employed to assess the formation of HAp layer above the glass surface. All these results confirmed the formation of HAp layer. Further, drug loading and antibacterial studies were carried out to investigate the application of glass samples as drug delivery vehicles and antibacterial agents, respectively. These investigations proved that the as-prepared glass samples have high drug loading efficiency and antibacterial activity.

**Key words:** Glasses, biomass derived materials, hydroxyapatite, drug loading efficiency, antibacterial activity.
1. Introduction

The conservation of the environment is a big issue with growing population. Food bio-wastes are a major source of environment pollution. It causes human illness and diseases. This requires higher attention to manage these wastes. The recycling of these waste materials leads to productivity and sustainability. Rice is one of the popular food all over the globe. According to a recent study rice is approximately 50% of total dietary caloric intake [1]. The global rice demand reached up to 700 million tons. The rice husk produced from paddy is generally used in the thermal power plants [2]. Rice husk (RH) is a major source of silica [3]. Therefore, it can be employed in the formation of value added product like silica which can be used in various applications like glass decorative items, display devices, industrial materials and energy storage devices [4–6]. Other common food wastes are eggshells, which are also available everywhere. Eggshells are a rich source of Calcium carbonate (CaCO$_3$) which on calcination produce Calcium oxide (CaO) [7]. CaO is very important component in the preparation of glasses [8]. These products are considered non-toxic and environmentally favourable [9–11].

Recently Sanjeev et al. [12] have evaluated the bacterial compatibility of SiO$_2$ extracted from rice husk and found them significant for biomedical, clinical, and biological applications. Another article by Jafari et al. [13] reported the loading of Ag$_2$S with rice husk based MCM-41 nanoparticles. Their study assessed the antimicrobial character of as-prepared samples against gram-positive and negative bacteria and found significant inhibition zones. Prokopowicz et al. [14] studied the in-vitro drug loading properties of mesoporous silica microparticles combined with calcium oxide. Chen et al. [15] provided the drug loading properties of rice husk derived mesoporous glasses grafted with folic acid. However, all these studies are mainly focussed on the materials derived utilizing RH. The present report aims to
utilize RH based SiO$_2$ with CaO extracted from egg shells and other required constituents to make biocompatible glasses.

Bioactive glasses (BG’s) are being used in clinical applications since more than a decade as dental filler materials, bone grafts and scaffold formation due to their ability of bonding to bone, osteoinductivity, biodegradation and biocompatibility [16–18]. Hench was the first scientist who discovered 45S5 labelled as Bioglass [19]. The main constituents of BG’s are silica (Si), phosphorus (P), calcium (Ca), magnesium (Mg), and sodium (Na) which are naturally present in the body. BG’s are capable to connect the bone by simply forming hydroxyapatite layer on their surface. This layer works as a channel which interact with collagen fibrils of damaged bone that increases osteoinductivity and forms a new bone [20]. The surface reactivity of BG’s improves osteoblast properties and gives strength to bond the bone with BG implants [21]. Apart from this, BG’s have exhibited antibacterial effects [22,23] and enhanced angiogenesis and osteogenesis [20].

After Hench’s discovery of bioglass, a variety of compositions have been studied with some modifications. However, all these studies lack in comparison studies of agro-waste derived silicate and phosphate glasses in terms of in-vitro reactivity and antibacterial studies. To the best of our knowledge, agro-waste derived bioactive glasses containing more than 20% of MgO on molar percentage basis are not reported yet. Therefore, in order to fulfil this gap, in the present report two glasses with SiO$_2$ and P$_2$O$_5$ as network formers have been taken under present investigation along with other constituents CaO and MgO. The variation in CaO and MgO content was done to see their influence on in-vitro bioactivity, drug loading properties, physical and thermal parameters.

2. Materials and methods

2.1 Synthesis of bioactive glasses
In the present work, bioactive glasses containing SiO$_2$ and P$_2$O$_5$ as glass formers have been synthesized by melt-quench technique. SiO$_2$ and CaO were extracted from agro-waste rice husk (RH) and chicken eggshells (CES), respectively. The details of their extraction process has been given in as earlier publications [24,25]. Agro-waste derived SiO$_2$ and CaO, with P$_2$O$_5$ (purity > 99.5%), MgO (purity > 99.5%) and CoO (purity > 99%) which are purchased from HIMEDIA Co. India were used. These agro-waste extracted minerals were further utilized along with P$_2$O$_5$ and MgO in the preparation of bioactive glasses. The raw materials were weighed appropriately and mixed homogeneously with the help of mortar and pestle. These mixed batches were transferred to alumina crucibles and melted at 1400 °C in an electric furnace. Further, the bubble free melt was poured on a copper plate and quenched with another copper plate. The as-quenched glasses were immediately shifted to a furnace at 500 °C for annealing. This was done to remove thermal stresses developed in the glasses during melting process. The obtained glasses when cooled down to room temperature were ground to powder for further characterization. Attempts were made to synthesis the similar glass composition using commercial SiO$_2$. Unfortunately, the glass could not melt even at 1550 °C for long holding time. Hence, the comparison study with commercial constituents is not given in the present work. The details of glass composition along with their label is given in table 1.

2.2 Physical properties of bioactive glasses

**Physical parameters:** The physical parameters like molar volume, molar mass and density have been calculated. The following equation is employed to calculate molar volume as a function of the mole fraction of each constituent:

$$V_m = \frac{\sum X_i M_i}{\rho}$$  \hspace{1cm} (1)
Where $X_i$ and $M_i$ represent mole fraction and molecular weight of the $i^{th}$ component, and $\rho$ is the density of the bioactive glass.

**Density**: For determining the density of glass samples Archimedes’ principle is employed, where benzene was taken as an immersion liquid with density 0.876 g/cm$^3$. All the measurement was done at room temperature. The following equation is employed to measure the density of bioactive glass samples [26],

$$Density \ (g/cm^3) = \frac{W_a}{W_a - W_b} \times \rho_b$$

(2)

Where $W_a$ and $W_b$ are the masses of sample in air and benzene, respectively and $\rho_b$ is the density of benzene.

**Vickers hardness**: The micro hardness of the bioglass samples was measured by using a micro hardness tester (Model: Mitutoyo micro-hardness tester) at room temperature by applying load of 500g for a dwell time of 15s.

**2.3 SBF preparation and In-vitro reactivity**

*In-vitro* reactivity was assessed by immersion of the as-synthesized glass samples in the SBF solution. The SBF solution was prepared by following a protocol given by Kokubo [27]. In a typical procedure, the fixed amount of NaCl, NaHCO$_3$, KCl, Na$_2$HPO$_4$, MgCl$_2$.6H$_2$O, CaCl$_2$.2H$_2$O, Na$_2$SO$_4$, Tris Buffer and HCl were taken. It is assumed that SBF has ion concentration equal to human blood plasma, therefore it is suitable for the determination of bioactivity of bioactive materials [27]. Further, for assessing the bioactivity, the appropriate amount of glass samples was soaked for two weeks with SBF solution. The pH of the solutions was measured every day for an interval of 14 days to observe the pH variations due to hydration of samples with pH meter Toledo pH meter (USA). After completion of time period, samples
were dried and characterized with XRD, FTIR, SEM, EDS, MP-AES to investigate the development of apatite layer.

2.4 Degradation studies

Weight loss or degradation behaviour of as-prepared glasses was investigated by measuring the weight of glass samples before and after immersion in the SBF solution. For measurement of weight, samples were taken out from the SBF solution after two weeks and dried for one day. The following equation was used to calculate weight loss:

\[
\text{Weight loss (\%) = } \frac{W_i - W_f}{W_i} \times 100
\]  

(3)

The calculated values of weight loss after two weeks are summarized in Table 2.

2.5 Drug loading

For assessing the loading efficiency of as-prepared glass samples, Vancomycin hydrochloride (VAN) was chosen as a model drug. Drug solution was prepared using distilled water as a solvent. A stock solution with a concentration of 100mg/mL was prepared which was used for drug loading studies. A standard curve was plotted for the assessment of loading efficiency. UV-Visible spectrophotometer was used for the determination of absorbance of drug solution before and after loading at a wavelength 240 nm.

2.6 Antibacterial studies

For detection of antibacterial performance, the different amount of BG powders were washed with ethyl alcohol and dried. Then the powders are mixed in Muller hinton broth (MHB) with the help of vortex for 1 minute before performing the experiment. Two bacterial cultures Staphylococcus aureus (devoted as S.aureus) and Escherichia coli (devoted as E. coli) were chosen to test the antibacterial activity. For this, the two bacteria were cultured overnight
and spread on petri plates using pour plate technique. Then filter paper discs loaded with glass particles were placed on these petri dishes. Further, these plates were incubated in a Biochemical oxygen demand (BOD) incubator for 24 hours. After this, the plates were examined for antibacterial activity and inhibition zones were measured. For quantitative analysis of bacterial resistance, the glass samples were loaded with bacterial strains in ELISA plate reader in triplicate.

3. Bioactive Glasses Characterization

The as-prepared glass samples were characterized with X-ray diffractometer (model Xpert Pro MPD Panalytical, Netherlands) in the range $2\theta = 10$ to $80^\circ$. For morphological and microstructure analysis before and after SBF treatment, scanning electron microscope (SEM) (JSM6510LV, JEOL/EO Tokyo, JAPAN) equipped with an energy dispersive X-ray spectroscopy (EDX) instrument (INCA x-act Energy 200, Oxford Instruments, UK) was used. Differential thermal analysis/ Thermo-gravimetric analysis (DTA/TGA) was carried out to determine different thermal parameters like glass transition temperature ($T_g$), onset crystallization ($T_x$), crystallization temperature ($T_c$), and thermal stability factor ($\Delta T$) with the help of differential thermal analyser (DTA) model SII 6300 EXSTAR. To analyse the changes in the ion concentration in SBF before and after immersion of glass samples, the microwave plasma atomic emission spectroscopy (MP-AES) (4100 MP-AES Agilent Inc., Santa Clara, CA, USA) was used. Fourier transform infrared (FTIR) spectra were recorded using KBr pellet technique using Fourier transform spectrometer (Perkin Elmer Model spectrum 2) to study the functional groups present in the as-prepared system and the new groups evolved after SBF treatment.

4. Results and Discussion

4.1 Density, Molar volume and weight loss of glass samples
Fig. 1 represents density and molar volume of bioactive glasses w.r.t molar concentration of cobalt oxide (CoO). It is clear from the figure that the density shows an opposite behaviour between silicate and phosphate based bioactive glasses. In the case of BGS1 and BGS2, there is a decrease observed with increase in the CoO concentration whereas for BGP1 and BGP2 density is increasing. As density and molar volume have an inverse relation, therefore molar volume increases in silicate system and decreases for phosphate system. The molar volume is calculated by the formula reported in and given in eq. 1 [28].

Table 2 shows that the density for silicate glasses (BGS1 and BGS2) decreases from 3.84 to 3.78 (g/cm$^3$) and in phosphate glasses (BGP1 and BGP2) it increases from 2.85 to 2.90 (g/cm$^3$). This might be due to partial replacement of lighter MgO (3.58 g/cm$^3$) with heavier CoO (6.11 g/cm$^3$) [29]. The increase in molar volume for silicate glasses can be explained in terms of glass structure. The introduction of oxides in the glass modifies its structure and leads to changes in the molar volume [30]. Another possible reason for increase in molar volume in silicate glasses might be explained by the variation in bond length where shorter bond of Mg-O (1.82 Å) is replaced by longer bond length of Co-O (2.13 Å). In the case of phosphate glasses, the bond length of P=O bond is (1.51 Å) [31]. The bond length of Co-O bond is 2.13 Å which is larger than P=O, but the bond strength is greater which give a strong network. Therefore, the molar volume is reduced.

The weight loss profiles after SBF treatment for both glass systems are given in Fig. 2. It can be observed that weight loss is less in silicate glasses as compared to phosphate glasses. In case of phosphate glasses, the mechanical strength is low. Hence, these glasses dissolve more as compared to silicate glass system. Also, weight loss increases with addition of CoO in both systems.

4.2 Network connectivity and hardness studies
Network connectivity (NC) is an important parameter while investigating bioactive behaviour of glasses. Also, it plays a significant role to predict the glass structure which is favourable for bioactivity properties. This parameter defines the physiological feasibility for the development of HAp layer when it lies close to 2.0 [32]. NC is calculated with the help of following equation:

\[ NC = \frac{4 \times SiO_2 + 6 \times P_2 O_5 - 2 \times (MgO + CaO + CoO)}{SiO_2} \]  (4)

The obtained NC values are enlisted in Table 2. These values show an ascending trend in both glass systems with the inclusion of CoO. It can be observed from the Table 2 that the NC values increase with inclusion of CoO. These values indicate the feasibility of HAp layer formation with CoO. Hence, insertion of CoO in both the systems enhances NC but is more effective in the case of phosphate glasses. The reason might be the low mechanical properties of phosphate network [33].

Hardness values determine the mechanical strength of the glass system which has a direct relation with dissolution rates. In order to study this, the Vickers hardness values are obtained using following relation:

\[ H = \frac{1.854P}{d^2} \]  (5)

Where ‘P’ is load applied and ‘d’ is length of diagonal of the indentation. The calculated values are given in Table 2. It is observed from the table that these values are higher for silicate-based glasses. However, with CoO addition there is no change in the hardness of the glasses. On the other hand, the phosphate glasses exhibit less hardness because these glasses are mechanically weak as compared to silicate glasses [34].

4.3 Thermal analysis
For evaluating different thermal parameters such as glass transition ($T_g$), onset crystallization ($T_x$), crystallization temperature ($T_c$) and thermal stability factor ($\Delta T$) and the effect of CoO inclusion in both glass systems, DTA studies were performed. DTA thermograms are given in Fig. 3. All the values for thermal parameters are represented in Table 2. It can be clearly observed from the Table 2 that silicate glasses possess higher glass transition as compared to phosphate glasses. Furthermore, with increase in the concentration of cobalt, the $T_g$ values increases but there is no significant increase in $T_x$ and $T_c$. The increase in the $T_g$ after CoO insertion is due to better network connectivity, which brings compactness and need more enthalpy to break the bonds in the glass network. This increase in enthalpy further increases $T_g$ [35]. Hence the prepared silicate glasses are more stable than phosphate glasses. These glasses can be used in bone tissue engineering applications.

4.4 XRD analysis

Fig. 4 shows the diffraction patterns for glass samples before and after immersion in the SBF solution. It is clear from the Fig. 4 that the untreated samples exhibit a broad hump at 20 values in between 20 to 30°. Further with SBF treatment some peaks are observed. In case of BGS1, the peaks are observed at $2\theta = 21.88$ and $30.99^\circ$ after immersion in the SBF for a week, which are corroborated with International Centre of Diffraction Data (ICDD) card no. 00-040-0008 of apatite phase. Further, with the longer immersion period, a new peak arises at $2\theta = 19.22^\circ$.

In BGS2, peaks appear at $2\theta = 23.78$, 30.07, and $32.35^\circ$ which are well matched with ICDD card no. 01-076-1822. After a period of two weeks of SBF treatment, a new peak evolved at $2\theta = 33.49^\circ$ which again matched with above mentioned reference pattern. BGP1 shows appearance of two peaks at 25.62 and $33^\circ$ after two weeks. The evolution of peaks in BGP2 at $2\theta = 25.62$, 33° after one week and further after two weeks with the SBF treatment, at $2\theta = 25.62$, 31.82, and $33^\circ$ occurred. These peaks are well matched with ICDD card no. 01-076-1822.
All the prepared glasses exhibit evolution of HAp peaks after a prolonged immersion in SBF. It can be observed that both silicate and phosphate glasses develop HAp phase after soaking in SBF solution exhibiting the physiological change in the glass system. Nawaz et al. [36] studied bioactivity of different SiO\textsubscript{2} based glasses. The bioactivity evaluation was carried out with SBF and found a few HAp peaks after this treatment. Another study carried out by Vyas et al. [35] with CoO doped silicate bioactive glasses did not show any significant change in XRD results even after SBF treatment. It is reported earlier that inclusion of phosphate in bioactive glasses does not enhance the crystallization of apatite phase [37,38]. However, in the present study all the samples exhibited the development of HAp layer. The reason might be the employment of biomass derived components which include SiO\textsubscript{2} and CaO in the development of glasses. The use of these constituents successfully reduced the melt temperature and made the network viable for deposition of Ca-P species on the surface of glass samples. Therefore, while interaction with SBF solution these glasses developed a Ca-P rich HAp layer. This observation is significant as compared to previous studies discussed above [35,36].

4.5 SEM-EDS analysis

Fig. 5 shows the SEM images of untreated and SBF treated bioactive glasses. It is very clear from the Fig. 5 (a-d) that the surface of glass samples, without SBF treatment is clear. Further, on having a look on the sample surface after the SBF treatment, it is observed that all the samples surface is covered with a homogeneous cauliflower like HAp layer. In case of samples BGS1 and BGS2 the formed layer is more compact as shown in Fig. 5 (e and f) as compared to BGP1 and BGP2 which is given in Fig. 5 (g and h). The corresponding EDS spectra represent the composition of constituent particles. The article reported in literature by Vikas et al. [29] observed the HAp layer development with SEM and compared it with 45S5 glass. The present study shows more homogeneous HAp particle distribution as compared to their study. This might be possible due to use of agro-waste materials.
4.6 pH analysis

The pH variation provides the information about the development of HAp layer above the glass surface. This pH variation is shown in Fig. 6. The initial rise in pH variations indicates the rapid release of alkaline ions (Ca$^{2+}$ and Mg$^{2+}$) from the glass samples into the SBF solution. These ions get exchanged themselves easily with H$^+$ ions present in the SBF solution according to steps proposed by Hench [39]. These steps include the exchange of modifiers ions with H$^+$ ions, which gives rise to formation of silanol groups. Further, these silanol groups increase the pH of the SBF solution. This increased pH then interrupt O-Si-O bonds present with OH$^-$ groups and break the silica network. This broken silica then dissolves in the SBF solution and glass surface comes in a close contact with the SBF solution. After this, the agglomeration of silanol groups forms a silica rich layer which is deficient in alkaline ions. The obtained silica layer works as a connecting environment between glass surface and SBF solution. This connection is responsible for the exchange of hydroxyl and carbonated species and a layer rich in Ca and P form above the glass surface which can be assessed with the help of SEM-EDS and XRD techniques.

Once all the ions are leached out from glass surface to SBF solution, a decline in the pH is observed. Therefore, pH either become constant with time or reduced. It can be observed from the graph that pH for BGS1 and BGS2 has shown more variation whereas BGP1 and BGP2 show a less but visible variation. Hence, pH studies for all samples exhibited the variation which supports the formation of HAp layer in these samples. These results further support the findings observed in XRD and SEM-EDS results.

4.7 Quantitative ion analysis

Fig. 7 shows the variation in the ion concentration of Si, Ca, P, Mg and Co. The change in the concentration of Si from original SBF shows the dissolution of as-prepared glasses. This
confirms the formation of thin layer of silica. It is the first step towards the formation of HAp layer. The concentration variation is directly proportional to Si concentration present in the as-prepared glass samples. In case of BGS1 and BGS2 the concentration of Si is more. Hence, its release is more in these two samples. Whereas, in case of BGP1 and BGP2 the quantity of Si is less, so release is also less. The release of Si ions is beneficial in the formation of osteoblast which makes bone [40]. The data shows that the release of Ca$^{2+}$ ions is more for all the glass samples as compared to other ions. The leaching of alkaline ions shows the replacement of these ions with H$^+$ ion which is an important part for bonding and helps in formation of bones. BGS1 and BGS2 release more Si ion as compared to BGP1 and BGP2 because higher content of the phosphate ions inhibit the release of other ions from sample surface to SBF which may lead to delay in the osteogenesis process [41]. However, the data shows that P ion is released in SBF solution in larger quantity in case of BGP1 and BGP2, which is directly related to its concentration in the parent glasses. Therefore, all the glasses show an exchange of ions that occur with variation in the constituent quantity.

4.8 Band gap studies

Energy band gap is calculated in order to observe the development of apatite layer on glass samples. The band gap variation in bioactive glasses before and after SBF treatment are shown in Fig. 8. The band gap of as-prepared BG’s and after dipping in the SBF solution are calculated by Tauc’s method using following equation [42].

$$\alpha h\nu = A(h\nu-E)^n$$

Where $E$ = energy bandgap, $A$ = constant, $h\nu$ = photon energy and $n$=1/2. The band gap is determined by extrapolating the linear portion of the graph to the x-axis where $(\alpha h\nu)^2 = 0$. Here, indirect band gap is calculated because the transitions of apatite like materials are
supposed to be indirect [43]. The calculated values of band gap are given in Table 2. This table clearly shows that there is a change observed in the band gap values after the SBF treatment. The change in band gap values can be explained in terms of entrance of CoO in the glass network. As CoO enters on the expense of MgO in both systems, due to structural units balance with modifier ions, there is no creation of non-bridging oxygens (NBO’s) [8]. Also, compactness of structure increases and hence more energy is required for the movement of electrons leading to increased band gap values. Another reason for these changes might be due to formation process of HAp layer, during which breaking and making of bonds takes place which leads to structural changes. Thus, band gap values get modified after insertion of CoO.

It is reported earlier that the band gap values of HAp layer lies from 6 eV to down 3.95 eV [44,45]. The band gap values observed is approximately near to this range. There are various studies reported on bioactive properties of Co-doped glasses but these studies did not provide any band gap related information [35,46,47]. Hence, present study is providing a sight for HAp development and are consistent with previous studies done for HAp band gap.

4.9 FTIR analysis

Fig. 9 shows the FTIR spectra for all the prepared glasses without and with SBF treatment given for different time intervals. In the case of BGS1, the major bands are evolved at wavenumber 632, 698, 1041, and 1513 cm$^{-1}$. The band appeared at 632 cm$^{-1}$ is attributed towards P–O asymmetric bending motion in PO$_4^{3-}$ group [48]. The evolution of a band at 698 cm$^{-1}$ correspond to symmetric Si-O-Si bending vibrations [49]. The band observed at 1041 cm$^{-1}$ is assigned to asymmetric stretching mode of vibration of Si-O-Si group in the tetrahedral network of silicate [49]. The band observed at 1513 cm$^{-1}$ is due to C-O bond which entered in the glass structure during melting process. Further, as the time of SBF treatment increases to seven days, the band appeared at 632 cm$^{-1}$ is getting split into two small weak bands
(highlighted in a rectangle) at 617 and 636 cm\(^{-1}\) which confirms the formation of HAp layer [50]. This is an indication for the modification in the glass surface after the SBF treatment. A further increase in immersion time the bands become more pronounced at 627 and 656 cm\(^{-1}\). Moreover, the changes in the position of all bands are very clear. A shift towards higher wavenumber in case of intense band appeared at 1041 cm\(^{-1}\) confirms the formation of new bonds which is related to the development steps of silica rich layer. This layer serves as the progression step for the formation of bone like layer i.e., HAp layer. In the BGS2 glass, before SBF immersion the small but visible peaks are observed at 632, 701, 730 cm\(^{-1}\) and a most intense band at 1037 cm\(^{-1}\). The band appeared at 632 cm\(^{-1}\) is assigned to asymmetric vibrations of phosphate group. Further, the bands evolved at 701 and 730 cm\(^{-1}\) corresponds to the symmetric Si-O-Si bending vibrations [49]. The band observed at 1037 cm\(^{-1}\) is assigned to asymmetric stretching mode of vibration of Si-O-Si group as discussed in previous paragraph. With the increase in the immersion time, the change in the intensity of bands is clearly visible. After seven days, the band at 632 cm\(^{-1}\) gets shifted into two bands i.e. 623 and 642 cm\(^{-1}\) which belongs to asymmetric vibrations of phosphate in PO\(_4^{3-}\) group [51]. This division of bands indicates the development of Ca-P rich layer. Also, the most intense band splits with prolonged immersion time which indicate the structural changes while entering the HAp layer in the structure [52]. Further, for BGP1 two small peaks are evolved at 657 and 759 cm\(^{-1}\). These bands can be assigned to symmetric stretching vibrations of P-O-P in phosphate groups [33]. Further, more bands are visible at 900, 1070 and 1287 cm\(^{-1}\). These bands can be attributed to the presence of asymmetric vibration of P-O-P metaphosphates units present in the glass structure [33]. However, with increase in the immersion time there are only few bands evolved, a variation in the band intensity is also observed. Moreover, a significant shift of all the bands towards higher wavenumber is observed. In BGP2, the two little humps are observed at 700 and 789 cm\(^{-1}\). These bands are attributed to symmetric stretching vibrations of P–O–P bonding
The other bands appeared at 918 and 1081 cm\(^{-1}\) correspond to vibrations of asymmetric stretching of PO\(_2\) groups. Another band appeared at 1258 cm\(^{-1}\) can be linked with asymmetric stretching of doubly bonded oxygen vibrations (P = O) modes [53]. It can be observed that with the increase in the immersion time in the SBF, the new peaks are evolved at 614 and 681 cm\(^{-1}\). The band appeared at 614 cm\(^{-1}\) is due to presence of P–O asymmetric bending motion in the PO\(_4^{3-}\) group. Hence all these finding conclude that some changes are occurring in the glass structure due to formation of HAp like layer. These results are in agreement with all the findings from XRD, SEM-EDS, pH, MP-AES and confirmed the development of HAp layer above the bioactive glasses.

### 4.10 Drug loading efficiency (DLE)

Vancomycin hydrochloride is an amphoteric glycopeptide antibiotic. It is used against gram positive bacterial infection when the other antibiotics become non-effective. Therefore, it is chosen as a model drug to find Drug loading efficiency (DLE) when loaded with glass samples. DLE may be defined as the amount of drug loaded per unit mass of the glass particles. It is calculated using the following equation:

\[
\text{Drug loading efficiency (DLE in \%) } = \frac{C_i - C_f}{C_i} \times 100
\]  

(7)

The DLE of all samples at different drug concentrations is represented in Fig. 10. It can be observed from the graph that for the least quantity of drug, DLE is higher for BGP1 which is 64.83 %. As the concentration of drug increases, the DLE values vary for different samples. At 20 mg/mL the DLE values for BGS1, BGS2, BGP1 and BGP2 are 31%, 19.86%, 64.83% and 5.12%, respectively. The phosphate glass samples showed higher DLE as compared to silicate glasses. This can be explained on the basis of the hydrogen bonding which is stronger in phosphate glasses as compared to silicate glasses as depicted in Fig. 11. For the highest
concentration of drug i.e., 100 mg/mL the DLE values increased for all the samples and is found 88.57%, 95.5%, 84.09%, and 94.72% for BGS1, BGS2, BGP1 and BGP2, respectively. From this observation, it can be concluded that with 2% CoO, both silicate and phosphate glasses show higher DLE which might be due to partial replacement of Co$^{2+}$ for Mg$^{2+}$. The ionic radius of Co$^{2+}$ is 70 pm and Mg$^{2+}$ is 72 pm, the smaller Co$^{2+}$ ion provides free volume to accommodate drug molecules. Therefore, higher concentration of drug can be delivered and bioactive glasses can be used as drug delivery vehicles. Here, it is very important to note that the higher drug concentration can be effective in desired location where small quantities can be inactivated by enzymes or can excrete via renal filtration [54].

4.11 Antibacterial analysis

The infections in the human body are spreading very fast. The primary cause of these infections is bacteria. There are a variety of bacteria present everywhere. Some are good, and some are bad. There are two types of bacteria gram-positive and gram-negative. Gram-positive bacteria lead to infections like food poisoning, infection in the stomach and intestines, urinary tract, lungs infection and wound infections. Gram-negative bacteria cause diseases, including pneumonia, bloodstream infections, wound or surgical site infections, and meningitis in healthcare settings. However, while designing a bioactive glass composition, it is necessary to observe its resistivity against bacteria. Hence, in the present study, to assess the antibacterial activity, S. aureus and E. coli, gram-positive and gram-negative bacteria, respectively, are used. The observed inhibition zones are shown in Fig. 12. It is observed from this graph that both samples show enhanced antibacterial activity against S. aureus and E. coli. Further, Fig. 13 reveals the quantitative growth inhibition against S. aureus, E. coli and B. subtilis. In case of BGS2, the bacterial growth inhibition values are 69.04, 62.05 and 66.37 % for S. aureus, E. coli and B. subtilis, respectively. For BGP2, these values are 98.46, 55.2 and 58.02% for S. aureus, E. coli and B. subtilis, respectively. This inhibition can be explained on the basis of
Co$^{2+}$ inclusion in the system. When these ions get released from glasses, interact with negatively charged cell membrane. Through this mutual interaction Co$^{2+}$ penetrates inside cell wall and damage the cell membrane which lead to cell death [55]. Hence, the introduction of CoO inside the glass system improves it against bacterial growth.

5. Conclusion

Two glass series with two different glass formers SiO$_2$ and P$_2$O$_5$ were prepared by melt-quench route using agro-waste materials. The SBF treated glass samples demonstrated the HAp layer development which is confirmed from XRD, pH variation, SEM-EDS, MP-AES and FTIR studies. The incorporation of cobalt oxide did not show any negative impact on the formation of HAp layer. The variation in different physical and thermal parameters was observed with variation in the concentration of CoO in the prepared glasses. Further, drug loading and antibacterial studies were enhanced with the insertion of CoO which clearly indicates the positive influence of CoO in both glass systems. All these findings suggested that agro-waste derived materials can be utilized to prepare bioactive glasses with SiO$_2$ and P$_2$O$_5$ as glass formers. These glasses can be utilized in the applications of bone tissue engineering field.

Availability of Data and Material

All the data and material incorporated in the present manuscript will be made available whenever required.

Author’s contribution

Damandeep Kaur: Conceptualization, design of study, data optimization, analysis, manuscript writing.

O. P. Pandey: Supervision, Validation, Writing - review & editing.
Disclosure of potential conflict of interest

Authors do not have any conflict of interest including any financial, personal or other relationships with other people or organizations.

Compliance with ethical standards

Formal consent is not compulsory for the above type of work.

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Figure 1

Density and molar volume of glass samples as a function of CoO (mol%)
Figure 2

Weight loss profiles of SBF treated samples
Figure 3

DTA thermograms of as-prepared glasses
Figure 4

XRD diffraction patterns of all the glass samples before and after soaking in SBF solution
Figure 5

SEM along with EDS spectra of unsoaked samples a) BGS1, b) BGS2, c) BGP1 and d) BGP2, respectively and after 14 days SBF treatment. e) BGS1, f) BGS2, g) BGP1 and h) BGP2, respectively and the corresponding EDS spectra of SBF soaked glasses are shown in (i), (j), (k) and (l), respectively.
Figure 6

pH variations of SBF interaction with glass samples at different time intervals
Figure 7

Different ion concentration (mg/mL) in SBF before and after 14 days immersion of glass samples
Figure 8

Band gap variation in bioactive glasses before and after SBF treatment.
Figure 9

FTIR spectra of glass samples before and after immersion in the SBF solution
Figure 10

Drug loading efficiency for all glasses at different drug concentrations
Figure 11

VANCO (Drug molecules) interaction with as-prepared glasses in the presence of cobalt oxide.
Inhibition zones for glasses against S. aureus and E. coli

Figure 12
Figure 13

Cell growth inhibition (%) against S. aureus, E. coli and B. subtilis

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