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Preparation of Bioactive Glass 77S for Bone Tissue Engineering Applications

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Abstract

Bioactive glasses are promising alternatives for repairing bone and dental defects due to their merit in enhancing bone regeneration while offering compatibility and non-toxicity. In the current investigation, bioactive glass was synthesized and the effect of thermal treatment was scrutinized on various phases formed in the structure. To this end, the three-component bioactive glass 77S with a composition 77%SiO_2-16%CaO-4%P_2O_5 was chosen and synthesized by sol-gel method. Then, STA and XRD experiments were conducted in order to study the thermal properties and phase behaviour, respectively. The effect of thermal treatment on different phases formed in the sample was assessed at 450, 510, 590, 675 and 740°C that chose by STA analysis. The phase structures of thermally treated samples were evaluated using XRD experiments for all the above mentioned temperatures. Phase analysis results suggested that the synthesized samples had been amorphous while indicating the presence of metastable crystalline phases decomposing by temperature. Moreover, crystalline phases of calcium silicate and calcium phosphate were detected in the structure as the temperature was increased. Surface area of bioactive glass were characterized by BET surface area analysis. Using sol-gel method produces surface areas and pore volumes significantly much higher than glasses obtained by melt, this method presents narrow pore distribution in the mesoscopic scale (2–10 nm).

Keywords: Bioactive glass, bone tissue engineering, sol-gel, thermal treatment.

Introduction:

Bone defects and damages are among the most important issues in human health and bone grafts are viable solutions to the former injuries. Bone regeneration using bone grafts has been long used in medical sciences. In fact, bone grafting is a method to replace the lost bone tissue which might have been due to its breakage as an example. Bone grafting is widely employed in case of severe breakages, non-union broken bones, inborn bone defects, tumors, and infections [Ngoc Hung, 2012].

Bioactive glasses are particularly popular as they form chemical bonds with the adjacent tissues. One class of the glasses capable of forming chemical bonds with bone tissues are those based on CaO-P_2O_5-SiO_2. Once in biological environment a hydroxyapatite layer forms on the bioactive glass. This feature has widespread application in clinical treatments where bone regeneration is sought for [Kokubo and Takadama, 2006; Nabian, 2011].

Sol-gel process is a wet chemical method as an alternative to melting method in the preparation of bioactive glasses. In this method, the necessary precursors (generally metallic alkoxides) are dissolved in aqueous or alcoholic environments and are finally converted to the gel. Then, the gel is dried and thermally treated for calcination [R.Ravarian and F.Moztarzadeh, 2010].

It is possible to produce high specific surface area bioactive glasses enhancing the bone regeneration as well as bioactive glass degradation [Clark and L.L. Hench,1991].

Materials and methods

Raw materials and synthesis procedures

The raw materials used in the current work include tetraethyl orthosilicate (TEOS) as the precursor to silicon oxide provided by Merck, 99% (8.00658.1000), triethyl phosphate (TEP) as the precursor to phosphorus oxide provided by Aldrich, 98% (122.52.1), and calcium nitrate tetrahydrate as the precursor to calcium oxide provided by Applichem, 98% (13477-34-4). Additionally deionized water and ethanol were used as solvents and nitric acid as the catalyst.

The first step in the preparation of bioactive glasses is to form a stable sol from the precursor materials. To this end, water, ethanol, and acid were mixed in a beaker and subsequently TEOS, TEP, and calcium nitrate were added, keeping intervals between each addition, followed by agitation for 3 hours until a transparent and stable solution was formed. Gelation was induced at the desired temperature and time. At the end, the gel was dried at 120°C.

Characterization

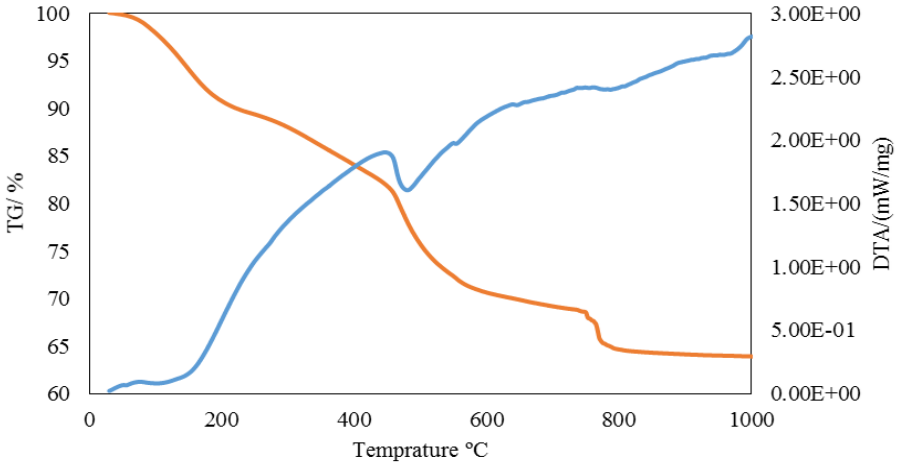
Simultaneous thermal analysis (STA) was achieved on the dried powder using the relevant equipment (BÄHR 503, Germany) at a ramp of 10°C/min up to a temperature of 1000°C so as to investigate the thermal behavior of the produced materials. Phase and structure studies on the thermally treated glass powders were also done on a D500, Siemens (Germany) x-ray diffraction apparatus using a CuKα wavelength x-rays (1.54056 Å) in the range of $10 \leq 2\theta \leq 80$ at 40 kV. BET surface area measurements were carried out with Belsorp-mini Ⅱ（×1） BEL JAPAN, Inc. for calcined sample of bioactive glass.

Results

Thermal behavior

Figure 1 depicts the results from TG/DTA analysis on the sol-gel synthesized bioactive glass. The first peak at 100-200 ºC corresponding to a weight loss of ~ 10% is most possibly due to water removal from the sample. The second peak in the range of 200-400 ºC associated with 8% weight loss could be attributed to the removal of residual water molecules along with organic compounds. Between 400-700 ºC the 14% weight loss corresponding to endothermic peak at 480 ºC is due to nitrate removal from the samples. After the thermal decomposition of nitrates, there is no more weight change implying no more decomposition reactions (responsible for ~ 30% weight loss). After further increasing the temperature we observe a peak at 750 ºC as a result of structure crystallization. Considering the thermal analysis graph for the glass, 560 ºC was chosen as the glass transition temperature.

Figure 1. TGA/DSC Analysis of the bioactive glass



Other researchers reported similar results. For instance, Ashouri and coworkers [1391] synthesized bioactive glass through sol-gel approach and showed that the majority of nitrate decomposition in STA experiments would be in the range of 525-550 ºC. Further, the glass transition temperature occurring after complete nitrate decomposition and prior to crystallization reactions was chosen to be 700 ºC.

In another research, Tofighi and Saatchi [1394] determined glass transition and crystallization temperatures to be 600 and 730 ºC, respectively, for a sol-gel-synthesized bioactive glass.

Other works include that of Ribeiro and coworkers. In their studies, based on STA data collected from a sol-gel-synthesized glass, the temperature ranges at which water and ethyl groups emerged were reported to be 26-128 ºC and 128-262 ºC, respectively. The former is followed by the decomposition of the constituting elements at 262-465 ºC and finally the dehydroxylation of silanol groups (Si-OH) in the range of 445-604 ºC.

In another research on thermally treated gels from sol-gel synthesis for the production of glass, Montazerian et al. studied the thermal behavior of the dried gel using differential thermal analysis (DTA). According to their observation there has been three weight loss steps in the sample. Water removal

occurred from room temperature up to 154 ºC. In the range 154-375 ºC a weight loss of 13% was attributed to the emergence of molecular water along with organic compounds. Finally, nitrates were removed at 375-703 ºC giving rise to a 17% weight loss summing to an overall of 41% weight loss from the glass.

Hence, all four studies mentioned above testify our STA results collected on bioactive glass 77S.

XRD Analysis

In order to precisely characterize the phases formed in the thermally treated bioactive glass, five temperatures were chosen and studied. In this respect, five samples were treated each for an hour at 450, 510, 590, and 675 ºC, respectively. Then, XRD analysis was done on each sample and the data were introduced in Xpert HighScore software for phase characterization.

Figure 2 depicts the diffraction pattern from the sample treated at 450, 510, 590, 675 ºC. As it is obvious at 450 ºC the base structure was amorphous and merely phases consisting nitrogen with peaks at 34.521 and 34.500 could be attributed to calcium phosphorus nitride (Ca_2PN_3: card number 01-081-1161) and calcium silicon nitrides ($Ca_5Si_2N_6$: card number 00-021-0160, $CaSiN_2$: card number 00-020-0230), respectively. Furthermore, peaks at 33.280, 42.195, 51.596, and 54.584 imply the presence of Ca_2SiO_4. The former phases are metastable structures and decompose by temperature.

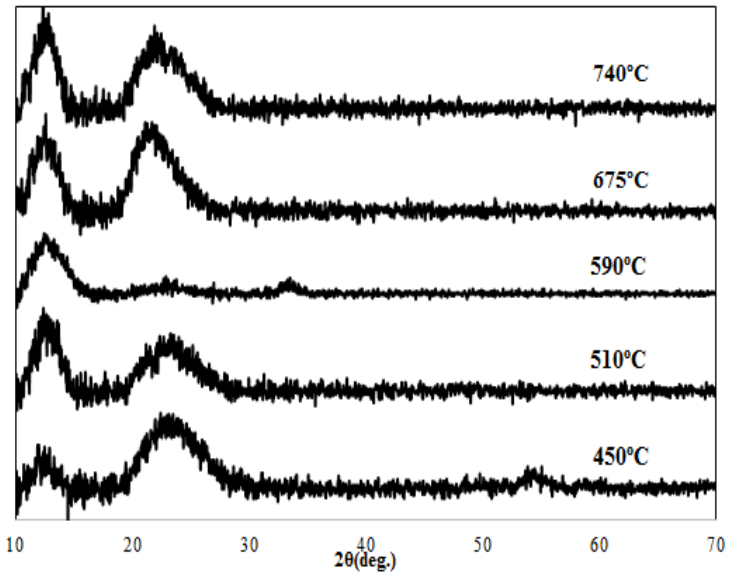
At 510ºC the base structure is amorphous. Beyond that, peaks produced at 25.502 and 26.268 are indicative of calcium nitrate ($Ca(NO_3)_2$: card number 00-047-0642). Aside from that, silicon phosphate (SiP_2O_7: card number 0755-025-00) may be distinguished by the peaks at 24.033 and 24.503. Finally, peaks at 12.101, 22,890, 30.710, and 32.090 could be ascribed to calcium phosphate ($Ca_3(PO_4)_2$: card number 00-029-0359).

Silicon phosphate and calcium phosphate phases have already been reported in similar studies on bioactive glasses. The presence of calcium phosphate phase may signify partial crystallization of hydroxyapatite. In an article by Vallet-Regı the possibility of apatite phase crystallization in the glass bed has been scrutinized by FTIR and a couple of other characterization techniques [2003]. In addition to this study, hydroxyapatite crystallization in glass structure has been investigated by Montazerian et al. and also Ribeiro et al.

At 590ºC, the nitrates have already been removed from the structure which could produce space inside the structure and by further increasing the temperature, the former gives rise to the formation of crystalline phases so 590ºC is crystallization temperature.

At 675ºC crystalline phases is discernible, In this regard, peaks at 31.02, 33.55, and 29.25 belong to calcium silicate (card number 00-029-0370). Also peaks at 22.72, 24.09, 30.75, and 34.18 imply the formation of calcium phosphate (card number 00-009-0348) and at 740ºC , By comparing the peaks evolved at this temperature and those at 675 ºC it becomes clear that by increasing temperature, the peak intensities increase implying enhanced crystallization in the structure.

Figure 2. Diffraction pattern for the sample thermally treated at 450, 510, 590, 675, 740 ºC.



To summarize, the five diffraction patterns indicate that at 450 ºC and 510 ºC nitrate phases are present in the structure and the former would decompose by increasing the temperature to 590 ºC, implying nitrate removal during the thermal treatment. By increasing the temperature further to 675 ºC one can distinguish the presence of calcium phosphate and calcium silicate phases.

Among the relevant studies, both in an article by Bellucci [2010] and in another work by Arstila and coworkers [2008], at low temperatures (below glass transition temperature) the structure is amorophous. By increasing the temperature there is a tendency to form crystalline phases in the structure. Moreover, the formation of calcium silicate and calcium phosphate phases by thermal treatment, and also their enhanced intensity by temperature has been reported extensively in the literature [J. Ma et al, 2010; ZHOU Zhi-hua et al, 2007].

surface area analysis

Surface area of calcined bioactive glass was measured and the specific surface area versus the mesoporous volume properties of the bioactive glass sample is shown in Table 1.

Table 1: The BET surface area and pore volume of bioactive glass powders.

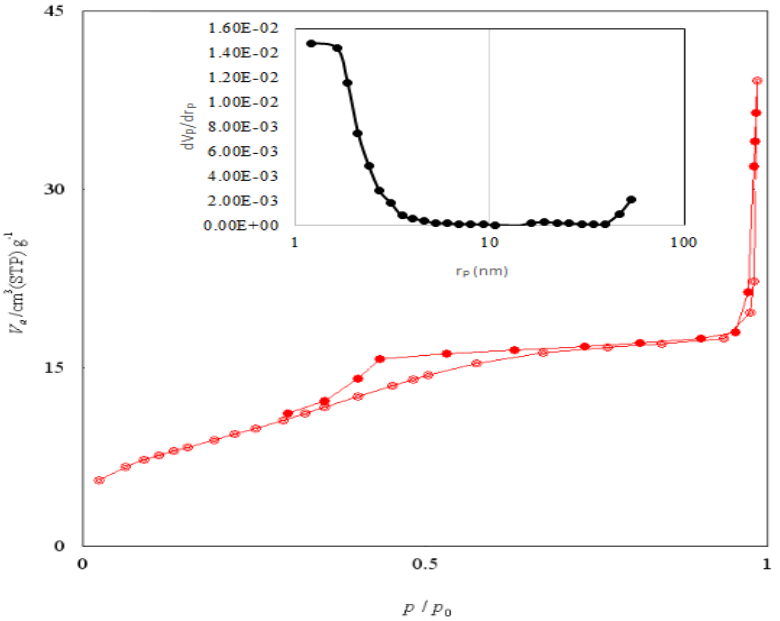
	S_{BET} (m^2/g)	V_p (Cm^3/g)
77S	33.66	0.044

S_{BET}: The BET surface area of powders.
V_p: The pore volume of powders.

Figure 3 shows adsorption-desorption isotherms and pore size distribution for bioactive glass nanostructures prepared by sol-gel method. Figure 3 can be ascribed as Type IV, which is associated to

capillary condensation according to IUPAC classification with Type H3 hysteresis that is characteristics of the mesoporous material with slit-shape pores.

Figure 3. Nitrogen adsorption-desorption isotherms and Barret-Joyner-Halenda (BJH) pore size distribution of bioactive glass



In an article, Iyyappan and Wilson [2013] synthesized hydroxyapatite (HA) particles by using $Ca(NO3)_2$, $4H_2O$ and $(NH_4)2HPO_4$ with and without using surfactant. BET surface area of calcined HA was measured and for the sample without using surfactant, surface area was measured 37.8(m^2/gr).

In another article, Chi-Jen Shih et al. [2014] compared the responses of fibroblasts and osteoblasts to bioglass(BG) and bioglass-containing mesoporous structure (BG-M) powders. The BG-M powders exhibited specific surface areas approximately three times larger than those of the BG powders. pore volume of bioglass was 0.13(cm^3/gr).

Discussion and conclusions

Bioactive glasses have central importance in medical applications due their capability to enhance bone regeneration. Synthesis of bioactive glasses by sol-gel method has several advantages over melting techniques. For instance, sol-gel synthesis is amenable to the synthesis of high-specific surface area glasses that have essential significance in biological and medical applications.

In the current study, glass 77S was synthesized successfully using a sol-gel approach. STA characterization was used to determine the glass transition temperature, being 560 ºC .

Phase studies performed on various samples treated at different temperatures confirmed the amorphousness of the synthesized sample in the sense that no crystalline phases was discernible below the glass transition temperature and only some metastable crystalline phases were present that decomposed with temperature. Calcium silicate and calcium phosphate phases formed upon nitrate removal from the structure at temperatures in excess of crystallization temperature. Additionally, by

further increasing the temperature no new phases were formed and merely, the former phases gained enhanced intensities. BET analysis showed porosity that occur because of sol-gel method. The sample show hysteresis loop, indicating the presence of mesopores. The hysteresis loop ascribed as Type IV.

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