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(54) Title: BIOCONVERSION OF TITANIUM ADDED FLUOROPHOSPHATE GLASSES AND METHOD OF MAKING THEREOF

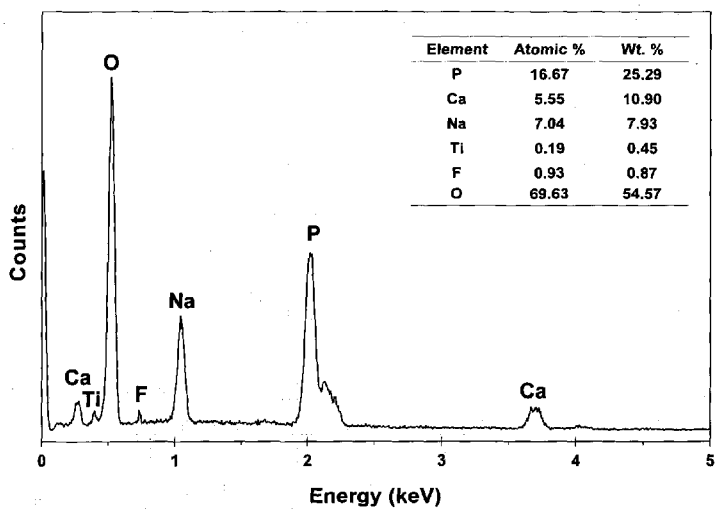

Fig. 6

(57) Abstract: Abstract The present invention discloses different composition of titanium added fluorophosphate glasses prepared using melt-quench method. The physico-chemical and bio conversion of titanium added fluorophosphate glasses were accessed using density measurements, ultrasonic measurements to determine elastic moduli, XRD patterns, FTIR spectra, XPS spectrograph, pH variations during 21 days of in vitro studies in SBF solution, SEM images and EDS spectra. Bioconversion was also accessed by in vivo studies of implantation of the optimized glass into animal bone for 10 weeks followed by SEM images, EDS spectra and CLSM images. The result obtained before, after in vitro and in vivo studies are discussed in terms of structure, stability, mechanical properties, bone bonding ability and bioconversion of the prepared glass samples. The sample TiFP4 is found to be more ideal and better than other samples for future clinical use.

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FORM 2

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COMPLETE SPECIFICATION

(See section 10 and rule 13)

Bioconversion of titanium added fluorophosphate glasses and method of making thereof

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The following specification particularly describes the invention and the manner in which it is to be made

DESCRIPTION

Field of invention

The present invention describes the composition of titanium added fluorophosphate glasses by melt quenching technique for different bio-medical applications.

Background of invention with regard to the drawback of associated known art

A number of materials have been examined for their ability to regenerate new bone. Currently, autologous bone remains the preferred material in bone graft and regenerative procedures. This bone, taken from a secondary site within the body is often excised and reimplanted. It contains both the inorganic mineral hydroxyapatite as well as the cell characteristics of bone. Even this is not a living material, but can remodel into new and functional bone. Autografts may be combined with supplementary agents such as growth factors or synthetic bone replacement materials. The disadvantage of autologous bone usage is the creation of a secondary trauma site that has to heal. Further, its usage is limited by availability. The materials taken from cadavers (allograft) are not constrained by supply. The above material is often demineralized, leaving behind a collagenous scaffold for the growth of a new bone. Further, it fails to function as an osteoinductive but only as osteoconductive material. These materials always carry the risk of disease transmission with them.

The third generation of materials used for the regenerating new bone is bioactive glass. These materials have the unique ability to form a chemical bond with the host tissue when implanted. Thus, it allows to retain the structural integrity and to resist movement at the implantation site. The chemical bond that occurs through the formation of hydroxyapatite on the glass surface converts the glass into bone rich material. The beneficial effects of bioactive glasses on cellular growth also substantiate to their use.

Attempts were made to synthesis artificial bone graft material which is suitable for different biomedical applications. L.L. Hench in Imperial college, London succeeded in developing silica based material, named Bioglass, in the year of 1960. But, it was hard to get good compatibility with biological tissues because of their relative insoluble nature. Silica-free phosphate glasses, namely bioactive glasses, were made in the year 1996. These glasses show better bioactivity due to their chemical composition, which is compatible with that of natural bone. But still the slow rate of biological conversion and poor mechanical strength of bioactive glasses hampers its clinical application. To circumvent these pitfalls a better composition of bioactive glass with higher porosity, elastic moduli, mechanical strength, bioconversion and controlled rate of dissolution near that of natural bone is continuously searched.

Object of invention

It is interesting to note that the simple phosphate glasses do not have high chemical stability when compared with multicomponent phosphate glasses. The poor mechanical strength of bioactive glass is a restrictive factor for load-bearing applications. In recent years, several attempts have been made to improve physico-chemical properties of glasses by adding metal oxide as a network modifier. Attempts are made to study the degradation of TiO_2-stabilised phosphate glasses. TiO_2 is a bioinert metal and it does not develop any allergic/inflammatory reaction on contact with body tissues. Hence, TiO_2 is used to induce calcium phosphate surface nucleation in calcium phosphate glass systems. The addition of TiO_2 enhances chemical durability due to the presence of Ti—O—P bonds rather than P—O—P bonds. Excessive fluorine on prolonged use is known to produce soft tissue ossification. But on optimised low doses it helps in new bone formation. To assess the optimal dose of fluorine in-improving bioconversion, it is added in incremental doses and analysed.

The present invention discloses the ideal composition of titanium added fluorophosphate glasses P_2O_5—CaO—Na_2O—TiO_2—CaF_2 prepared by keeping the ratio of P/Ca, as a constant. The addition of fluorine to titanium added phosphate base glass system induces better surface nucleation. The dissolution of glass is not the only important factor for the bone-bonding ability of glass, but the composition of glass also plays an important role. The present invention discloses the improved bioactivity by adding fluoride molecules and enhanced mechanical strength, load bearing capability, elastic moduli etc. by adding titanium.

Statement of invention

1. The protocol for melt quenching method to produce titanium added fluorophosphate glasses.
2. The addition of titanium with fluorophosphate yield better mechanical strength.
3. Physico-chemical properties of titanium added fluorophosphate glasses are accessed.
4. The bioconversion of titanium added fluorophosphate glass is ascertained.
5. No cytotoxicity is observed in titanium added fluorophosphate glasses up to the addition of 2 mol% of titanium content.

A summary of invention

The present invention discloses the composition with different contents of P_2O_5–CaO–Na_2O–TiO_2–CaF_2 (TiFP1, TiFP2, TiFP3, TiFP4 and TiFP5) glass systems are prepared using normal melt-quench method by keeping the ratio of P/Ca as a constant. The physico-chemical and bioactivity of the titanium added fluorophosphate were assessed using density measurements, ultrasonic velocity measurements, x-ray diffraction (XRD) patterns, Fourier transform infrared spectra (FTIR), pH variation of the sample glasses soaked 21 days in the laboratory prepared simulated body fluid (SBF) solution, scanning electron microscope (SEM) images, energy dispersive x-ray spectra (EDS) and x-ray photo electron spectrograph (XPS). Toxic nature of the selected glass sample was assessed using cytotoxicity study in cell culture lines. Bioconversion was also accessed by *in vivo* studies of implantation of the glass into animal bone followed by confocal laser scanning micrograph (CLSM) images. The results obtained before, after *in vitro* and *in vivo* studies are discussed in terms of change in structure, stability, mechanical properties, bone-bonding ability and bioconversion of the prepared glass samples.

Detailed Description

Titanium added fluorophosphate glass composition P_2O_5–CaO–Na_2O–TiO_2–CaF_2, methods of preparation and use thereof are disclosed. The glasses can be used for different bio-medical applications such as prosthetic implants, stents, screws, plates, tubes, controlled drug delivery etc. The addition of fluorine is made at the expense of Na_2O content in the glass composition and keeping the P/Ca ratio as constant. The glasses were prepared in the mentioned compositions include various salts in mol% with the following ranges:

S. No.	Salt	Ratio in mol%
1.	P_2O_5	36–65
2.	CaO	22–36
3.	Na_2O	11–32
4.	TiO_2	0.01–10
5.	CaF_2	0.01–15

To access the variation if any, the following chemicals were alternatively used as per its proportionate molecular weight:

S.No.	Required salt	Alternatively used
1.	P_2O_5	Ammonium di hydrogen phosphate, Phosphorous chloride, ammonium phosphate, calcium phosphate, sodium phosphate, silver phosphate etc.
2.	CaO	Calcium sulphate, calcium carbonate, calcium fluoride, calcium fluorophosphate, calcium chloride, calcium caseinate, calcium bicarbonate etc.
3.	Na_2O	Sodium carbonate, sodium citrate.
4.	TiO_2	Titanium fluoride, Titanium carbonate, Titanium phosphate, Titanium chloride etc.
5.	CaF_2	Calcium di fluoride, calcium tri fluoride, calcium fluorophosphate, sodium fluoride etc.

The influence of fluorine on titanium added phosphate glass system is studied in terms of pH variations during *in vitro* studies, FTIR spectra, XRD etc. The structural role of titanium in loose packing of glass network is noticed. The hydroxyapatite (HAp) forming ability of prepared glasses is carried through *in vitro* studies in simulated body fluid (SBF). The scanning electron microscopy (SEM) images before and after *in vitro* studies show the formation of HAp in all glass surfaces, while a higher rate of formation of HAp is evidenced on fluorine added glasses rather than fluorine free glass. FTIR spectra and XRD patterns that are observed support its higher bioactivity. Further, the cell viability test showed no cytotoxicity on MIT assays and hence these glasses are suitable for synthetic bone graft material for human use. The *in vivo* studies were done on rabbit by implanting the optimised sample TiFP4 into the femoral condyle. SEM and CLSM studies on the un-decalcified sectioning of the implant after 10 weeks confirm the bioconversion of glass into bone.

Materials and method

Titanium added fluorophosphate glasses were prepared by melting the homogeneous mixture of phosphate, calcium, sodium, titanium and fluoride salts followed by sudden quenching of the melt. The derivatives of phosphate, calcium, sodium, titanium and fluoride salts were weighed accurately and ground using mortar and pestle/ planetary ball mill. The mixture was fed into alumina crucible and then preheated at a temperature ranging from 140 ^{o}C to 190 ^{o}C for 1 h in a closed furnace and cooled to room temperature at a rate of 1 ^{o}C per minute. The preheated mixture was ground using mortar and pestle/ planetary ball mill. The mixture was taken in a platinum crucible and melted at the temperature ranging from 1050 ^{o}C to 1400 ^{o}C for 1 h. The temperature in the furnace was kept constant throughout the process. The melt was poured in a preheated graphite/ steel mould and cooled to room temperature. In this method, the entire synthesis protocol is successfully done without the usage of any toxic chemicals.

The method for preparing titanium added fluorophosphate glasses comprise the following steps:

a) The calculated quantities of the chemicals were weighed accurately using an electronic balance.

b) The weighed chemicals were ground using mortar and pestle/ planetary ball mill for 1 h to obtain a homogeneous mixture.

c) The mixture was preheated at a temperature ranging from 140 ^{o}C to 190 ^{o}C for 1 h in a closed furnace and cooled to room temperature at a rate of 1 ^{o}C per minute.

d) The preheated mixture was ground using mortar and pestle/ planetary ball mill to obtain a homogeneous powder.

e) The obtained homogeneous powder was taken in a platinum crucible (10% Rhodium doped) and melted at the temperature ranging from 1050 ^{o}C to 1400 ^{o}C for 1 h in electric furnace.

f) The melt was suddenly quenched in a preheated steel/ graphite mould of temperature 300 ^{o}C – 600 ^{o}C and then cooled to room temperature.

g) The obtained solid glass sample is annealed at 300 ^{o}C – 500 ^{o}C for 1 h and cooled at the rate of 0.5 ^{o}C - 2 ^{o}C per minute.

h) The prepared glass sample was cut into required shape and size using a diamond cutter for different characterisation studies.

Example 1:

21.3221 g of P_2O_5, 4.7899 g of CaO, 2.7383 g of Na_2O and 1.1497 g of TiO_2 pure chemicals were taken in agate mortar/ planetary ball mill. Ethanol was added with the mixture and ground for 1 h to obtain a homogeneous mixture. The mixture was dried at 100 ^oC -200 ^oC for 1 h and ground using agate mortar/ planetary ball mill to obtain a fine powder. The powdered mixture was melted in platinum doped with 10% Rhodium crucible and heated at the temperature of 1050 ^oC – 1400 ^oC for 1–4 h and then quenched in a preheated stainless steel/ graphite mould at a temperature of 300 ^oC - 600 ^oC and then cooled. The prepared glass sample was annealed at 300 ^oC– 500 ^oC for 1 h then cooled at the rate of 0.5 ^oC – 2 ^oC per minute to release the stress in the glass sample. The prepared glass sample was cut into required size and shape using diamond cutter. The code for the present sample is called as TiFP1.

Example 2:

20.4572 g of P_2O_5, 5.3879 g of CaO, 2.9774 g of Na_2O, 0.2398 g of TiO_2 g of 0.9376 CaF_2 and pure chemicals were taken in agate mortar/ planetary ball mill. Ethanol was added with the mixture and ground for 1 h to obtain a homogeneous mixture. The mixture was dried at 100 ^oC -200 ^oC for 1 h and ground using agate mortar/ planetary ball mill to obtain a fine powder. The powdered mixture was melted in platinum doped with 10% Rhodium crucible and heated at the temperature of 1050 ^oC – 1400 ^oC for 1–4 h and then quenched in a preheated stainless steel/ graphite mould at a temperature of 300 ^oC – 600 ^oC and then cooled. The prepared glass sample was annealed at 300 ^oC– 500 ^oC for 1 h and then cooled at the rate of 0.5 ^oC – 2 ^oC per minute to release the stress in the glass sample. The prepared glass sample was cut into required size and shape using diamond cutter. The code for the present sample is called as TiFP2.

Example 3:

20.5664 g of P_2O_5, 5.4166 g of CaO, 2.7127 g of Na_2O 0.3616 g of TiO_2, and 0.9426 g of CaF_2 pure chemicals were taken in agate mortar/ planetary ball mill. Ethanol was added with the mixture and ground for 1 h to obtain the homogeneous mixture. The mixture was dried at 100 ^oC – 200 ^oC for 1 h and ground using agate mortar/ planetary ball mill to obtain a fine powder. The powdered mixture was melted in platinum doped with 10% Rhodium crucible and heated at the temperature of 1050 ^oC – 1400 ^oC for 1 – 4 h and then quenched in a preheated stainless steel/ graphite mould at a temperature of 300 ^oC – 600 ^oC then cooled. The prepared glass sample was annealed at 300 ^oC– 500 ^oC for 1 h and then cooled at the rate of 0.5 ^oC – 2 ^oC per minute to release the stress in the glass sample. The prepared glass sample was cut into required size and shape using diamond cutter. The code for the present sample is called as TiFP3.

Example 4:

20.5571 g of P_2O_5, 5.4142 g of CaO, 2.6648 g of Na_2O, 0.4217 g of TiO_2, and 0.9422 g of CaF_2 pure chemicals were taken in agate mortar/ planetary ball mill. Ethanol was added with the mixture and ground for 1 h to obtain the homogeneous mixture. The mixture was dried at 100 ^oC – 200 ^oC for 1 h and ground using agate mortar/ planetary ball mill to obtain the fine powder. The powdered mixture was melted in platinum doped with 10% Rhodium crucible and heated at the temperature 1050 ^oC – 1400 ^oC for 1 – 4 h then quenched in a preheated stainless steel/ graphite mould of temperature 300 ^oC - 600 ^oC then cooled. The prepared glass sample was annealed at 300 ^oC – 500 ^oC for 1 h then cooled at the rate of 0.5 ^oC – 2 ^oC per minute to release the stress in the glass sample. The prepared glass sample was cut into required size and shape using diamond cutter. The code for the present sample is called as TiFP4.

Example 5:

20.6767 g of P_2O_5, 5.4457 g of CaO, 2.4452 g of Na_2O, 0.9477 g of CaF_2 and 0.4847 g of TiO_2 pure chemicals were taken in agate mortar/ planetary ball mill. Ethanol was added with the mixture and ground for 1 h to obtain a homogeneous mixture. The mixture was dried at 100 ^oC – 200 ^oC for 1 h and ground powder using agate mortar/ planetary ball mill to obtain a fine powder. The powdered mixture was melted in platinum doped with 10% Rhodium crucible and heated at the temperature ranging from 1050 ^oC to 1400 ^oC for 1– 4 h and then quenched in a preheated stainless steel/ graphite mould at a temperature ranging from 300 ^oC to 600 ^oC and then cooled. The prepared glass sample was annealed at 300 ^oC – 500 ^oC for 1 h then cooled at the rate of 0.5 ^oC – 2 ^oC per minute to release the stress in the glass sample. The prepared glass sample was cut into required size and shape using diamond cutter. The code for the present sample is called as TiFP5.

Physico-chemical and *in vitro* studies

1. Density Measurements

The density of the prepared glass samples was measured using Archimedes' principle with water as a buoyant and the relation, $\rho = \frac{W_a}{W_a - W_b} \times \rho_b$, where W_a is weight in air, W_b is the weight in water, and ρ_b is the density of water. A digital balance (model BSA224S-CW; Sartorius, Goettingen, Germany) with an accuracy of ±0.0001 g was used for weight measurements. The measurements were repeated five times to find an average and an accurate value. The overall accuracy in density measurement is ±0.5 kgm^{-3}. The percentage error in the measurement of density is ±0.05.

2. Ultrasonic Measurements

Ultrasonic velocities (U_L, longitudinal and U_S, shear) and attenuations (U_L, longitudinal and U_S, shear) measurements were carried out using pulse echo method and cross-correlation technique. The measurement system consists of an ultrasonic process control system (model FUII050; Fallon Ultrasonics Inc. Ltd., ON, Canada), a 100-MHz digital storage oscilloscope (model 54600B; Hewlett Packard, Palo Alto, CA), and a computer. The measurements were carried out by generating longitudinal and shear waves using X- and Y- cut transducers operated at a fundamental frequency of 5 MHz.

3. X-ray Photoelectron Spectroscopy

Elemental analysis of the prepared glass sample was done using X-ray photo electron spectroscopy (model AXIS Ultra DLD; Kratos, Kyoto, Japan) with Al $K\alpha$ source operating at 210 W. The glass sample was ground using planetary ball mill (model PM 100; Retsch, Haan, Germany) and the powdered glass sample was used for XPS analysis. A survey spectrum (0–1200 eV) was recorded and high-resolution spectra for C1s and N1s band were obtained. X-ray as the excitation radiation was used for the XPS measurements. The spectra were collected in a fixed retarding ratio mode with bandpass energy of about 10 eV.

4. Fourier Transform Infrared Analysis

Infrared absorption of powdered glass samples after *in vitro* studies were analyzed from FTIR spectra. The FTIR absorption spectra were recorded at room temperature using an FTIR from 4000 to 400 cm^{-1}, (model 8700; Shimatzu, Tokyo, Japan) spectrometer. A 2.0 mg sample was mixed with 200 mg KBr in an agate mortar and then pressed under a pressure of 100 $kgcm^{-1}$. It gave a pellet of 13 mm diameter. For each sample, FTIR spectrum was normalized with blank KBr pellet.

5. X-ray Diffraction Analysis

To confirm the amorphous nature of prepared glasses and the presence of HAp layer on the surface of glass samples, XRD studies were carried out on each glass sample. An X-ray diffractometer (model PW 1700; Philips, Eindhoven, The Netherlands) was used with $CuK\alpha$ as a radiation source to obtain the XRD pattern in the range of a scanning angle between 20° and 80°. The glass samples were removed from SBF solution after 21 days *in vitro* studies and then washed gently with double distilled water. The washed glass samples were dried at room temperature. The dried glass was subjected to obtain the XRD pattern as discussed above.

6. pH Measurements

The prepared glass samples were soaked for 21 days in laboratory prepared SBF solution and kept in CO_2 incubator at the temperature of 37 ^{o}C in 6% CO_2. The variation in pH values of simulated body fluid (SBF) solutions was measured on all the 21 days using a pH meter (model 3-star; Thermo Orion, Beverly, MA) for all glasses under identical conditions. The pH electrode was calibrated using the standard buffer solution with a pH value of 4.01, 7.00, and 10.01 before taking the measurements. The percentage error in the measurement of pH is ±0.005.

7. Scanning Electron Microscopy

The surface morphology of the prepared glass sample was analysed using SEM studies. The glass samples were gently washed with double-distilled water and dried at room temperature. A thin layer of a gold film was coated on the surface of glass sample using sputtering technique. The SEM (model Ultra 55; Zeiss, Oberkochen, Germany) was used to obtain a surface image of all glass samples before and after *in vitro* studies to analyse their surface morphology.

8. Energy Dispersive X-ray spectroscopy

Energy dispersive X–ray spectrograph (EDS) was taken for all the prepared glass samples before and after *in vitro* studies using EDS (model X-max 50 mm^2; Oxford, Abingdon, England) for obtaining semi quantitative elemental information of the surface of samples. The percentage of error associated with the elemental composition analysis is ±0.1.

9. Cell culture and cytotoxicity assay

Nontoxic nature of the selected glass sample was assessed using cytotoxicity study in cell culture lines. Human gastric adenocarcinoma (AGS) cell line (ATCC-1739) was obtained from the National Centre for Cell Science, Pune, India. The cells were grown and maintained in Dulbecco's modified Eagle's medium (DMEM)/nutrient mixture F-12 HAM (1:1) with 2 mM L^{-1} glutamine supplemented with 10% fetal bovine serum, 45 IU ml^{-1} penicillin and 45 IU ml^{-1} streptomycin. Growth ingredients were also added and incubated in a humidified atmosphere at 37 ^{o}C in 5% CO_2. After a number of passaging, the pure confluent AGS cell lines were obtained and cells at a density of 10^3 were used to evaluate

the cytotoxicity at a concentration of 100 mg ml^{-1} for the selected bioactive glass samples. The morphology of AGS cell lines was observed regularly under binocular inverted microscope. After 48 h of incubation, MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay was performed to evaluate the viability of the bioactive glass treated AGS cells. The percentage of cell viability from triplicates of the bioactive glass treated and non-treated cells was calculated using optical density (OD 590 nm) as follows:

$$Cell\ viability\ \% = \frac{OD\ of\ the\ glass\ particles\ \ treated\ cells}{OD\ of\ the\ cells} \times 100$$

***In vivo* animal studies**

Animal studies were done after getting the approval from animal ethical committee (Approval number IAEC-LDC/8/14/1 dated 14th February 2014). Young rabbits of weight about 1.600 Kg were purchased from King Institute, Chennai as per CPCSEA guidelines. Under ketamine anaesthesia, one centimetre incision was made over the medical epicondyle of femur under image control. Under microscopic magnification, periosteum was opened and a 2 mm drill hole was made. The selected sample of fluorophosphate glass rod of diameter 2 mm was pegged into the hole. After saline wash, wound was closed using single layer 3-0 ethilon sutures and then 250 mg of ceftriaxone was given intra-muscularly.

Rabbit was allowed to move freely immediately after recovery from anesthesia. Fluorescent calcein (Sigma Aldrich, Japan) (10 $mgkg^{-1}$) was administrated intramuscularly on the day of surgery and then every week until 2 days before euthanising to label newly formed bone continuously. After 10 weeks, the animals were euthanised using a lethal dose of ketamine and the lower end of femur harvested and preserved in 10% formalin and used for SEM, EDS, CLSM studies. Un-decalcified sectioning was done on the harvested femur perpendicular to the implant rod using microtome (Model SP1600; Leica, Nussloch, Germany) to get slices of 1 mm thickness.

10. Histomorphological studies

The un-decalcified section of the implant was dried at room temperature using desiccator. A thin layer of a gold film was coated on the surface of glass sample using sputtering technique. SEM and EDS studies were done on the dried slice to find the formation of HAp at the glass-bone interface.

11. Histophysiological analysis

Calcein fluorescence was used to examine the newly formed bone using a confocal laser scanning microscope (model: LSM 510 META; Zeiss, Gottingen, Germany). The excitation wavelength was set at 488nm (Ar laser). Calcein fluorescence was detected through a BP 515 -565 nm bandpass filter.

Details obtained from the Figures:

Fig. 1 illustrates the protocol used for the synthesis of titanium added fluorophosphate glass samples with different contents of fluorine.

The density variation of the prepared glass samples as a function of added CaF_2 content is shown in **Fig. 2**. A small decrease in the density from 2634.5 kgm^{-3} to 2632.8 kgm^{-3} due to the initial addition of calcium fluoride is noted. An increase in the density value to 2646.4 kgm^{-3} is noted for 1.25 mol% of CaF_2 content. Further addition of calcium fluoride leads to negligible decrease of the density value to 2643 kgm^{-3} for 2.5 mol% of CaF_2 content. Beyond this, the density increases to 2659.9 kgm^{-3} is noted with the further addition of CaF_2 content. The observed behaviour explains the alteration of glass network by fluorine atom. **Fig. 3a and 3b** shows the variation of elastic moduli of all the prepared glass samples as a function of CaF_2 content. Elastic moduli such as longitudinal, shear, young's and bulk modulus shows the same trend with the density variations during the addition of CaF_2 content. The glass containing 3.75 mol% of CaF_2 showed the maximum moduli value than the other CaF_2 added glasses.

Fig. 4 shows the elemental composition of the glass sample TiFP4 using XPS. The observed intensity at the binding energies at 28 eV, 118.2 eV, 135.2 eV, 187.7 eV, 349.8 eV, 523 eV, 533.5 eV, 561 eV, 1072.5 eV, shows respectively the presence of CaF_2, P, P_2O_5,, P, CaO, NaPO3, O, Ti and Na_2O the glass sample TiFP4. SEM image of the prepared glass sample TiFP4 is showed in **Fig. 5.** The smooth surface of the glass sample TiFP4 obtained from SEM image exhibits the amorphous nature of the sample. **Fig.6** shows EDS spectrograph of the glass sample TiFP4. A close agreement is noted between experimental and nominal composition of the glass sample TiFP4. Fig. 5 and Fig. 6 confirm the presence of titanium and fluorine atoms in the glass network.

21 days of *in vitro* studies were made on all the prepared glass samples. The observed pH variations during 21 days *in vitro* studies help to assess the bioactivity of the prepared glasses. The pH variations of all the prepared glasses during *in vitro* studies are given in **Fig. 7.** The initial release of phosphate ions lead to form phosphoric acid resulting in sudden decrease in pH value of all the glass samples. At the end of second day of immersion, sodium ions are released and hence the increase in pH value. At the end of 21 days in vitro studies, the sample TiFP4 shows higher pH value than all the other samples.

FTIR spectrograph of all the prepared glass samples after *in vitro* studies is shown in the **Fig. 8**. The FTIR absorption assignment band at 478 cm^{-1}, 530 cm^{-1}, 720 cm^{-1}, 870 cm^{-1}, 1016 cm^{-1}, 1100 cm^{-1}, 1275 cm^{-1}, 1630 cm^{-1}, 3430 cm^{-1} are respectively of vibration bands of PO_4^{3-}, HAp, P—O—P (symmetric mode), C—O, P—O (stretching mode), α—$Ca_2P_2O_7$, PO_2 (asymmetric mode), hydrogen bonding mode of CaH_2PO_4, and water associated in HAp. The presence of HAp confirms the bone bonding ability of from all the glass samples.

XRD patterns of all the glass after *in vitro* studies are shown in **Fig. 9**. In the obtained XRD shoulder peak at 31.912^{O} and a weak one at 31.792^{O} in the sample TiFP4 shows respectively the rich concentration of HAp and Weak concentration of FAp.

Fig. 10 shows the SEM image of the glass sample TiFP4 after *in vitro* studies. The SEM image confirms the rich deposit of Ca-P layer on TiFP4 glass surface. The size of the deposited particles is in the order of 20-100 nm. **Fig. 11** shows the elemental composition of the deposited precipitate using EDS. The Ca/P ratio of the deposit is about 1.6 indicating the deposit is HAp. But the presence of fluorine molecules in the surface precipitate confirms the existence of fluoroapatite. The EDS spectrograph clearly indicates the presence of hydroxyapatite and fluoroapatite on the surface of the glass sample TiFP4.

Fig. 12 shows the optical microscope image of cell viability test for the sample TiFP4. The image clearly shows there is no cytotoxicity observed in the glass sample TiFP4. **Fig. 13(a)** shows the SEM image at 100X magnification of un-decalcified section of the femoral condyle of the rabbit after 10 weeks of glass implanting. There is a definitive distinct layer of transformations all around the glass where it was in contact with the bone. Fig. **13(B)** shows the SEM image at 1000X magnification where the distinctive interface between bone and glass is well seen**. Fig.14** shows the EDS spectra at the interface that it is transforming to HAp and this proves the bond the glass has made with the bone.

Fig15 shows the CLSM magnified view of the bone and the newly formed interface. When illuminated by Ar laser, there is brilliant fluorescence over the ring of the bone and interface. On super imposition of both it is clearly made out active apatite formation has extended to a depth of 427 μm in the glass form the periphery confirming the bioconversion of titanium added fluorophosphate glass.

We claim:

1. A fluorophosphate glass having the composition of (35-65)phosphorus pentoxide – (22-36)calcium oxide – (11-32)sodium oxide – (0.01-10)titanium oxide – (0.01–15)calcium fluoride, said percentages being molar percentages.
2. The composition for prosthetic device or fluorophosphate glass or coating as claimed in claim 1, wherein phosphorus pentoxide is substituted by any of the following: ammonium di hydrogen phosphate, Phosphorus chloride, ammonium phosphate, calcium phosphate, sodium phosphate, and silver phosphate.
3. The composition for prosthetic device or fluorophosphate glass or coating as claimed in claim 1, wherein calcium oxide is substituted by any of the following: calcium sulphate, calcium carbonate, calcium fluoride, calcium fluorophosphate, calcium chloride, calcium caseinate, and calcium bicarbonate.
4. The composition for prosthetic device or fluorophosphate glass or coating as claimed in claim 1, wherein sodium oxide is substituted by any of the following: sodium carbonate, sodium citrate and sodium fluoride.
5. The composition for prosthetic device or fluorophosphate glass or coating as claimed in claim 1, wherein titanium oxide is substituted by any of the following: titanium fluoride, titanium carbonate, titanium phosphate, titanium chloride instead of titanium oxide.
6. The composition for prosthetic device or fluorophosphate glass or coating as claimed in claim 1, wherein calcium fluoride is substituted by any of the following: calcium di fluoride, calcium tri fluoride, calcium fluorophosphate, and sodium fluoride.
7. The composition of claim 1, wherein the bioactive glass has a composition by either molar percentage or weight percentage:

Compound	Mol%	Wt.%
P_2O_5	35–65	60–70
CaO	22–36	10–20
Na_2O	11–32	06–20
TiO_2	0.01–10	0.1–12
CaF_2	0.01–20	0.1–10

8. Any prosthetic device or implant or bone substitute containing the composition of claim 1wherein said device is made essentially of said fluorophosphate glass or coated with said fluorophosphate glass.

Signed on dated **9th December 2014**

FOR PANDIAN BIOMEDICAL RESEARCH CENTRE

DIRECTOR

To
The Controller of Patents
The Patent Office
Intellectual Property Rights Buildings
G.S.T. Road, Guindy
Chennai – 600 032

Detailed Description of the preferred embodiments:

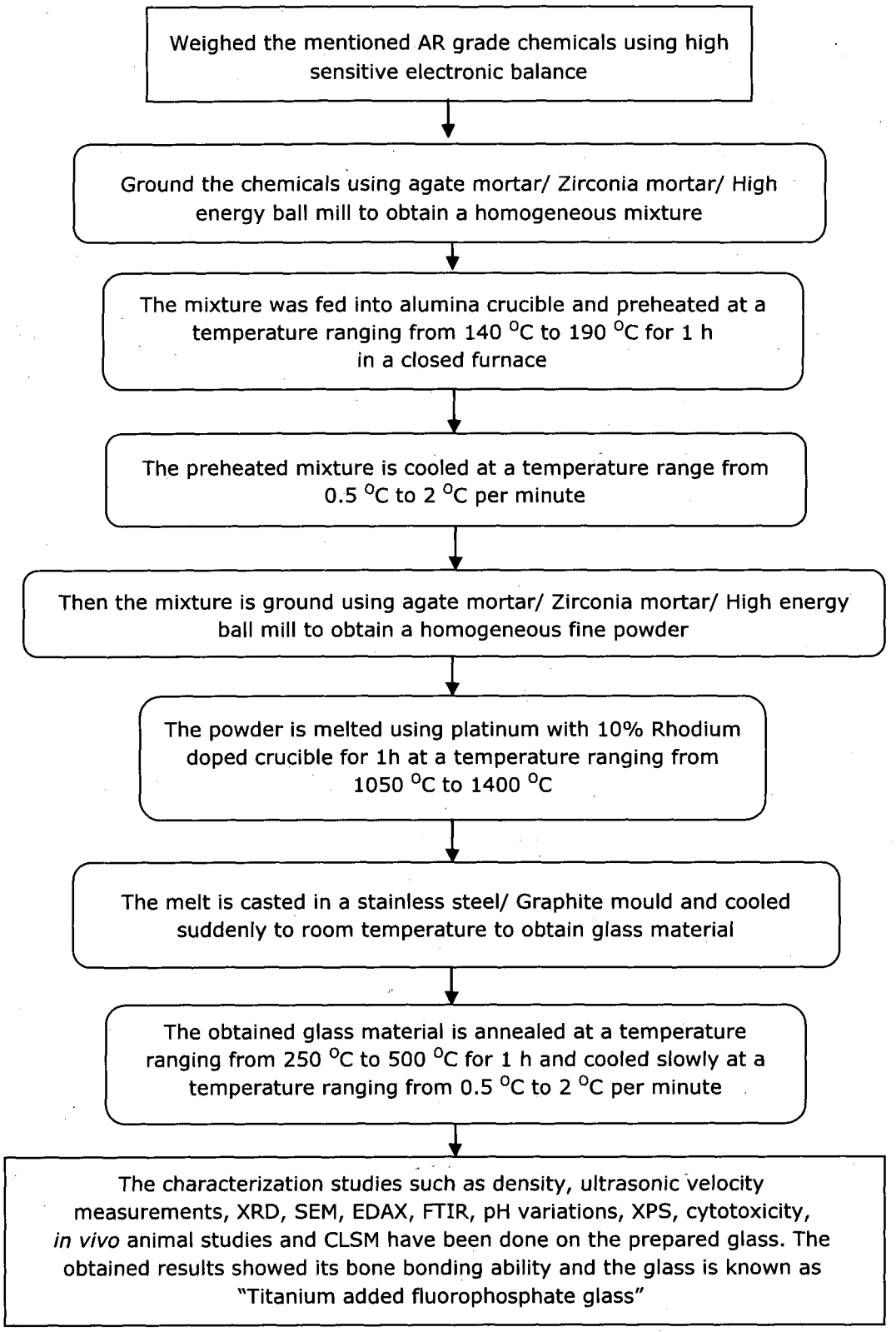

Fig. 1

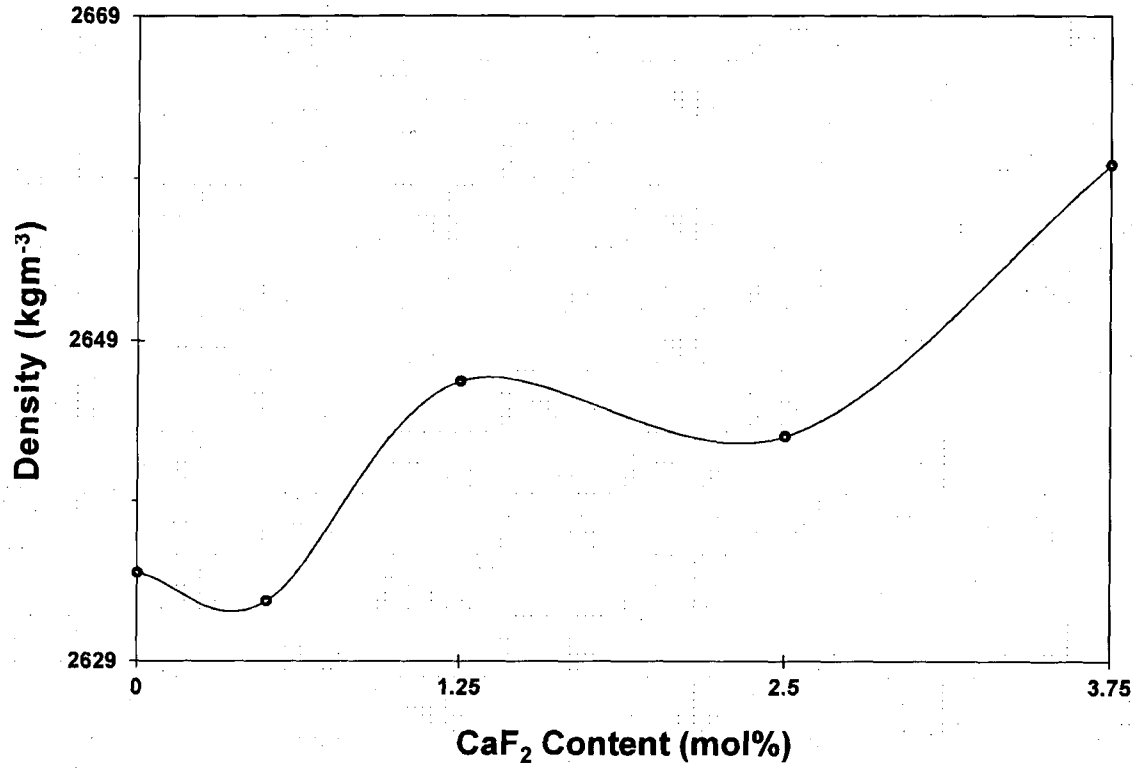
2669
2649
2629
Density (kgm-3)
0
1.25
2.5
3.75
CaF2 Content (mol%)

Fig. 2

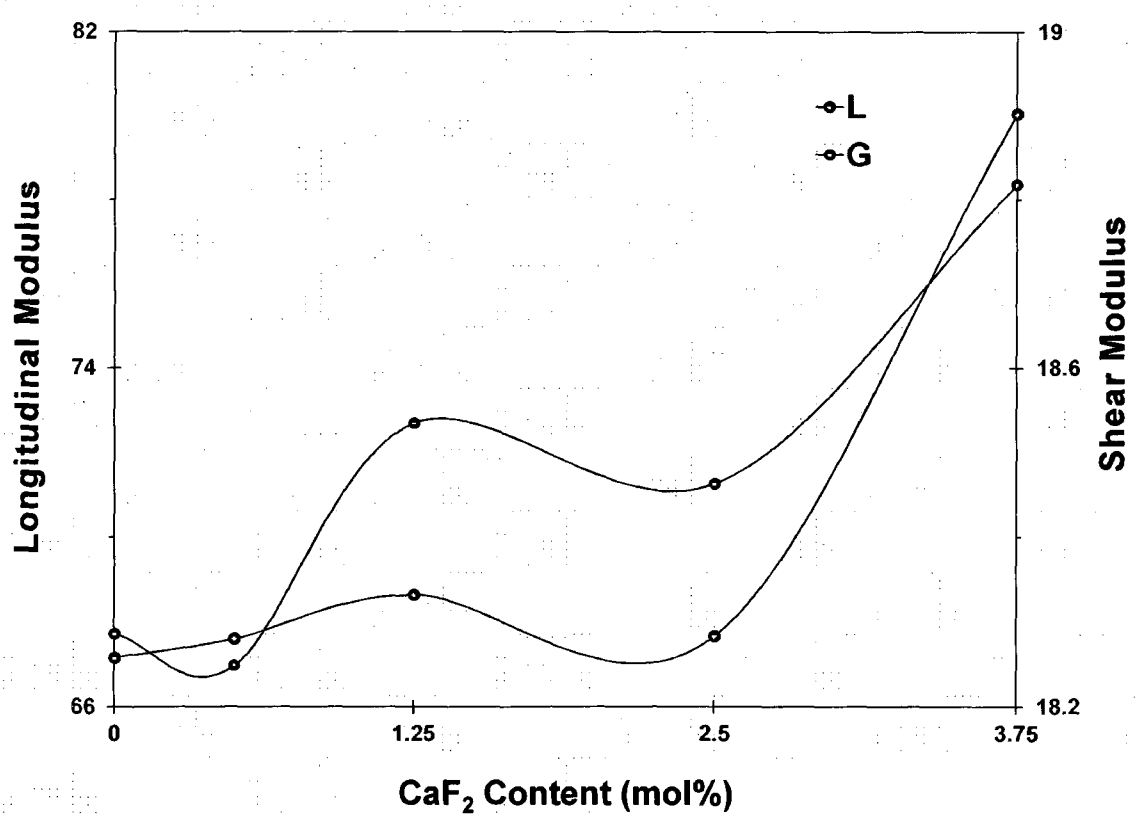
82
74
66
Longitudinal Modulus
19
18.6
18.2
Shear Modulus
L
G
0
1.25
2.5
3.75
CaF2 Content (mol%)

Fig. 3a

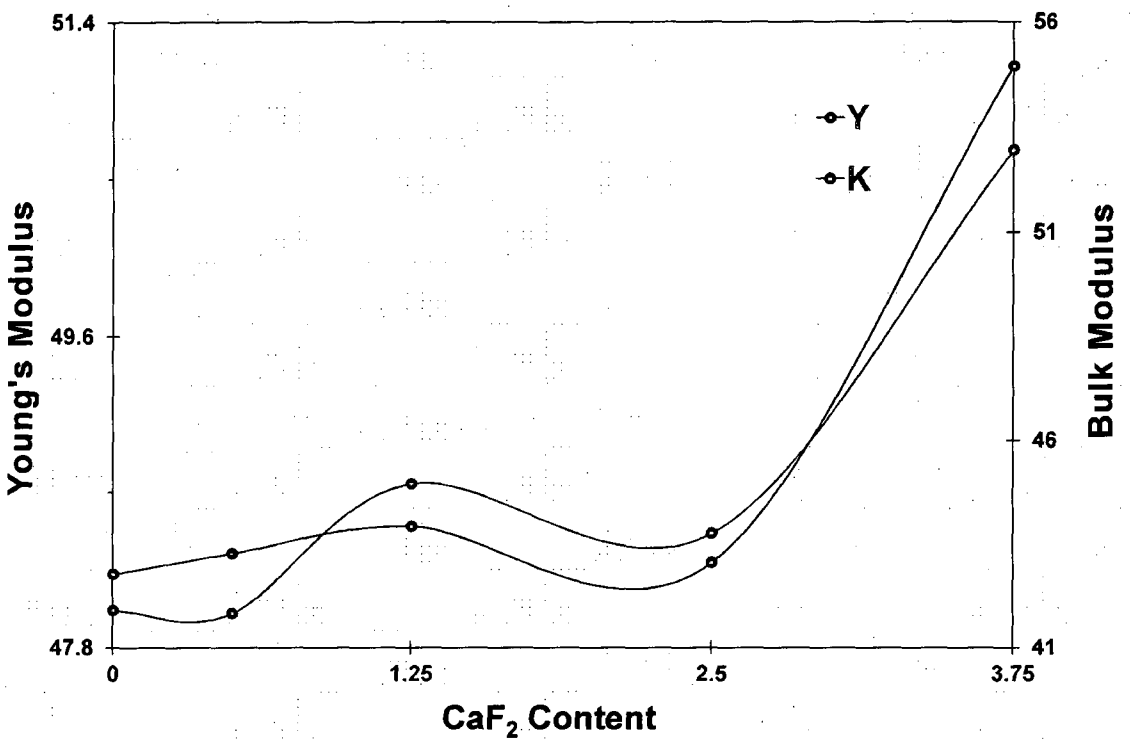
51.4
49.6
47.8
Young's Modulus
56
51
46
41
Bulk Modulus
Y
K
0
1.25
2.5
3.75
CaF2 Content

Fig. 3b

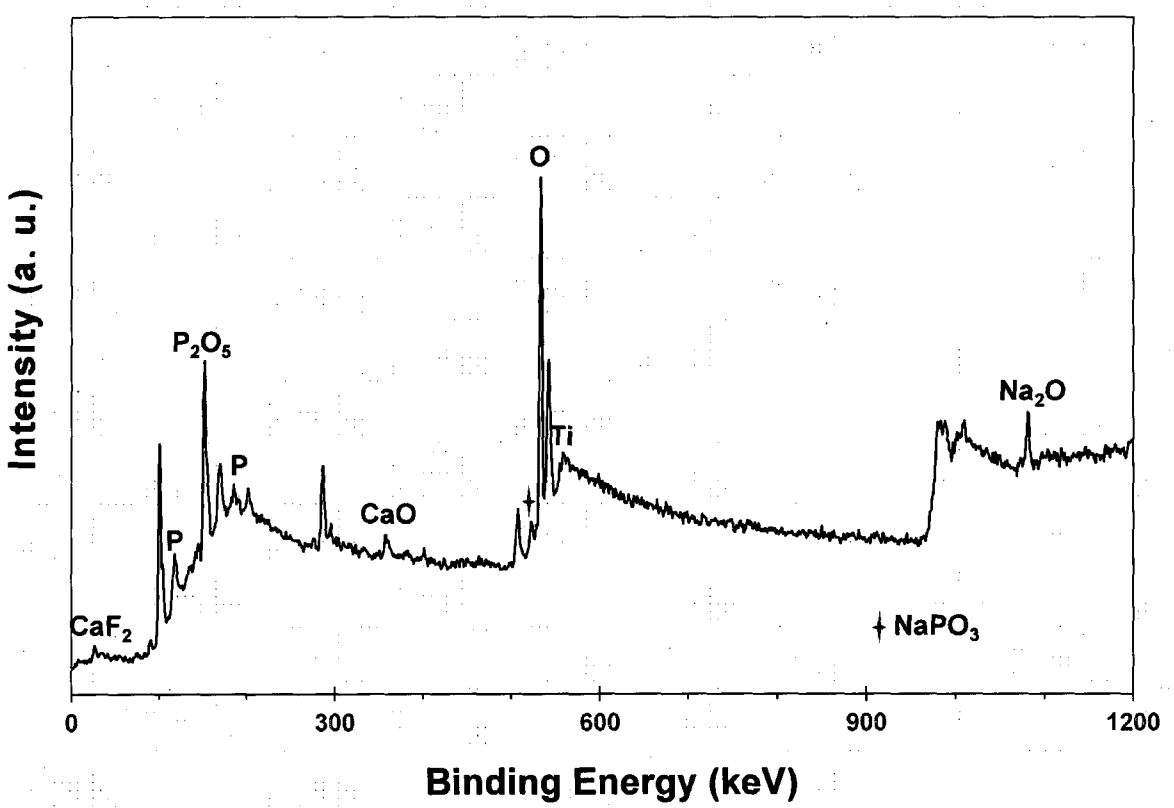
Intensity (a. u.)
O
P2O5
P
P
CaO
Ti
Na2O
CaF2
NaPO3
0
300
600
900
1200
Binding Energy (keV)

Fig. 4

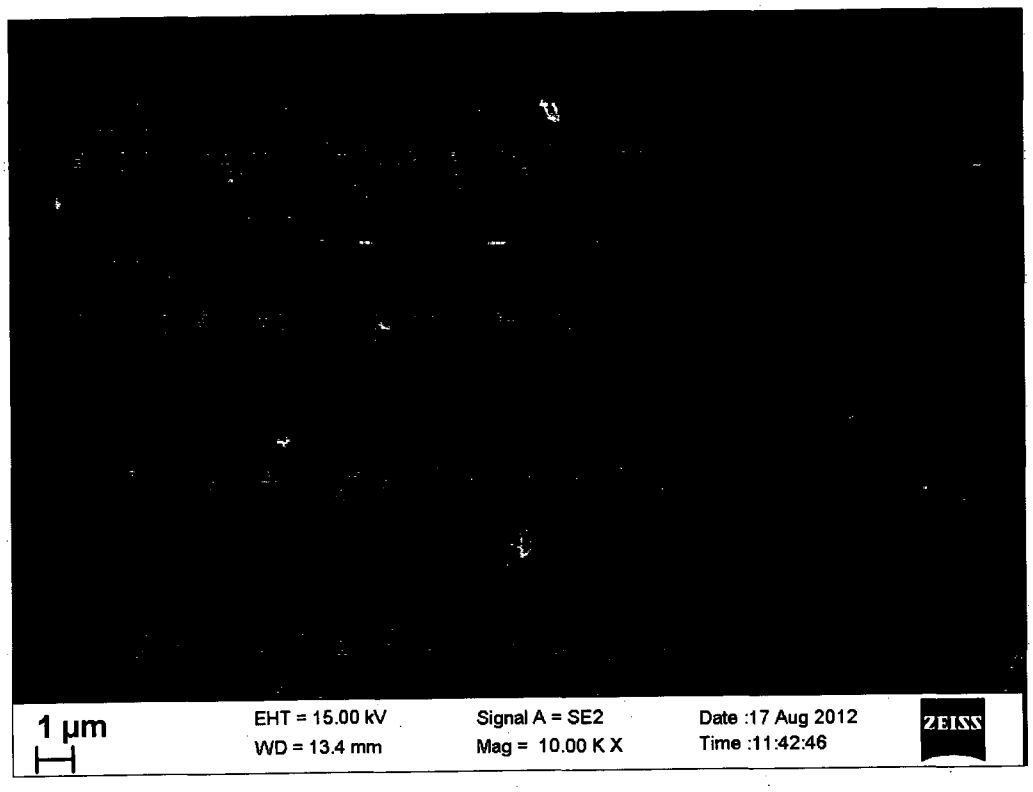
1 µm
EHT = 15.00 kV
WD = 13.4 mm
Signal A = SE2
Mag = 10.00 K X
Date :17 Aug 2012
Time :11:42:46
ZEISS

Fig. 5

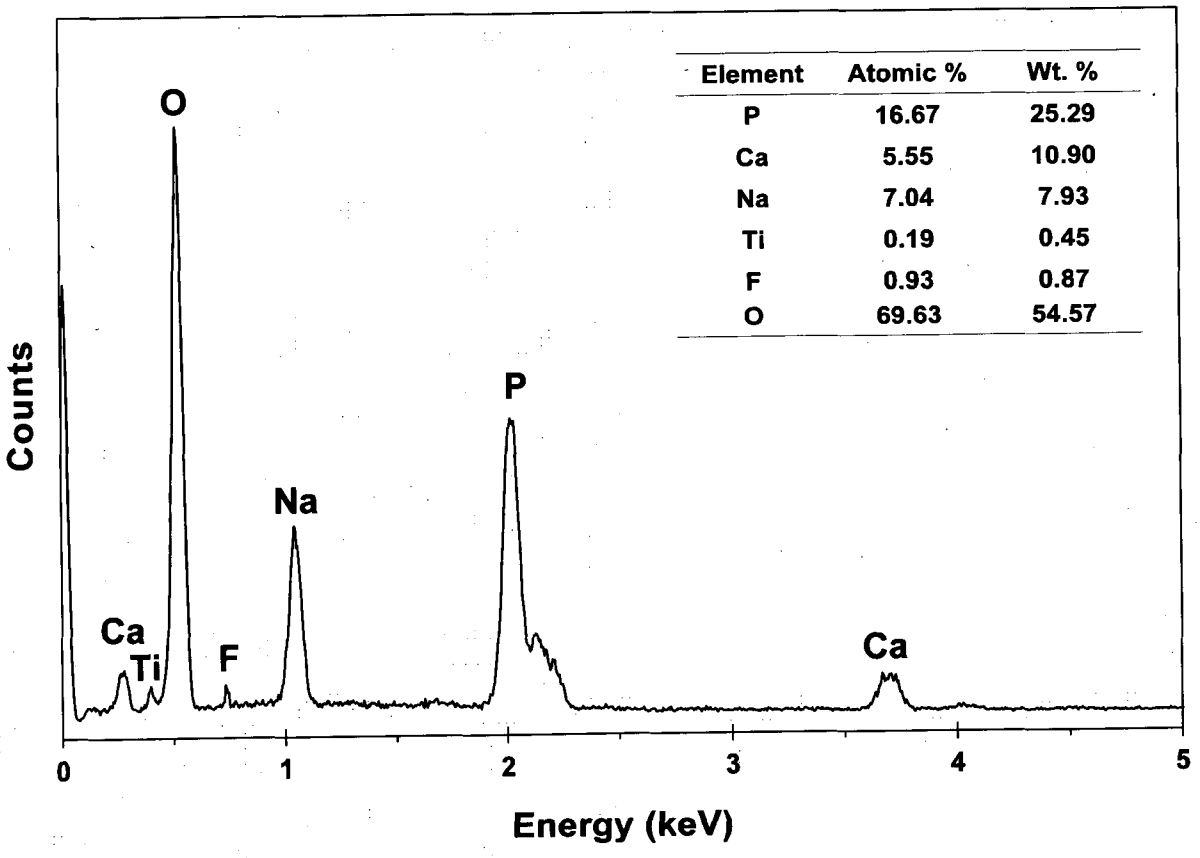
Element Atomic % Wt. %
P 16.67 25.29
Ca 5.55 10.90
Na 7.04 7.93
Ti 0.19 0.45
F 0.93 0.87
O 69.63 54.57
Counts
O
P
Na
Ca
Ti
F
Ca
0
1
2
3
4
5
Energy (keV)

Fig. 6

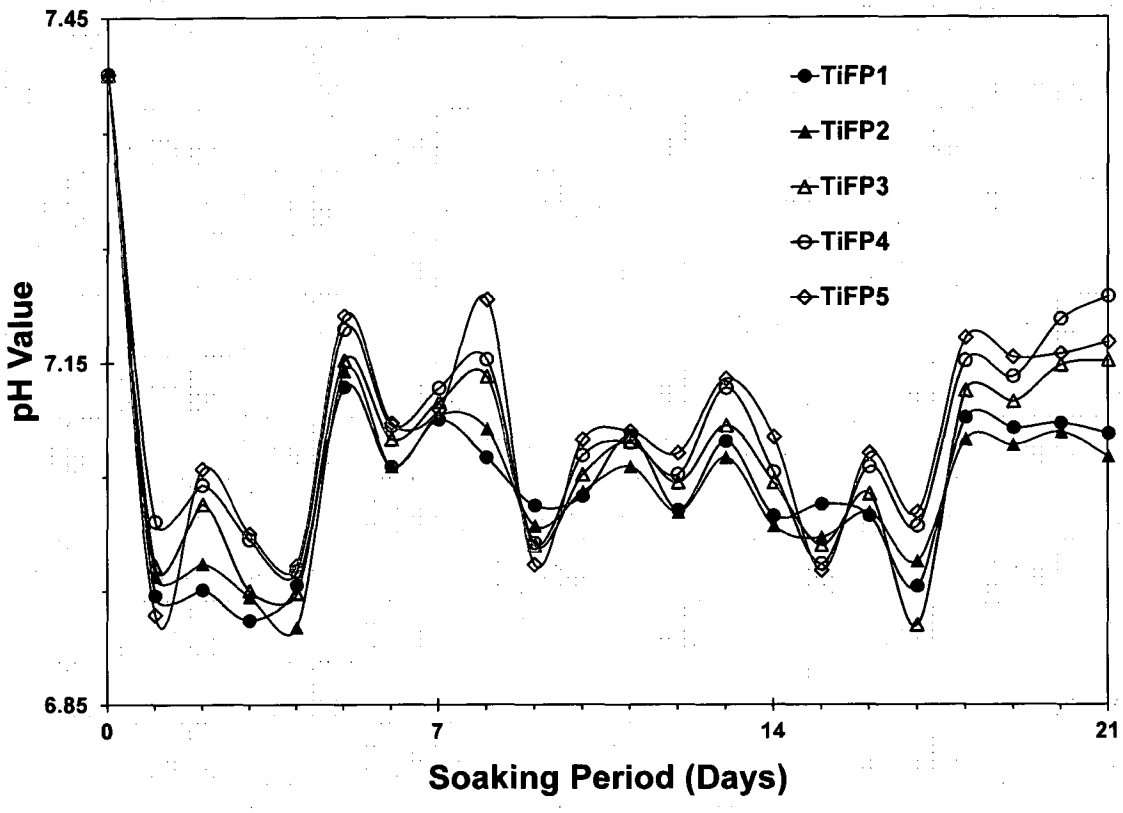
7.45
7.15
6.85
pH Value
0
7
14
21
Soaking Period (Days)
TiFP1
TiFP2
TiFP3
TiFP4
TiFP5

Fig. 7

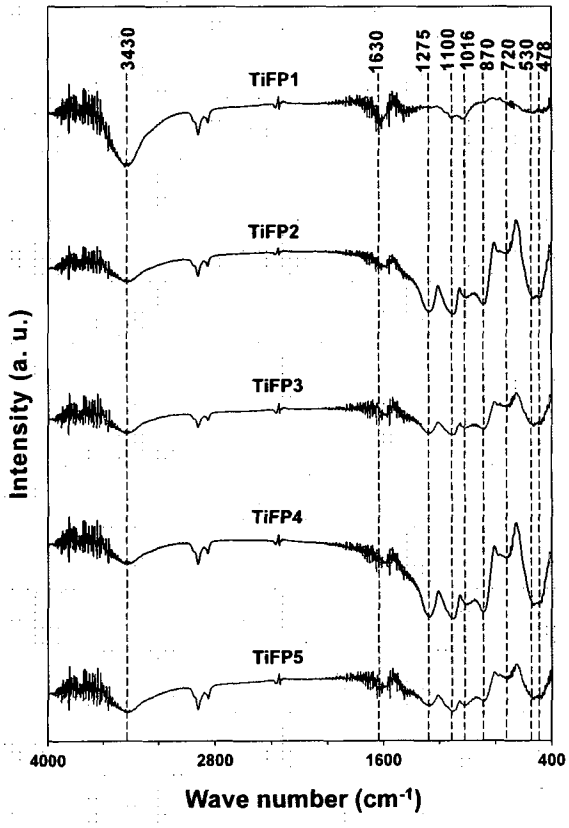
3430
1630
1275
1100
1016
870
720
530
478
TiFP1
TiFP2
TiFP3
TiFP4
TiFP5
Intensity (a. u.)
4000
2800
1600
400
Wave number (cm-1)

Fig. 8

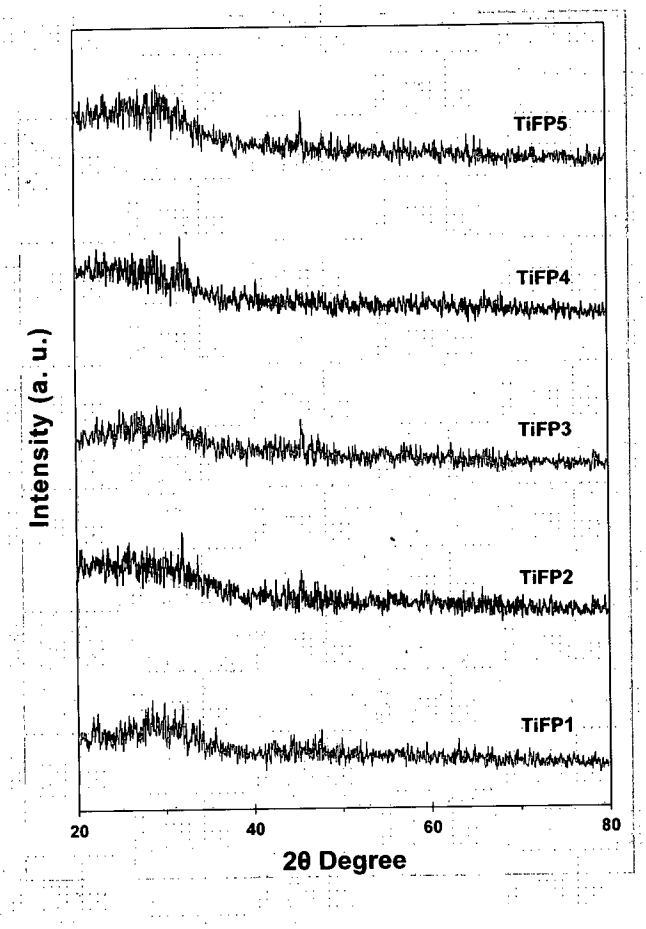
TiFP5
TiFP4
TiFP3
TiFP2
TiFP1
Intensity (a. u.)
20
40
60
80
2θ Degree

Fig. 9

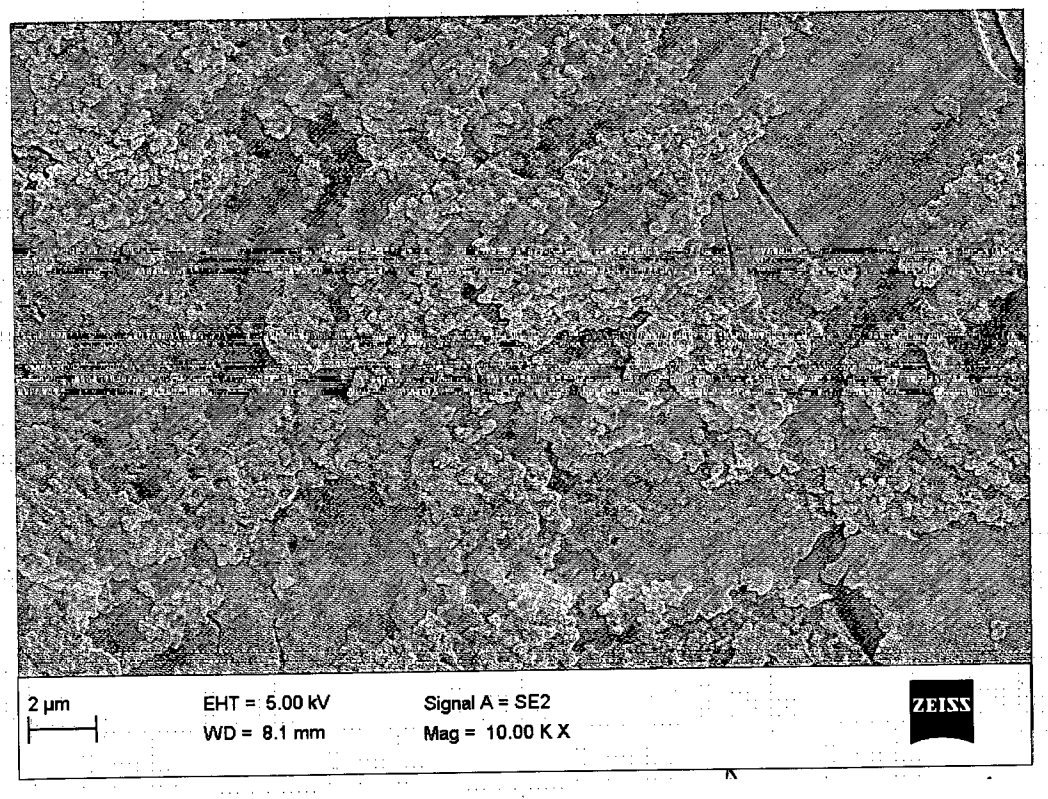
2 µm
EHT = 5.00 kV
WD = 8.1 mm
Signal A = SE2
Mag = 10.00 K X
ZEISS

Fig. 10

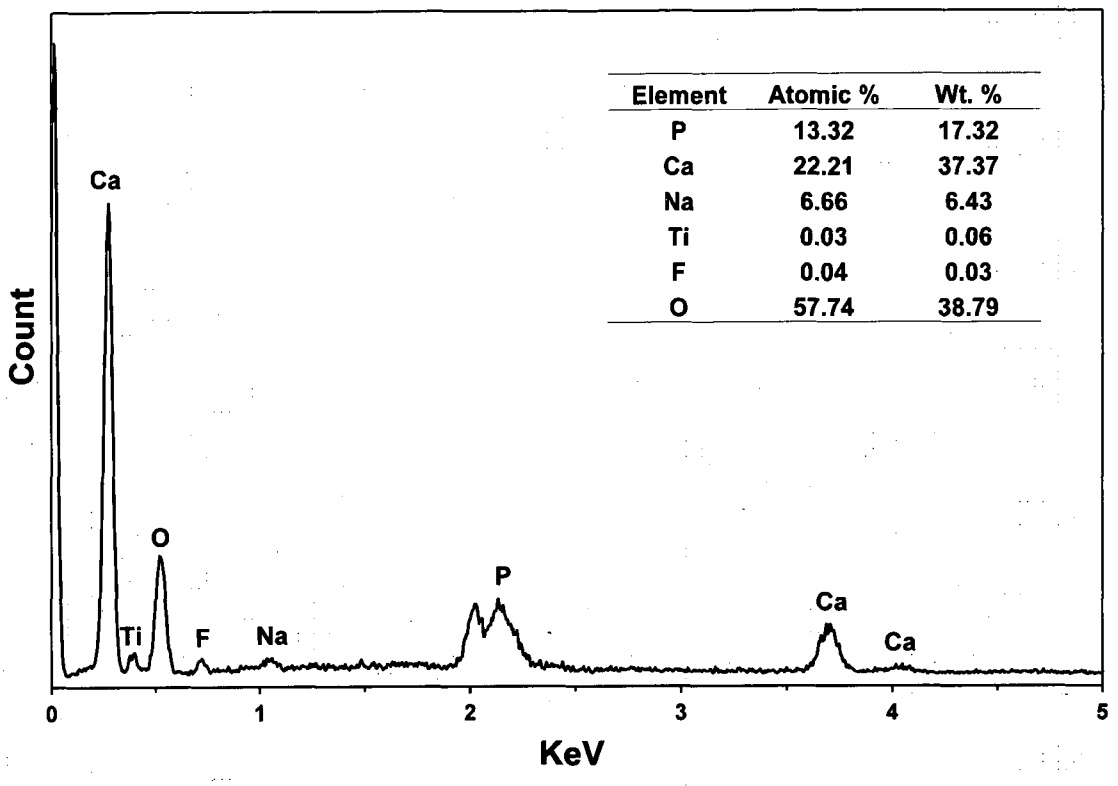
Element Atomic % Wt. %
P 13.32 17.32
Ca 22.21 37.37
Na 6.66 6.43
Ti 0.03 0.06
F 0.04 0.03
O 57.74 38.79
Count
Ca
O
Ti
F
Na
P
Ca
Ca
0
1
2
3
4
5
KeV

Fig. 11

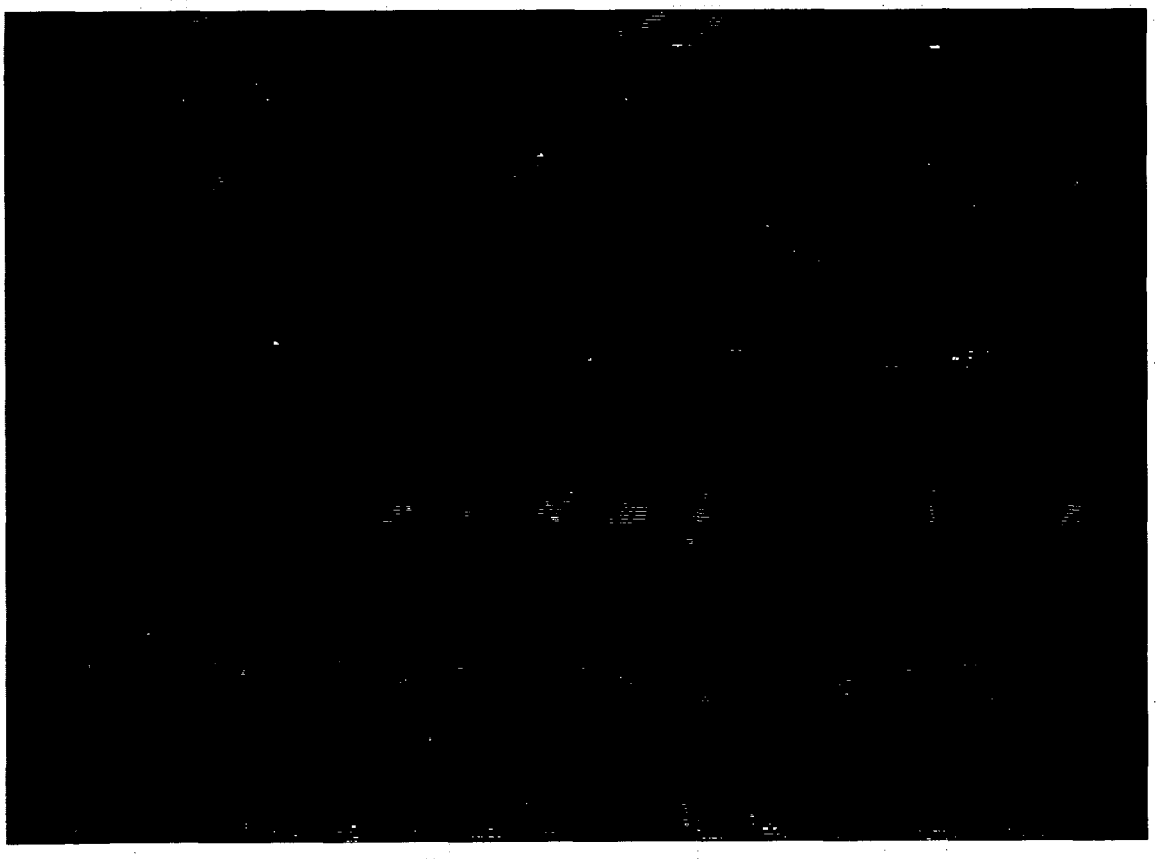

Fig. 12

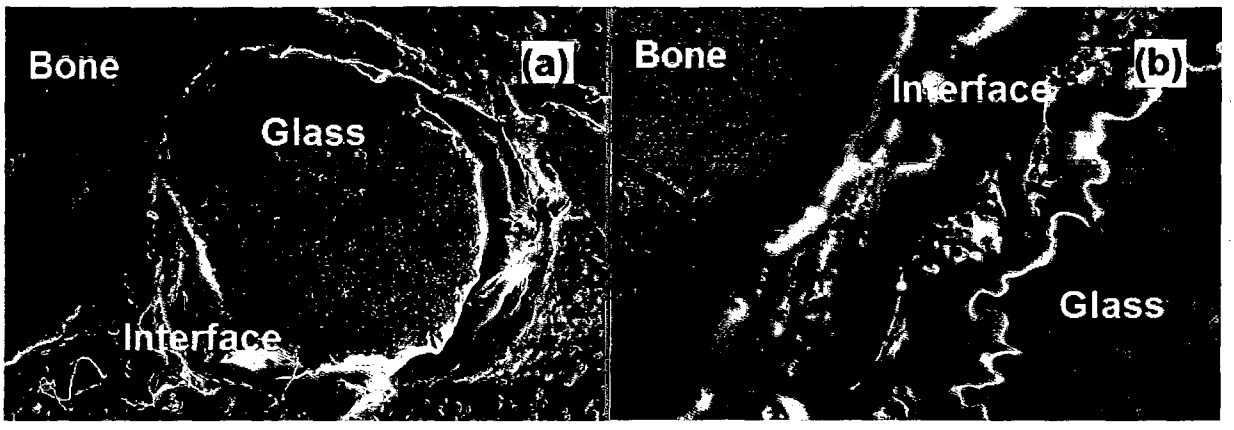

Fig. 13

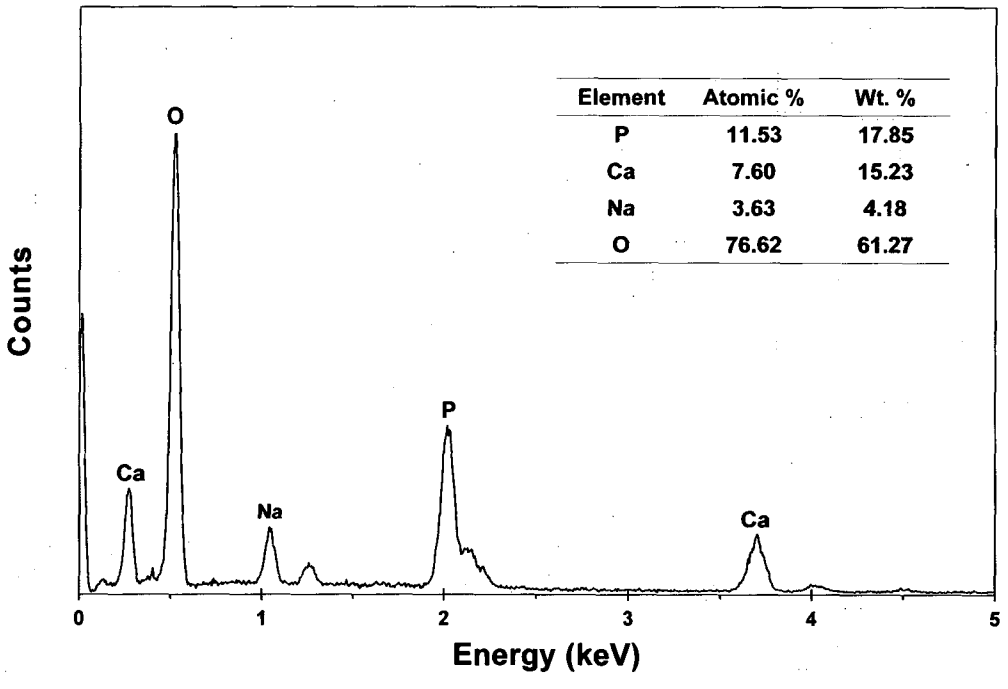

Fig. 14

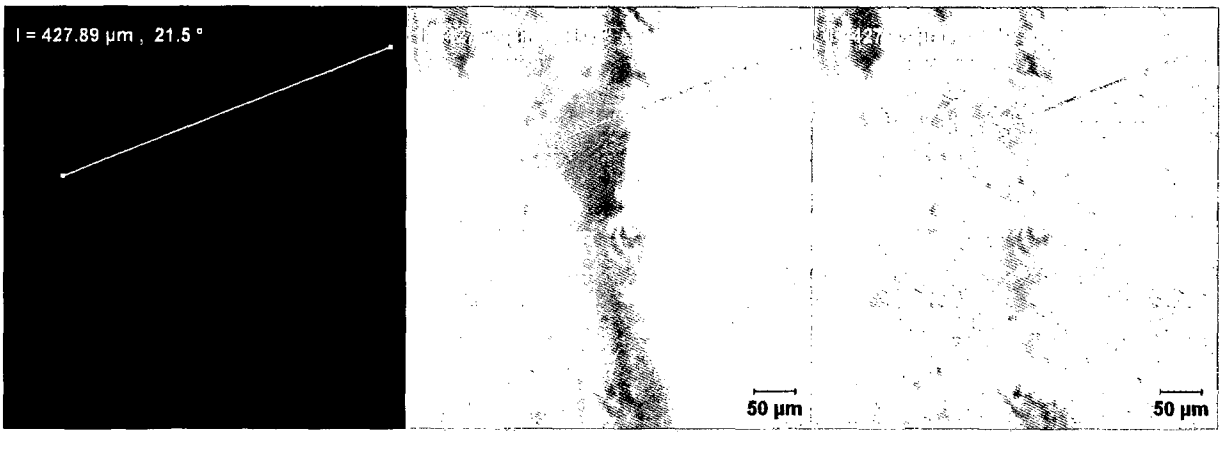

Fig. 15

INTERNATIONAL SEARCH REPORT

International application No
PCT/IN2014/000761

A. CLASSIFICATION OF SUBJECT MATTER
INV. C03C3/247 A61L27/10 A61L27/12 C03C4/00
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C03C A61L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2013/093101 A1 (QUEEN MARY & WESTFIELD COLLEGE [GB]) 27 June 2013 (2013-06-27) page 3, line 15 - page 5, line 17; claims 1-29; tables 1b,1c	1-8
Y	ABOU NEEL E A ET AL: "Physical and biocompatibility studies of novel titanium dioxide doped phosphate-based glasses for bone tissue engineering applications", JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE, KLUWER ACADEMIC PUBLISHERS, BO, vol. 19, no. 1, 3 July 2007 (2007-07-03), pages 377-386, XP019575555, ISSN: 1573-4838 the whole document	1-8

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 April 2015	06/05/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Wrba, Jürgen

INTERNATIONAL SEARCH REPORT

International application No
PCT/IN2014/000761

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ENSANYA ALI ABOU NEEL ET AL: "Control of surface free energy in titanium doped phosphate based glasses by co-doping with zinc", JOURNAL OF BIOMEDICAL MATERIALS RESEARCH PART B: APPLIED BIOMATERIALS, vol. 89B, no. 2, 1 May 2009 (2009-05-01), pages 392-407, XP055184556, ISSN: 1552-4973, DOI: 10.1002/jbm.b.31227 the whole document	1-8
Y	KNOWLES J C: "Phosphate based glasses for biomedical application", JOURNAL OF MATERIALS CHEMISTRY, ROYAL SOCIETY OF CHEMISTRY, GB, vol. 13, 14 August 2003 (2003-08-14), pages 2395-2401, XP002633096, ISSN: 0959-9428, DOI: 10.1039/B307119G [retrieved on 2003-08-14] the whole document	1-8
Y	ENSANYA A. ABOU NEEL ET AL: "Bioactive functional materials: a perspective on phosphate-based glasses", JOURNAL OF MATERIALS CHEMISTRY, vol. 19, no. 6, 1 January 2009 (2009-01-01), pages 690-701, XP055178396, ISSN: 0959-9428, DOI: 10.1039/B810675D the whole document	1-8

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IN2014/000761

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013093101 A1	27-06-2013	EP 2794503 A1	29-10-2014
		WO 2013093101 A1	27-06-2013