Synthesis of Magnesium Doped Calcium Phosphate Glasses with Controllable Degradability

FINAL REPORT

SUBMITTED TO

University Grants Commission

By

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Complete Technical Report

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F. No : MRP-5514/15(SERO/UGC) dated Jan. 2015

Origination

Immediately after receiving sanction order from SERO-UGC, the proposed work has been initiated. Firstly, review of literature at National and International scenario in relation with degradability of calcium phosphate glasses embedded with magnesium ions has been completed. Meanwhile, request for quotations for required glassware, chemicals and equipment (high temperature melting furnace and annealing furnace) have been made to avoid the time delay.

Procurement of Equipment & Separate Lab Set Up

Separate research lab is made available by the college in order to keep project related equipment (high temperature furnaces) and other essential chemicals, computer etc. Duly procured high temperature melting and annealing furnaces have been installed in the lab that allows me to start preparing glass materials. Later, in trial & error technique along with literature knowledge, lot of attempts have been made to find out the suitable and optimum composition to mix magnesium ions in calcium phosphate based bioactive glasses.

Composition Details

For the proposed project work, a particular glass composition (58-x) P_2O_5-22 CaO-17 Na_2O-3 K_2O: x MgO ($0.5 \leq x \leq 2.0$ mol %) is chosen. The details of the composition and corresponding nomenclature of different samples are given below.

MG_5: 59.5 P_2O_5-20 CaO-17 Na_2O- 3 K_2O: 0.5 MgO

MG_{10}: 59.0 P_2O_5-20 CaO-17 Na_2O- 3 K_2O: 1.0 MgO

MG_{15}: 58.5 P_2O_5-20 CaO-17 Na_2O- 3 K_2O: 1.5 MgO

MG_{20}: 58.0 P_2O_5-20 CaO-17 Na_2O- 3 K_2O: 2.0 MgO

Methodology

a) Sample Preparation

Phosphorous pentoxide (P_2O_5) was added in the form of ammonium dihydrogen orthophosphate ($NH_4H_2PO_4$) whereas sodium, potassium, calcium and magnesium were introduced in the form of their respective anhydrous carbonates. All the raw materials of chemicals were of analytical grade and were used without further purification. All reagents were thoroughly mixed in an agate mortar and melted in a platinum crucible in the temperature range of 1100 to 1200 °C in a PID temperature controlled furnace for about half an hour. The resultant melts were rotated several times 30 min apart to achieve homogeneity. The homogeneous resultant bubble free melts were cast into preheated stainless steel moulds of the required dimensions. The prepared samples were directly transferred to a regulated muffle furnace at 500 °C for annealing. After 1 h, the muffle furnace was left to cool to room temperature at a rate of 30 °Ch^{-1}. The samples prepared were ground and optical polished to the dimensions of 2 cm × 2 cm × 0.2 cm.

b) Density Measurements & Estimation of Physical Parameters

The density "d" of the bulk samples was determined to an accuracy of (± 0.0001) by the standard principle of Archimedes' using *o*-xylene (99.99% pure) as the buoyant liquid.

The density (ρ) is calculated using the relation,

$$\rho = \frac{a}{a-b}\rho_w$$

Where ρ_w is the density of water, a is the mass of the glass sample in air and b is the mass of the glass sample in water. The masses of the glass samples in air and water will be measured using a digital balance.

Table 1 Physical parameters of P_2O_5- CaO- Na_2O- K_2O: MgO glass samples.

Sample	Avg. Mol. Wt. (g/mol)	Density (g/cm^3)	Conc. of 'Mg' ions N_i ($10^{21}/cm^3$)	Inter ionic distance of 'Mg' ions $r_{i\,(A°)}$	Polaron radius $r_{p\,(A°)}$
MG_5	194.2	2.533	3.92	0.63	0.25
MG_{10}	193.2	2.539	7.91	0.50	0.20
MG_{15}	192.3	2.545	11.95	0.43	0.17
Mg_{20}	191.2	2.571	12.62	0.34	0.14

Using the measured density values and other fundamental data, physical parameters viz., such as magnesium ion concentration N_i, mean magnesium ion separation r_i, polaron radius r_p have been evaluated and same shown are presented in Table 1.

From the Table 1, it is very clear that the molecular weight of glass samples is found to be decreased with increase in the content of MgO which may be due to larger molecular weight of P_2O_5 than that of magnesium oxide; whereas the density of glass samples is found to be increased with the rise in the content of MgO. A slight increase in density'd' with increase in MgO content has been observed that can be associated to the replacement of P_2O_5 with MgO. The increase in density behaviour very indicates that the addition of MgO extended the structure of loose glass network. In general, the degrees of structural compactness, the modification of the geometrical configuration of the glassy network, change in the coordination of the glass forming ions and the fluctuations in the

dimensions of the interstitial holes are the some of the factors that influence the density of the glass material. Further, it is also noticed that with increase in the content of MgO, the polaron radius and interionic distance found to be decreased which also supports the variation trend of density.

c) Preparation of Simulation Body Fluid (SBF) for *in-vitro* bioactivity test

The SBF solution is prepared by dissolving reagent grade chemicals of NaCl, $NaHCO_3$, KCl, $K_2HPO_4.3H_2O$, $MgCl_2.6H_2O$, $CaCl_2$ and Na_2SO_4 in ion distilled water as per the standard Kokubo method. The temperature of the solution has to be maintained at 37 °C throughout the experiment to simulate a human physiological environment. The pH of the solution will be buffered at 7.4 with tris–hydroxymethyl-aminomethane $[(CH_2OH)_3CNH_3]$ and hydrochloric acid. The glass samples immersed in SBF solution will be taken out, washed with deionized water, air-dried and finally stored in a vacuum desiccator. In order to investigate the bioactivity, each sample was soaked in tris-buffered simulated body fluid (SBF) solution, which resembles the human blood plasma, at 37°C, for different intervals of time. The SBF solution has ion concentration and pH nearly equal to those of human blood plasma: Na^+: 142, K^+: 5.0, Mg^{2+}: 1.5, Ca^{2+}: 2.5, Cl^-: 147.8, HCO_3^-: 4.2, HPO_4^{2-}: 1.0 and SO_4^{2-}: 0.5 m*M*, buffered at pH 7.4 with $(CH_2OH)_3CNH_3$/HCl.

d) Characterization Techniques

First and foremost, it is highly essential to check whether the samples are of amorphous or crystalline in nature after fine polishing to the required dimensions. The amorphous nature of samples was identified using Rigaku D/Max ULTIMA III X-ray diffractometer with CuK_α radiation. Scanning electron microscopy studies were also carried out on these samples to observe the crystallinity using HITACHI S-3400N Scanning Electron Microscope.

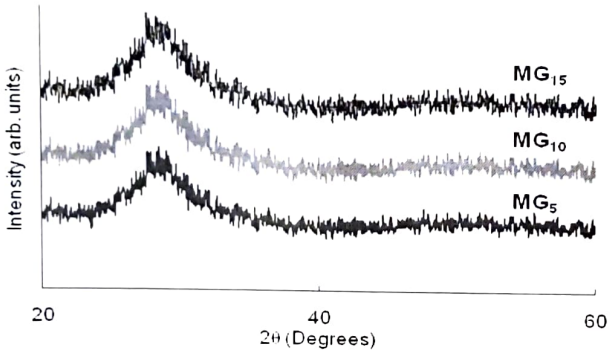

Fig. 1. XRD patterns of P_2O_5- CaO- Na_2O- K_2O: MgO glasses.

Fig. 1 shows X-ray diffraction spectra of P_2O_5-CaO-Na_2O-K_2O: MgO glasses. The spectra clearly indicated the absence of peaks which confirm prepared glasses were of amorphous. It is also an indication for the absence of sign of crystallinity that might be possible during the heating procedure. Patterns of all samples have exhibited broad halo that clearly confirmed the amorphous nature to all the glass samples. The observed broad halo may be an indication for initializing development of different crystalline phases among all glasses. This wide halo found to be very slightly increased with the content of MgO.

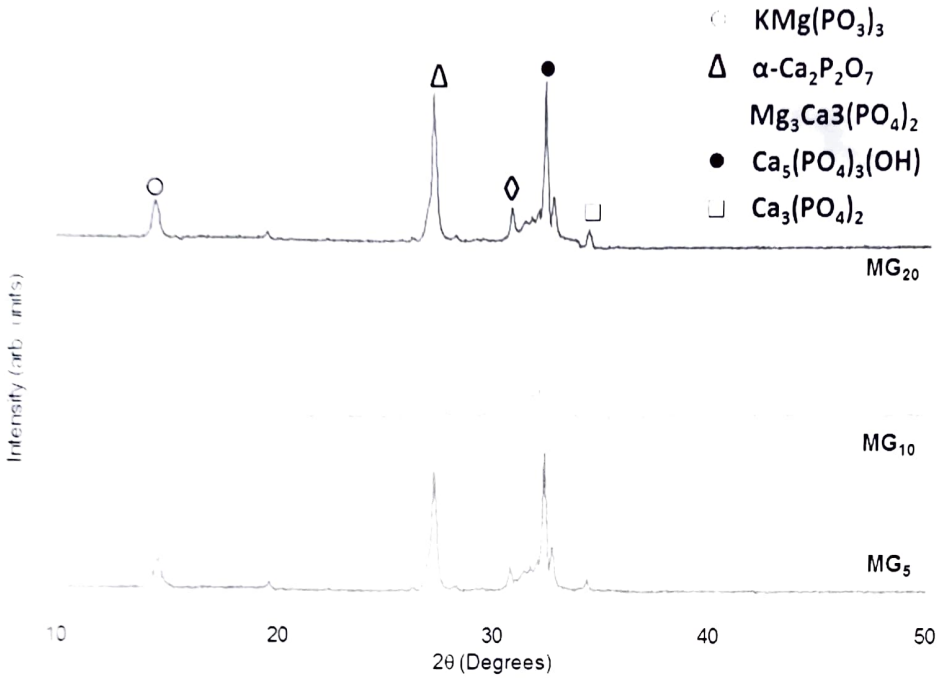

Fig. 2. XRD patterns of P_2O_5-CaO-Na_2O-K_2O: MgO glass samples.

Intensities and peak heights corresponding to crystallites $K_2Mg(PO_3)_4$, $Ca_7Mg_2P_6O_{24}$, $Na_2MgP_2O_7$, $Ca_3(PO_4)_2$ and $Ca_5(PO_4)_3(OH)$are increased while intensities and peak heights of remaining crystalline phases found to decrease. Interesting observation is that after 8 days of soaking period, samples exhibited all crystalline phases evidenced from XRD patterns, whereas intensities of most of the peaks were found to decrease significantly after 15 days that confirms glass samples have biodegradable properties. It is very clear that the addition of MgO and soaking time resulted development of magnesium enriched crystalline phases in combination with phosphate, calcium, sodium & potassium which makes glass samples to possess good bioactive potentials. Thus, it allows us to conclude that increasing trend of bioactivity evidenced from hydroxyapatite formation with increase in immersion time. It is observed that the addition of magnesium oxide increases the formation and growth of hydroxyapatite layer. Gradual increase in

the intensities of crystalline phases $Mg_3Ca_3(PO_4)_4$, $KMg(PO_3)_3$peaking at 14.75° and 30.84° with the content of magnesium oxide.

Thus, it is clearly evident that the addition of MgO would significantly enhance the hydroxyapatite forming ability which in turn bioactivity. This increase in apatite forming ability might be associated with structural changes occurred due to cross linkages in the phosphate glass network formers, modifying cations, variations of metal oxygen bond strength and electronegativity of magnesium ions.

After immersion in SBF for 7 & 15 days, no such broad halo was observed in the XRD patterns that indicates formation of different crystalline phases.XRD patterns of glass samples after soaking in SBF for 7 & 15 days resembles the same with minor change i.e. increase in intensity for 15 days and patterns for 15 days soaking period were presented in Fig. 2. These patterns have clearly evidenced the formation of different crystalline phases$K_2Mg(PO_3)_4$,$Ca_2P_2O_7$, $Ca_7Mg_2P_6O_{24}$,$Na_2MgP_2O_7$, **$Ca_5(PO_4)_3(OH)$**, $Ca_3Mg(SiO_4)_2$ and$Ca_3(PO_4)_2$withmatched intensity & angle of diffractions for standard JCPDS card numbers 24-0877, 41-0489, 20-0348, 48-0574, 09-0432, 35-0591 and 32-0176 respectively.

Scanning electron microscopy (SEM) pictures have clearly confirmed that the glass samples consisting of non-uniform, well defined and fine-grained micro crystals irregularly dispersed within a residual glassy matrix. Addition of MgO causes increasing trend of micro-crystalline structural groups incorporated in to the glass matrix, which favors the phase separation tendency. Another interesting observation is that, there were no signs of cavities and cracks might be possible during crystallization. SEM images of glass MG_{15} after *in-vitro* test for 7 & 15 days are presented in Fig. 3. After 7 days of incubation, clear change in surface morphology is observed where the sample surface

was found to partially cover with snowflake shaped particles. After 15 days of soaking in SBF, sample has exhibited higher layer formation in turn high apatite-forming ability.

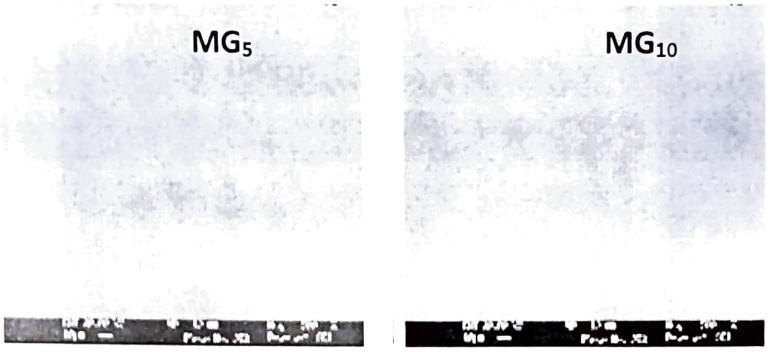

Fig. 2. SEM pictures for some of P_2O_5-CaO-Na_2O-K_2O: MgOglasses.

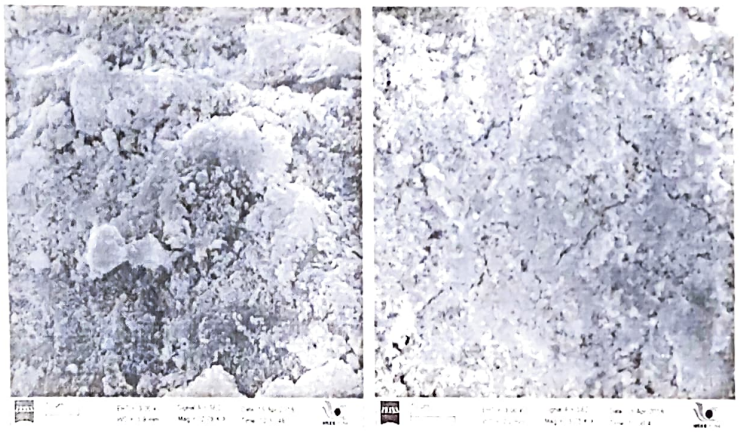

Fig. 3. SEM pictures of MG_{15} glasses after in-vitro test for 7 and 15 days.

e) FT IR Spectra

Prior to immersion and after immersion in SBF, infrared absorption spectra were recorded on a JASCO-FT/IR–5300 spectrophotometer up to a resolution of 0.1 cm^{-1} in the spectral range 400–4000 cm^{-1} using potassium bromide pellets (300 mg) containing pulverized sample (1.5 mg). These pellets were pressed in a vacuum die at ~680MPa.

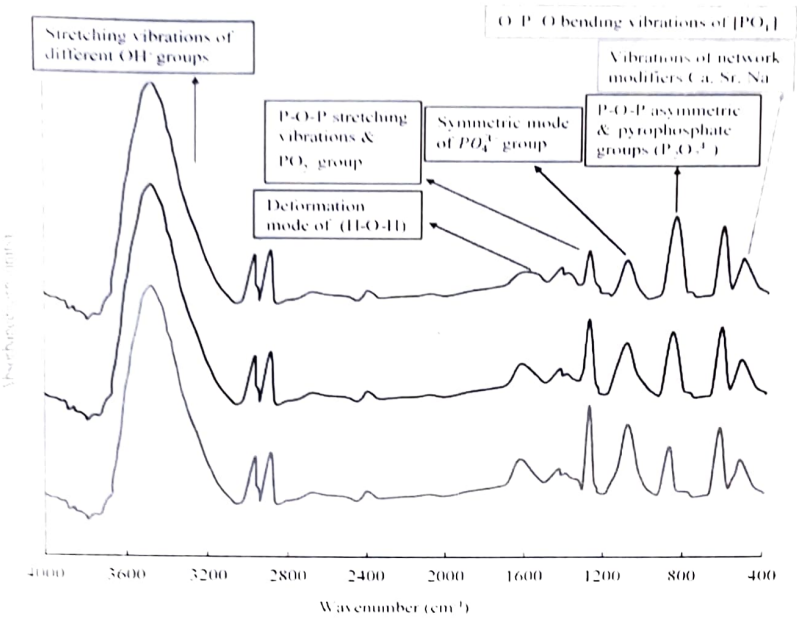

Fig.4. FT-IR absorption spectra of P_2O_5- CaO-Na_2O-K_2O: MgO glasses before SBF soaking.

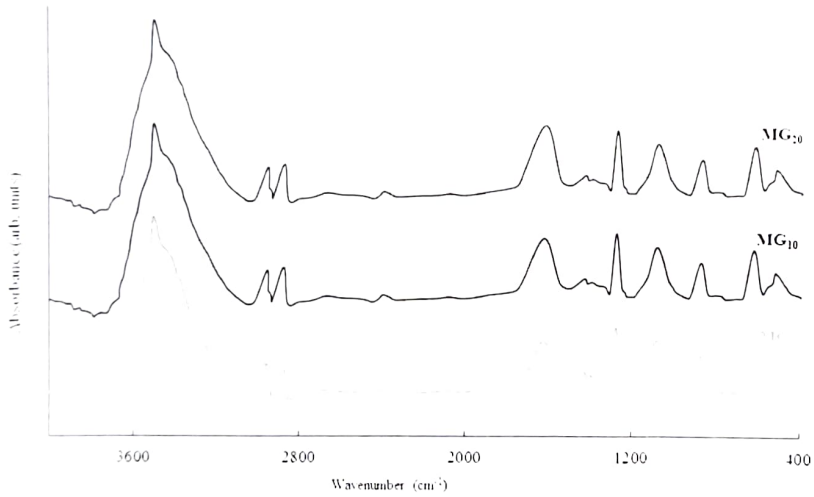

Fig.5. FT-IR absorption spectra of P_2O_5-CaO-Na_2O-K_2O: MgO glasses after SBF soaking for 15 days.

The bioactivity of glass materials completely depends on molecular structural changes in the glass network. More specifically, the bioactive properties of phosphate-based glasses depend on the presence of glass network modifying cations (Ca, Mg, Na) and the number of non-bridging oxygen (NBO) atoms.

After soaking in SBF for 7 days, the FTIR absorption spectra almost ensembles the similar spectra with minor changes in the intensities. Fig. 6 depicts the FTIR absorption spectra of glass samples after soaking time of 15 days. From the spectra, three significant prominent changes have been observed. First, splitting of O-P-O band into two peaks at about 550 and 620 cm^{-1} which is the characteristic of formation of hydroxyapatite. It is also observed that the intensities of these two peaks found to be increased slightly with the content of MgO. Second, the increase in the intensities and shifting of peaks towards lower wavenumber corresponding to modifier cations bands observed in the range 420–580 cm^{-1}. Third change is that increase in the intensities of PO_2^- vibrational bands and decrease in the PO_4^{3-} tetrahedral groups which confirms the growing degree of structural disorder. Thus it is clear that all the glass samples after exposing to SBF, intensities of bands due to PO_2^- increases at the expense of vibrational bands due to PO_4^{3-} tetrahedral groups that leading to hydroxyapatite formation become more dominant.

All bioactive glasses after soaking in SBF for 15 days have exhibited the formation of hydroxyapatite. As the immersion time is increased, the bands due to PO_2^- groups are observed to grow at the expense of the bands due to bending mode of PO_4^{3-} tetrahedral groups.It is also noteworthy that the addition of MgO content along with increase in immersion time might have shifted the peaks corresponding to O–P–O bending vibrations and bending mode of PO_4^{3-} tetrahedral groups towards lower wavenumber as structural degree of disorder around phosphate groups becomes increased.

Thus, it can be concluded that the addition of magnesium by replacing calcium would result in expanded and more loosely cross-linked phosphate glass network since the charge to size ratio of magnesium ion compared to calcium ion is slightly low. Out of all the glass samples, the sample MG_{20} found to exhibit high bioactivity since it has more network disorder with increase in immersion time.

Closing Remarks

P_2O_5-CaO-Na_2O-K_2O:MgO glass-ceramics were synthesized through conventional melt quenching and *in vitro* bioactivity test was carried out. Further, all glass samples were characterized by XRD, SEM and FTIR spectroscopic study. XRD patterns of glass samples do not show any sharp peaks, but they show broad halo that confirms the characteristic nature of glass. Formation of different micro-structural crystalline phases calcium magnesium phosphate, calcium phosphate, potassium magnesium phosphate has been clearly evidenced from the XRD patterns after soaking. The crystalline phase calcium phosphate hydroxyapatite indicating bioactivity was found to be more intense in the sample MG_{20}. SEM pictures of samples have clearly confirmed the presence of non-uniformly sized, well defined and fine-grained micro crystals irregularly dispersed within a residual glassy matrix. Out of all samples, MG_{20} is found to show high degree of disorder which also supports FTIR result. Infrared absorption spectra confirmed the presence various phosphate vibrational bands along with possible cross linkages. With increase in soaking time, there is reasonable increase in the intensities of PO_2^- vibrational bands and decrease in the PO_4^{3-} tetrahedral groups confirms the growing degree of structural disorder in the glass network. More specifically, splitting of O-P-O band into two peaks and slight increase in the intensities of both peaks confirm the formation of hydroxyapatite. From the analyses of all the results, it can be concluded that the glass MG_{20} found to exhibit high bioactivity due to more structural degree of disorder.