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Original article

# Removal of residual nitrate ion from bioactive calcium silicate through soaking

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ABSTRACT

Bioactive calcium silicates prepared by sol–gel routes mainly use calcium nitrate as the calcium precursor. However, the toxic nitrate ions are usually removed by calcination (*i.e.* 550 °C or over), which poses great challenge for the *in situ* preparation of inorganic/polymer composites, as polymer moieties could not survive such temperatures. In this study, we prepared 70Si30Ca (70 mol% $SiO_2$ and 30 mol% CaO) bioactive glass at low temperatures where polymer could survive (*i.e.* 200 °C and 350 °C), and proposed to remove the residual nitrate ions through soaking. Deionized water and simulated body fluid (SBF) were employed as the soaking medium. The results showed that the residual nitrate ions could be removed as quickly as 0.5 h while maintain the bioactivity of the samples. This technique may open the possibility of preparing sol–gel derived bioactive glass/polymer hybrids *in situ* with reduced potential toxicity.



## 1. Introduction

Bone grafts, including autografts, allografts, xenografts and artificial materials are extensively used in clinics to replace or to repair bone tissues in defect sites resulted from disease or trauma [1]. The demand for artificial bone grafts has been raised as the supply of qualified natural bone grafts has diminished.

Bioactive glasses have showed excellent bioactivity thus have been increasingly used in dental and orthopedic surgeries to deal with bone-related diseases. 45S5, with its composition 45% $SiO_2$, 24.5% CaO, 6% $P_2O_5$ and 24.5% $Na_2O$ (wt%), has been proven to bond both to bone or soft tissues through the formation of a bone-like hydroxycarbonate apatite (HCA) layer [2], thus becomes one of the most successful examples.

Although the melt-quenching-derived bioactive glasses have gained great commercial success, they are inherently brittle, which largely limits their application in weight bearing situations. Incorporation of polymeric moieties is expected to be a wise option. In order to get most use of both organic and inorganic moieties, a homogeneous mixing of them is a prerequisite. This can be in the form of either composite or hybrid materials. 45S5 is prepared through the melt-quenching method at high temperatures above 1700 °C [3,4], which can hardly satisfy the above criteria. Therefore, a low-temperature sol–gel preparation method will be needed, to generate well-controllable micro/nanoparticles using as bioactive fillers or to form bioactive glass phase in the presence of polymer matrix.

A typical sol–gel procedure for bioactive glass involves the formation of a clear sol solution by the hydrolysis and condensation of silicon and calcium precursors, sol to gel transition, and the following aging, drying and stabilization stages. Tetraethyl orthosilicate (TEOS) or tetramethylorthosilicate (TMOS) usually serves as the precursor of silicon. And calcium nitrate usually serves as the precursor of calcium since it is highly soluble, low cost and more importantly, less thermal stable, *i.e.* relatively easier to be decomposed by heating compared with other inorganic calcium salts [5–10]. Nevertheless, a minimal stabilization temperature over 550 °C is required to remove the potentially toxic nitrate ions [11,12], which is well above the decomposition temperature of most biomedical polymers. For example, Koh *et al.* [13] prepared CaO–$SiO_2$-PTMO hybrids at 40 °C only to find the existence of nitrate ions. The only strategy to fabricate *in situ* inorganic/polymer hybrids is to lower the stabilization temperature to what polymer can survive. Although another calcium precursor, calcium

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2-methoxyethoxide, has recently been explored with great success in preparing bioactive glass at lower temperature [14,15], however it is not stable and often needs to be used freshly, thus still need more investigation. So far, the best choice for calcium is still calcium nitrate. Therefore, it is of great interest to explore new method to remove the residual nitrate ions in the bioactive glasses made from calcium nitrate.

Sol–gel-derived 70Si30Ca calcium silicate (70 mol% $SiO_2$ and 30 mol% CaO) is a Class A biomaterial, which has great potential to be used as the third generation biomaterial just like the commercial 45S5 Bioglass® does [16]. Using this glass as a model system, we set to develop new method for nitrate ion removal. In this study, we propose to remove the residual nitrate ions in 70Si30Ca powders stabilized under low temperatures (*i.e.* 200 °C and 350 °C) by soaking. Deionized water and simulated body fluids (SBF) were selected as soaking medium separately to examine the validity of proposed method.

## 2. Experimental

### 2.1. Preparation of 70Si30Ca powders

Tetraethyl orthosilicate (TEOS, purity ≥99.0%), calcium nitrate tetrahydrate ($Ca(NO)_3{\cdot}4H_2O$, purity ≥99.0%) and concentrated nitric acid were purchased from Sigma–Aldrich (Shanghai, China) and used as received.

70Si30Ca bioactive calcium silicate was prepared by sol–gel route. Typical recipe is like the following: 10 mL TEOS was hydrolyzed for 30 min in the mixture of ethanol and water at room temperature with 2 mol/L $HNO_3$ as catalyst. 4.53 g calcium nitrate tetrahydrate was added gradually under continuous stirring. The molar ratio of water to TEOS (R ratio) was 12:1. The obtained clear sol solution was sealed in a polypropylene container and left to gel. The gel was aged at 60 °C for a week, followed by drying at 120 °C for another week and then stabilized at 200 °C, 350 °C and/or 600 °C for 2 h, which were referred as BG-200, BG-350, BG-600, respectively. The stabilized 70Si30Ca calcium silicates were then grounded into powders (<50 μm) for testing.

### 2.2. Thermal behavior and stabilization simulation

Differential thermal analysis (DTA) and thermal gravimetric analysis (TGA) were employed to study the thermal behavior of 70Si30Ca on a TA Q-600 instrument. The dried gels (obtained before the stabilization stage) were placed in alumina crucibles and measured under a nitrogen flow of 100 mL $min^{-1}$ using a heating rate of 10 °C $min^{-1}$.

Simulation of the stabilization process was carried out on a Pyris 1 instrument to monitor the mass changes in this stage. The dried gels were placed in platinum crucibles and measured under an air flow of 20 mL $min^{-1}$ with a heating rate of 10 °C $min^{-1}$. The temperature was kept at 200 °C, 350 °C and/or 600 °C for 2 h separately.

### 2.3. Immersion experiments

150 mg of accurately weighted 70Si30Ca powders were immersed in 100 mL water or simulation body fluid (SBF) at 36.5 ± 0.5 °C for 0.5 h, 2 h, 24 h, and 72 h, respectively, to remove the residual nitrate ions.

The nitrate ion remaining in the calcium silicates was determined by FTIR and Raman spectroscopy. FTIR was carried out with a Bruker Equinox 55 instrument in a wave number range from 400 $cm^{-1}$ to 4000 $cm^{-1}$ operating in the absorbance mode. Raman was carried out with a Renishaw in Via plus instrument in a laser with the wavelength of 663 nm.

### 2.4. Evaluation of bioactivity

150 mg of 70Si30Ca samples obtained after above immersion were immersed again in 100 mL SBF at 36.5 ± 0.5 °C for 24 h, 72 h and 168 h to examine their *in vitro* bioactivity. XRD of the powder was employed to examine the formation of hydroxycarbonate apatite (HCA). The solids were collected with filter papers followed by washing with pure water and then dried in vacuum overnight at 40 °C before the XRD measurements. The XRD experiments were performed with a Bruker D8 Advance Diffractometer using Cu-*Kα* radiation (λ = 1.54 Å) and operated at 40 kV and 200 mA, with a step size of 4° $min^{-1}$, a counting rate of 30 s per step, and 2*θ* values from 5° to 80°.

## 3. Results and discussion

Fig. 1a presents DTA and TGA traces of 70Si30Ca dried gels. DTA trace indicates the heat changes of the sample as a function of temperature in the range of 25–800 °C, and TGA trace reflects the weight changes. The DTA trace exhibited two obvious endothermic peaks. The first endothermic peak, which initiated at 55 °C, corresponds to the loss of physically absorbed water and liquid by-products resulted from the polycondensation reaction. All the water and liquid by-products were removed before 100 °C (8.8% weight loss). The second endothermic peak, which initiated at

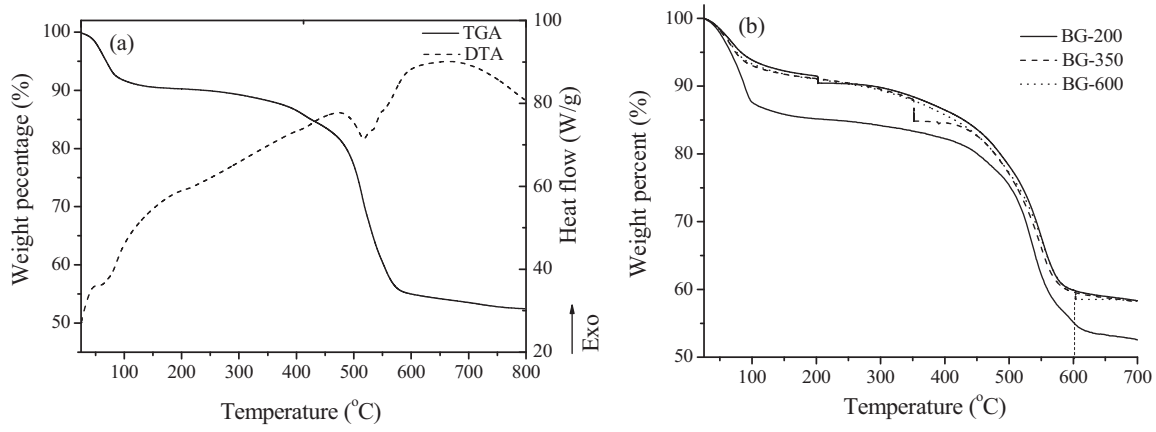


**Fig. 1.** (a) TGA and DTA traces of 70Si30Ca dried gels performed under $N_2$ protection; (b) simulation of the stabilization process of 70Si30Ca dried gels performed in air atmosphere.