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5. Characteristics of Bioactive Glass Produced with Phosphate Rock from Dange in Sokoto State

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ABSTRACT

Bioactive glasses are expensive common implant materials due to lack of local production create financial burden. Facile approaches involving the use of local sourced available low cost materials substitutes are a necessity for bioactive glass production in dental and bone regeneration therapy. The utilization of phosphate rock from Dange in Sokoto state in the production of bioactive glass is carried out in this research. Phosphate rock samples were collected and beneficiated for chemical composition. Quaternary SiO_2-Na_2O-CaO-P_2O_5 bioactive glass was prepared by the conventional melt quenched method from local source phosphate rock and sand. The glass material obtained was subjected to immersion studies in simulated body fluid (SBF) for 21 days. The surface morphology of the glass before and after immersion in SBF was studied using SEM, while pH analysis was used to monitor changes on the glass surface in SBF solution. FTIR was used to confirm apatite formation on the material. Results showed that the concentration of Ca, P and C increased on the surface of the glass sample as immersion time increased, which was attributed to the formation of carbonated hydroxyapatite (HCA). The material shows ability to bond to bone making it a promising material for bone repair; therefore, phosphate rock obtained from Dange in Sokoto State, North-West, Nigeria could serve as a useful and viable low cost material for production of large scale bioactive glass for commercialization.

KEYWORDS

Sokoto phosphate, bio-glass, bioactivity, hydroxyl carbonate apatite.

1. Introduction:

The quest for implant materials to be use in bone tissue engineering, have expanded at a tremendous rate in the previous year's using materials such as metals, plastics, polymers, ceramics, and composites (Jonathan, 2016). However, the challenge to obtained materials with excellent biocompatibility, no toxicity, wear and corrosion resistances, convenient mechanical properties, high porosity without implant failure and the risk of infection complication was a concern (Food and Drugs Administration, 2018).

The discovery of the biomaterials, which exhibit not only osteoconductivity but also osteoinductivity has attracted much interests in the field of bone tissue engineering. Bioactive glasses are class of materials that have capability to bond directly with the host bone (Fu *et al,* 2011). These materials can be easily assimilated by the body and are considered to be biodegradable. Researches have revealed that artificial bones made from hydroxyapatite or a combination of hydroxyapatite (HA) and tricalcium phosphate (TCP) is a perfect substitute for natural bone owing to its excellent biocompatibility and properties close to that of human bone. Bioglass made of hydroxyapatite (HA) are available in dense and porous forms (Abreeq et al, 2013).

Calcium phosphates produced from natural sources have been synthesized through several methods, including wet chemical precipitation, sol-gel, and hydrothermal synthesis procedure (Zhang & Santos, 2000; Sandra *et al,* 2016). Phosphate glass is studied and applied in various fields including composite materials, sealing materials, orthopedic and dental implant materials, and solid electrodes because it has a low transition temperature, lower melting temperature compared to silicate glass (Kokubo *et al*., 2003; Kang & Lee, 2017).

Major mineral components of the skeleton are calcium and phosphorus. The bones stores 80 percent of phosphorus and 90 percent of calcium of total body weight. The dietary inadequacies of calcium and phosphorus on short term basis gained from skeletal stores. The bones become weaken or even breakage occurred by long term deficiencies of calcium and phosphorus (Muhammad, *et al*, 2014). Nigeria is endowed with rocks having extensive deposits of phosphates occurrence in sedimentary basins. Nigeria phosphate rock from Sokoto state are composed of phosphate oxide (33.89%) and calcium oxide (48.35%) average composition along with biologically beneficial trace elements such as Mg, SiO_2, F, Na, Mn, Al, K, and Fe. They have wide application in fertilizer, allied chemicals and others industrial used (Okosun & Alkali, 2013; Christopher *et al*., 2016). Calcium phosphate products such as bioglass are considered better candidates for periodontal regeneration because they act both as a graft material and barrier membrane to promote guided bone regeneration and can also progressively resorb. These are products manufactured mainly from phosphate rock mineral (Kattimani *et al.* 2016).

The challenges of utilizing some of the implant materials especially bioactive glass are high cost of importation of these materials. This create financial burden as it lead to high treatment charges in hospitals, thereby making many accident victims with bone issues to resort to local medication centers for treatment (Bamidele *et al*, 2009; Raw Materials Research Development Council (RMRDC), 2016).

International trade administration, (2016) explained that over 30,000 Nigerians travel to other parts of the world annually on medical tourism; as local production of medical equipment and therapy is limited to peripheral items, such as hospital beds and some low end prescription remedies as such the country remains a net importer of medical implant materials and others. These create a continuous increase in foreign exchange rate on import goods in Nigerian. In continuation of our interest in the synthesis of bioactive glass suitable for bone tissue repair, we report herein the preparation of bioactive glass in the quarter-nary system containing SiO_2-CaO-Na_2O-P_2O_5 from inexpensive and readily available Nigerian phosphate rock as precursor via the conventional melt quenched process.

2. Materials and Methods:

2.1 Materials:

The phosphate rock used as starting material was obtained from Dan kilo-Dange in Sokoto State, North-West, Nigeria for calcium oxide and phosphorous pentaoxide content and had the composition shown in **Table 1**. The silica oxide content was obtained from Ilaro sand deposit in Egbado south, Ogun State, Nigeria, and had the composition shown in **Table 2**. Analytical grade reagents was used as obtained to include; sodium carbonate (Na_2CO_3) (Loba Chemicals, 58.48%), to synthesize the bioactive glass.

2.2 Preparation of Bioactive Glass:

General procedure for bioactive glass with composition (wt%) 45.00 SiO_2, 24.50 Na_2O, 24.50 CaO and 6.00 P_2O_5 is a modification of Hench's method of conventional melt derived Bioglass® 45S5 (Pascual, 2015). Fine grain of the phosphate powder of 0.25mm sieve size and others materials were weighed in there required quantities using ML204T/00 digital weighing balance and poured in a container for mixing to obtained a homogeneous mixed glass batch. The glass batch was poured in a crucible and melted for 6 hours 33 minutes at 1450°C in an XD-1700m electric furnace. The molten glass was removed in the furnace and air quenched by pouring in to 20mm x 20mm square mould to form cube. The glass was annealed at 300^0C for 60 minutes.

Table 1. Composition of the phosphate rock

Compound (oxides)	Conc. Unit (Wt %)
P_2O_5	15.8
Cl	0.654
CaO	72.72
TiO_2	0.28
V_2O_5	0.006
MnO	0.919
Fe_2O_3	8.96
Co_3O_4	0.054

Compound (oxides)	Conc. Unit (Wt %)
CuO	0.018
GeO_2	0.035
SrO	0.299
Y_2O_3	0.15
ZrO_2	0.047
CeO_2	0.01
HgO	0.005

2.3 Characterization:

The microstructure of the glass was characterized in a PRO: X: Phenonm world, model 800-07334, serial number: MVE01570775 scanning electron microscope (SEM) before and after immersion in simulated body fluid (SBF) for maximum of 21 days. The sample was carbon-coated and observed at an accelerating voltage of 15 kV. Fourier transform infrared (FTIR, Shimadzu 8400 S), with wave-number range of 4000-400 cm^{-1}, employing KBr pellets operating in a reflectance mode with a 4 cm^{-1} resolution was used to monitor the nature of bonds present in the glass network.

Table 2. Composition of the sand

Compound (oxides)	Conc. Unit (wt%)
SiO_2	97.7
Cl	1.0
TiO_2	0.429
MnO	0.014
Fe_2O_3	0.148
CuO	0.016
As_2O_3	0.0069
SeO_2	0.0047
ZrO_2	0.0852
RuO_2	0.33
Sb_2O_3	0.026
Pr_2O_3	0.066
Eu_2O_3	0.059
Er_2O_3	0.007
Au	0.025
Ti_2O_3	0.019
PbO	0.008

2.4 Bioactivity Test:

In 1991 Kokubo developed simulated body fluid (SBF), which has become the most widely used solution for *in vitro* investigation of bioactivity of bioactive materials by providing conditions very close to those found *in vivo.* The ion concentration of simulated body fluid is nearly equal to that of human blood plasma (Srivastava and Pyere, 2012). The simulated body fluid (SBF) solution was prepared by dissolving the required amounts of analytical reagent-grade chemicals such as; the sodium chloride (NaCl), sodium bicarbonate ($NaHCO_3$), potassium chloride (KCl), di-potassium hydrogen phosphate trihydrate ($K_2HPO_4 \cdot 3H_2O$), magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), calcium chloride ($CaCl_2$), sodium sulphate (Na_2SO_4), 1M - hydrochloric (HCl) acid and tris (hydroxymethyl) aminomethane [tris buffer, $NH_2C(CH_2OH)_3$] with ions concentrations shown in **Table 3** The *in vitro* studies was carried out by immersion of bioactive glass sample in 50 ml SBF solution for a given times of 7, 14 and 21 days in a plastic container and placed in GALLENKAMP incubator at a controlled temperature of 37 ^{0}C and pH of 7.40. The SBF solutions were not refreshed throughout the period of immersion. The pH of the solution was checked daily for 9 days using a pH meter. The samples were extracted from the SBF solution after given times of 7, 14 and 21 days, rinsed with deionized water and dried for SEM and FTIR.

Table 3. Ion concentrations (mM) in human plasma in comparison with SBF (Adams and Essien, 2015)

Ion	Simulate Body Fluid	Blood plasma
Na^+	142.0	142.0
K^+	5.0	5.0
Mg^{2+}	1.5	1.5
Ca^{2+}	2.5	2.5
Cl^-	148.8	103.0
HCO_3^-	4.2	27.0
HPO_4^{2-}	1.0	1.0
SO_4^{2-}	0.5	0.5

3. Results and Discussion:

3.1. SEM Observation of Bioactive Glass before and after Immersion in Simulated Body Fluid (SBF):

In **Figure 1**, is the SEM micrograph of the glass after melted at 1450°C is shown. The clarity of the glass is the confirmation that, there is presence of Si, Na, Ca and P in the glass sample as prepared and the glass was in amorphous nature as confirmed by FTIR. The material shows homogeneous surfaces of flaky particles structure after seven days of immersion in SBF, covering layers of hydroxyapatite (HA) was observed on the glass surface as shown in **Figure 2(a).**

This is an indication that the concentration of sodium in the bioactive glass decreases in agreement with the dissolution theory of bioactive glasses in physiological fluids. That means, there was increase in concentration of Ca and P in the material due to the formation of HA on the surface of the material.

After 14 days in SBF solution, as shown in **Figure 2(b)**. The HA out growth and develop a kind of crack-like shape. Smaller particles can be seen growing out from the apatite layer. This appearance may be due to the formation of crystalline HCA by incorporation of CO_3^{2-} from the SBF solution onto the glass surface. The apatite layer became flaky and aggregated and had almost completely covered the surface of the glass after 21 days as shown in **Figure 2(c)**. Formation of the apatite layer on a glass surface through bio mineralization is thought an essential step for a glass to bond to living tissue in vivo as stated in earlier work of (Essien *et al*, 2012).

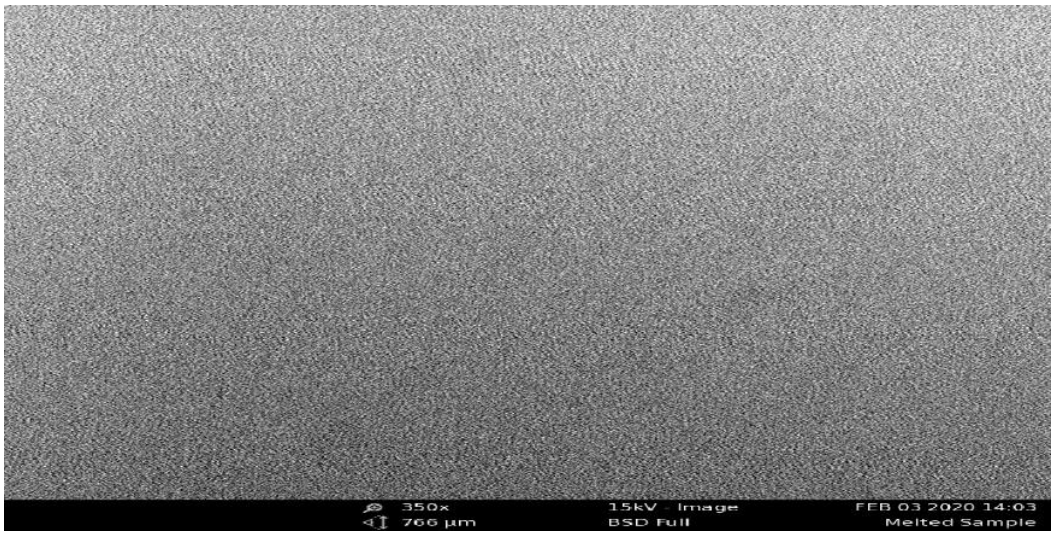
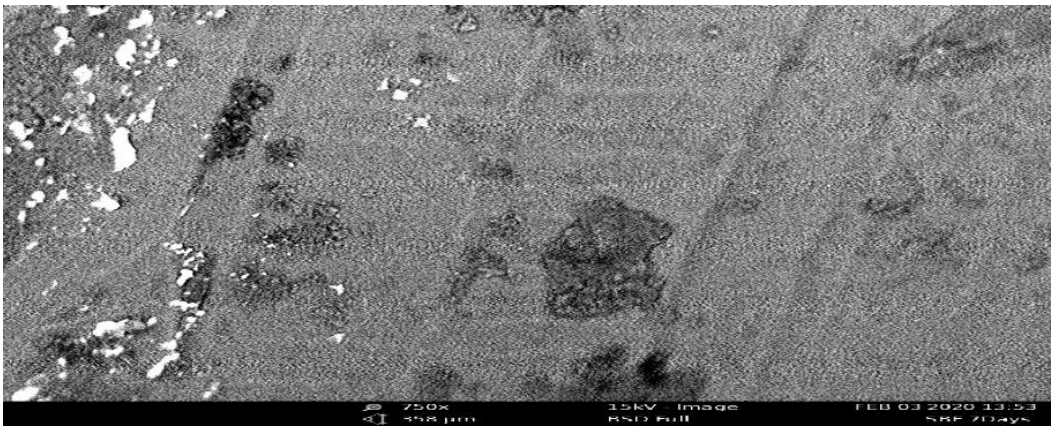
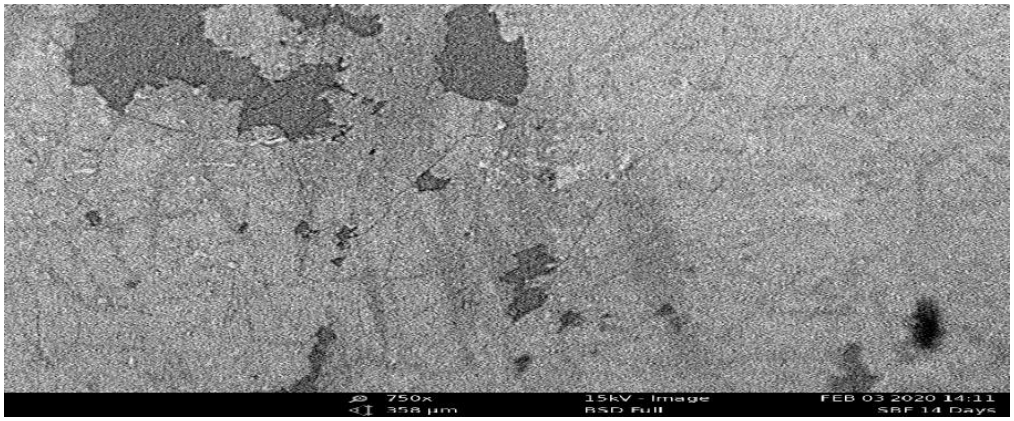


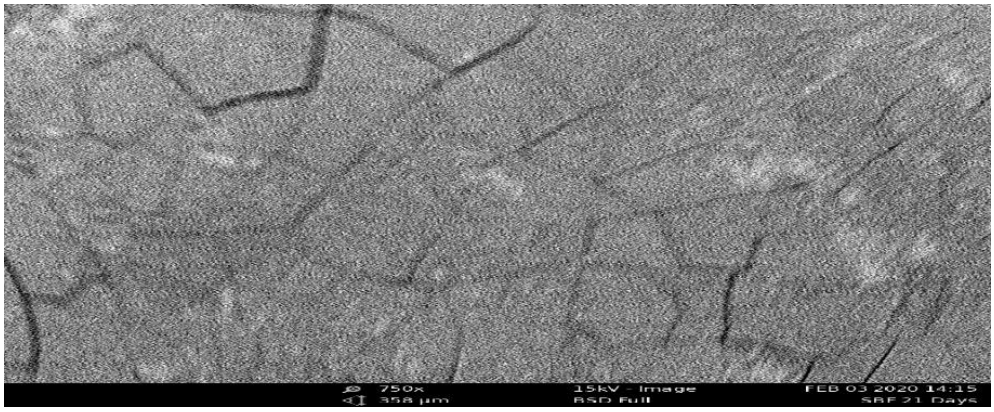
Figure 1. SEM micrograph of the bioactive glass as Melted



(a)



(b)



(c)

Figure 2. SEM micrographs of the bioactive glass after immersion in SBF for (a) 7, (b) 14 and (c) 21 days showing the growth of apatite.

3.2 pH Changes during Immersion in Simulated Body Fluid:

Figure 3 shows the changes in pH of the SBF solution after immersing the bioactive glass for the first 9 days. The pH of the solution increased sharply for the first two days reaching a value of 8.20 compared with the initial pH of 7.40; there after it remains constant until the 4th day. This is due to the release of Na^+ and Ca^{2+} ions into the surrounding solution through exchange with H^+ or H_3O^+ ions. The H^+ ions being replaced by cations, result in increase in hydroxyl concentration of the solution enabling formation of the silica glass network at the glass solution interface and attendant decrease in the pH. After day 4, the pH increases more gradually because part of the released calcium is used to form CaO-P_2O_5- rich layer, decreasing the Ca release kinetics. With prolonged immersion, the pH reached a saturated state (pH = 8.60). The pH variation of the bioactive glass is in agreement with previous studies on pH changes of SiO_2-CaO-Na_2O-P_2O_5 bioactive glass in biological fluids (Vyas *et al*, 2016).

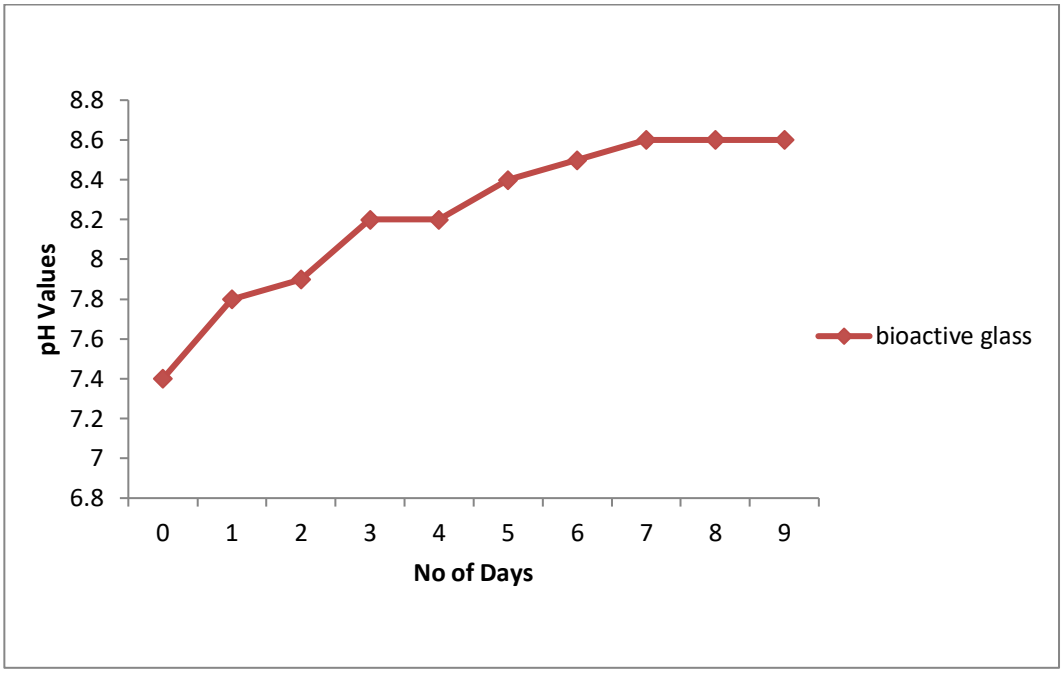


Figure 3. Days of bioactive glass immersion in SBF with pH from the initial pH = 7.4.

3.3 FTIR Evaluation of Bioactivity of the Glass:

A glass specimen is considered to exhibit bioactive behaviour if a calcium and phosphorus rich layer forms on its surface. Fourier Transform Infra-red Spectroscopy was therefore used to monitor the formation of the hydroxyl carbonate apatite (HCA) layer on the glasses immersed in simulated body fluid over a period of 0, 7, 14 and 21 days.

The FTIR spectra of the glass samples soaked in SBF for 0, 7, 14 and 21 days are shown in **Figure 4**. As observed, the spectrum of the parent glass before immersion reveals bands at 459.07 cm^{-1}, 597.95 cm^{-1}, 675.11 cm^{-1}, 746.48 cm^{-1}, 937.44 cm^{-1}, 1039.67 cm^{-1}, 1132.25 cm^{-1}, 1398.44 cm^{-1}, 1639.55 cm^{-1}, 2063.90 cm^{-1}, 2281.87 cm^{-1}, 2852.81 cm^{-1}, 2922.25 cm^{-1}, 3248.23 cm^{-1}, and 3406.40 cm^{-1}.

The prominent band at 3406 cm^{-1} and 3248 cm^{-1} is due to vibratory stretching of OH group of HA. The band at 2852cm^{-1} and 2922cm^{-1} is due to the stretching vibration of OH bond of adsorbed water molecule on the sample. The band at 2063 cm^{-1} and 2281 cm^{-1} is due to the carbonate which is absorbed in the sample may be during preparation. The band at 1639 cm^{-1} is due to the bending of OH bond of absorbed water molecule from silonol group (Si-O-Si) on the sample. The band at 1398 cm^{-1} is due to double bond of P=O stretching vibrational mode. The bands at 1039 cm^{-1} and 1132 cm^{-1} are associated with Si-O-Si and P-O vibration modes, due to asymmetric stretching vibration. The band at 937 cm^{-1} has been assigned to the network Si-O-NBO (non-bridging oxygen bonds). The band at 746 cm^{-1} is associated with Si-O-Si bending vibrations. The band at 675 cm^{-1} is assigned to OH bending mode in the sample. The band at 597 cm^{-1} assigned to PO_4^{3-} orthophosphate species within the glass; correspond to P-O bending mode due to the presence of amorphous phase in the sample.

The band at 459 cm^{-1} is associated with network Si-O-Si rocking modes. After 7 days of immersion a band appears in the region, at 939 cm^{-1} suggesting the disruption of the non-bridging oxygen due to leaching of Ca and dissolution of soluble silica at the glass interface during the period of immersion in SBF solution. Also, a new band emerged at 1404 cm^{-1} and at 2068 cm^{-1} the band split into two modes at 2045 cm^{-1} and 2021 cm^{-1} forming a twin band which attribute to the presence of CO_3^{2-}. This suggests the onset of incorporation of CO_3^{2-} in to hydroxyl carbonate apatite (HCA).

After 14 days of immersion, two band emerged 900 cm^{-1} and 1035 cm^{-1}, which may be due to increase in the concentration of Ca^{2+} on the glass surface as a result of uptake from SBF solution, the CO_3^{2-} band becomes broader, and develop new band at 1330-1454 cm^{-1}, the band at 746 cm^{-1} split in to two forming a twin mode at 740 cm^{-1}, and a band at 609 cm^{-1} emerge after the disappearance of 596 cm^{-1} band mode which are characteristic of apatite crystalline phase indicating that hydroxyl carbonate apatite (HCA) now dominate the apatite phase. As immersion days reached 21 days the bands at 1330-1454 cm^{-1} disappear while the twin band at 740 cm^{-1} fuse in to one, which was an indication of the precipitation of HCA on the glass surface in accordance with the earlier works carried out by (Nayak *et al*, 2010; Watt *et al*, 2010; Essien *et al*, 2012; Khan, *et al*, 2016; Vyas *et al*, 2016, Abifarin *et al* 2019).

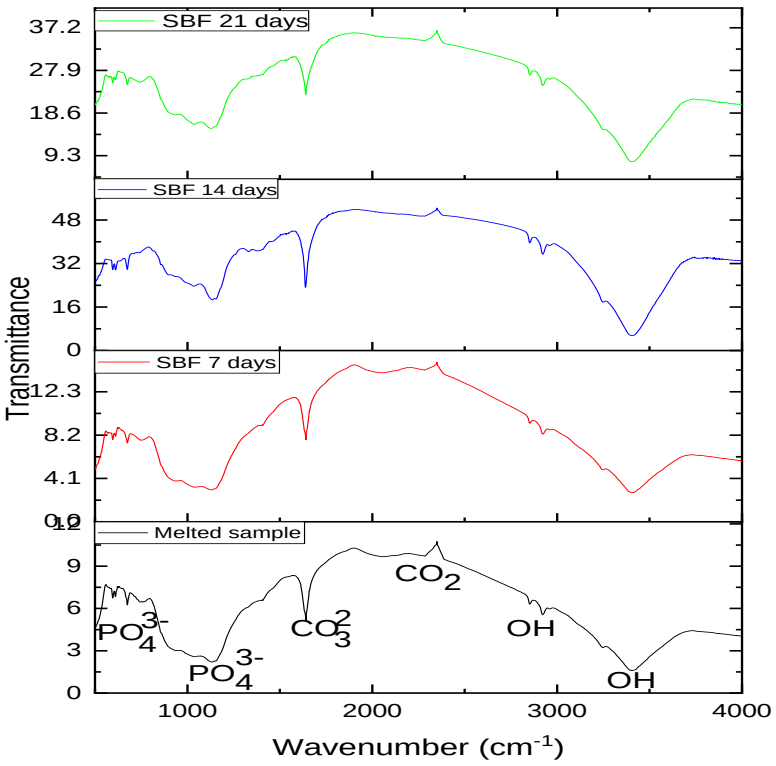


Figure 4: FTIR of melted and SBF samples

4. Conclusion:

A bioactive material composed of SiO_2-Na_2O-CaO-P_2O_5 has been prepared by the melt derived technique from phosphate rock obtained from Dange in Sokoto, Nigeria. The low-cost material is an appropriate method for preparation of a quaternary bioactive glass containing Na_2O. Immersion study shows that the pH changes in Simulated body fluid increased gradually to a value of 8.6 after 9 days, which allows us infer that the glass shows a controlled rate of degradation leading to hydroxyl carbonate apatite formation that may be useful in osteoconductivity. The levels of bioactivity, that is, the rate of apatite layer formation, and the apatite like layer thickness depend on the glass chemical composition and on the morphological parameters, such as surface area, pore size, and pore volume. The phosphate rock obtained from Dange in Sokoto State, North-West Nigeria could serve as a useful and viable low-cost material for preparing large scale calcium and phosphate source for the preparation of bioactive glasses with potentials for commercialization.

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