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Degradation Kinetics of Calcium Polyphosphate Bioceramic: an Experimental and Theoretical Study

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In this work, the degradation kinetics of calcium polyphosphate bioceramic was studied. Liquid state ^{31}P nuclear magnetic resonance (NMR), X-ray diffraction (XRD) and scanning electron microscope (SEM) were used to characterize the product. The in vitro degradation test was carried out at 37 °C for up to 48 hours for both the simulation solution and the extreme solution. The ion concentrations were measured and analyzed by establishing a mathematical model referring to the chemical reaction kinetics. The results indicated that the degradability of calcium polyphosphate increased with the decrease of pH value, and the sample showed a rapid loss of ion concentration within the initial period of immersion followed by a slower loss ratio. The relationship between ion concentration and the degradation time coincided with Boxlucas model.

Keywords: *calcium polyphosphate, degradation, mathematical model*

1. Introduction

Controlling degradation behavior is a critical property in bone repair materials research, and has been widely investigated to date[1]. In general, the degradation of the biomaterial should be controlled precisely to give enough time for the cells to lay down their own extracellular matrix and regenegrate the injured bone, and at the same time to ensure that the scaffold does not last longer than needed.

Calcium polyphosphate (CPP), with lower Ca: P ratio, was rediscoveried as a novel bone repair material in recent years. This kind of inorganic polymer with a long-chain polymer form can be easily either arranged to give amorphous phosphate glasses or crystalline structures, depending on the processing parameters and starting compounds use[2,3]. Many studies were demonstrated that calcium polyphosphate was a biodegradable materials[4-6], and the polymerization degree, preparation condition and degradation medium can affect the degradation rate of calcium polyphosphate[7,8].

The objective of this article was to investigate the controlled degradability of CPP in different degradation medium in vitro. And a polyphosphate degradation kinetics model was established basing on the chemical reaction kinetics. The obtained degradation data was analyzed to estimate the theoretical model in order to explain the degradation kinetics of CPP.

2. Materials and Methods

2.1. Preparation of calcium polyphosphate scaffold

The preparation of CPP powder has been described in detail by Qiu[9]. Briefly, according to the stoichiometric use, calcium phosphate monobasic monohydrate powder was sintered at 500 °C under atmospheric conditions, and held for 10 hours. Then this powder was sintered at 1200 °C to yield amorphous melt frit. The melted frit was promptly quenched into distilled water to avoid crystallization during cooling. The amorphous frit was milled and screened to powder in a size range of 48~75 μm.

The samples for immersion study were fabricated as follows. The sieved CPP powder (0.6 g) and stearic acid (0.4 g) was mixed and pressed uniaxially to form green bodies with dimension of $\Phi 10 \times 10$ mm. The green bodies were then heated in air to 800 °C at a heating rate of 5 °C/min. After holding for 3 hours at 800 °C, the specimens were furnace-cooled to room temperature.

2.2. Materials characterization

The polymerization degree of sample was quantified using solution ^{31}P nuclear magnetic resonance (NMR)[10]. For liquid state ^{31}P NMR measurement, the CPP powder was dissolved in a 3% solution of disodium ethylenediaminetetracetate dihydrate (Na2–EDTA) adjusted to a pH of 7.0-7.4 with sodium hydroxide[11]. Multiple NMR analyses were conducted at 300 K on a Bruker AV300 NMR spectrometer with ^{31}P probe operating at 121.49 MHz. 200 scans were collected with a 10.275 μs pulse duration width and a 6.00 μs pulse separation to obtain spectra, being referenced to an 85% solution of H_3PO_4 in H_2O[12].

X-ray diffraction (XRD) analysis was used to identify the crystalline phase. The calcium polyphosphate powder was analyzed for XRD experiment performed on the X'Pert Pro MPD X-ray diffractometer (Philips®, Netherlands). The voltage and anode current were 40 KV and 40 mA, respectively. The Cu Kα = 0.15405 nm and continuous scanning mode with 0.02 step size and 0.5 seconds of set time were used in XRD experiment for collecting the data of sample.

The percentage of open porosity, that accounts only for the penetrable volume of the scaffold (i.e. interconnected pores), was

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determined in known manner by impregnation with ethanol under vacuum at 20 °C, using triplicate samples. The percentage of open porosity was calculated as:

$$\text{open porosity}(\%) = \frac{W_{wet} - W_{dry}}{\rho_{liquid} . V_{scaffold}} .100 \quad (1)$$

where M_{dry} and M_{wet} are the mass of sample in air and in ethanol, respectively. ρ_{liquid} is the density of ethanol at room temperature, and $V_{scaffold}$ is the volume of the CPP scaffold.

2.3. Degradation behavior

2.3.1. In vitro degradation testing

The degradation testing was performed according to the ISO 10993-14 "Biological evaluation of medical devices – Part 14: Identification and quantification of degradation products from ceramics". And it consisted of two tests at different pH values. The first test was performed in Tris–HCl solution (pH 7.4) and is referred to as simulation solution testing; the second test was conducted at pH 3.0 using citric acid and is referred as extreme solution testing. Tests were carried out at 37 °C and with a mixing speed of 120 r. p.m. The samples were incubated for 4, 8, 12, 18, 24, 30, 36, 42 and 48 hours for both testing solutions, using triplicate samples.

2.3.2. Weight loss, ion concentration and pH value measurement in degradation medium

The sample weight before degradation was measured as W_o, the sample weight after degradation washed by distilled water was measured and dried as W_t, the weight loss:

$$\text{Weight loss } (\%) = [(\ Wo - Wt\ ) / Wo\ ] \times 100 \quad (2)$$

The ammonium molybdate ultraviolet-visible spectrophotometry (AMUVS) method was used to measure the phosphate ion concentration in degradation medium[13]. Briefly, phosphate was reacted with ammonium molybdic acid and further reduced by ascorbic acid to form a blue complex with a maximum absorbance between 700 nm using a UV/VIS Spectrophotometer. Each 0.1 mL of degraded medium was analyzed to determine the amount of free phosphate ion in solution.

Moreover, chemical method was used to measure calcium ion concentration in degradation solution. Since calcium ion in solution can chelated with disodium ethylenediaminetetracetate dihydrate (Na_2–EDTA)[14] and this chelated product can eliminate the characteristic chromogenic reaction between Ca^{2+} and calcein.

Calcein titration method was used to measure the Ca^{2+} concentration change in degraded solution. About 3 mg calcein was added to *v* mL degraded medium after solution color turning yellow-green fluorescence with stir. Standard Na_2 – EDTA solution (c/mol.L^{-1}) was titrated into solution until the fluorescence was disappearing. The cost volume (v_c/mL) was recorded. Then calculated Ca^{2+} concentration (X_1/ mg.L^{-1}) as follow:

$$X_1 = \frac{cV_c \times 0.04008}{V} \times 10^6 \quad (3)$$

The pH value was also be measured. Each measured sample contains three parallel test samples.

2.3.3 SEM analyse

The scaffolds before and after degradation were sputter coated with gold. The surface of the scaffolds was characterized by a JSM-5900LV scanning electron microscopy (Japan).

2.4. Mathematical model

In this paper, the obtained data were analyzed to estimate the theoretical model in order to explain the degradation kinetics of CPP, and the first order process was used to explore the degradation kinetics model. Due to the theoretical model and the data structures, the non-linear regression was manually performed in Origin 7.0.

3. Results and Discussion

3.1. Materials characterization

3.1.1. The polymerization degree of the sample

The liquid state ^{31}P NMR spectra of CPP was showed in Figure 1. Based on previous ^{31}P-NMR analysis of sodium and zinc polyphosphate glasses[15], comparative average polymerization degree is calculated by integrating standardized delimited areas under the respective peaks for the ortho groups (Q0), end groups (Q1) and internal groups (Q2):

$$\text{Polymerization Degree} = \frac{Q0 + Q1 + Q2}{Q0 + \frac{Q1}{2}} \quad (4)$$

As shown in Figure 1, the surface areas of Q0, Q1 and Q2 were 0.01, 10.06, and 89.93%, respectively. The polymerization degree of CPP was 20.

3.1.2. XRD analysis

Figure 2 was the XRD pattern of CPP scaffold. Compared with standard PDF card 77-1953, it was showed that characteristic peaks in CPP curve accorded with the standard curves, especially three characteristic calcium phosphate peaks between 20~30°, which indicated the crystal system of CPP was the same as β-CPP.

Previous study by Qiu et al.[7] explored that the degradation behavior of CPP was obviously affected by crystalline structure: the degradation rate of a-CPP, β-CPP and γ-CPP decreased in turn. Literature[16] reported that during the sintering process from room temperature to 1000 °C, γ-CPP was transformed to α-CPP. And the crystal system of CPP was β-CPP at sintering temperature ranges of 585 to 900 °C.

3.1.3. Microstructure and porosity of sample

As shown in Figure 3, the scaffold exhibited three-dimensionally interconnected pore structure and with a pore size of about 200-400 μm. For bone tissue engineering, ideal scaffold should have appropriate pore structure and pore size, to ensure a biological environment conducive to cell attachment, proliferation and flow transport of nutrients and

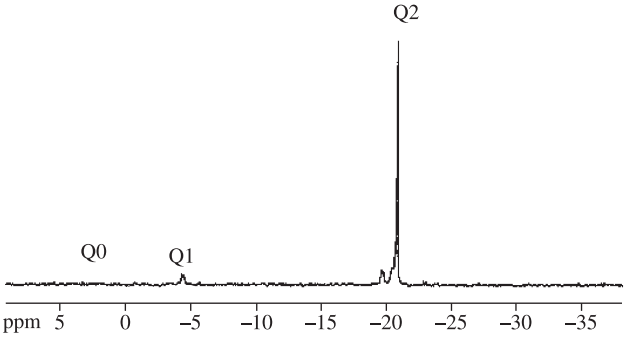

Figure 1. ^{31}P NMR spectra of the calcium polyphosphate.

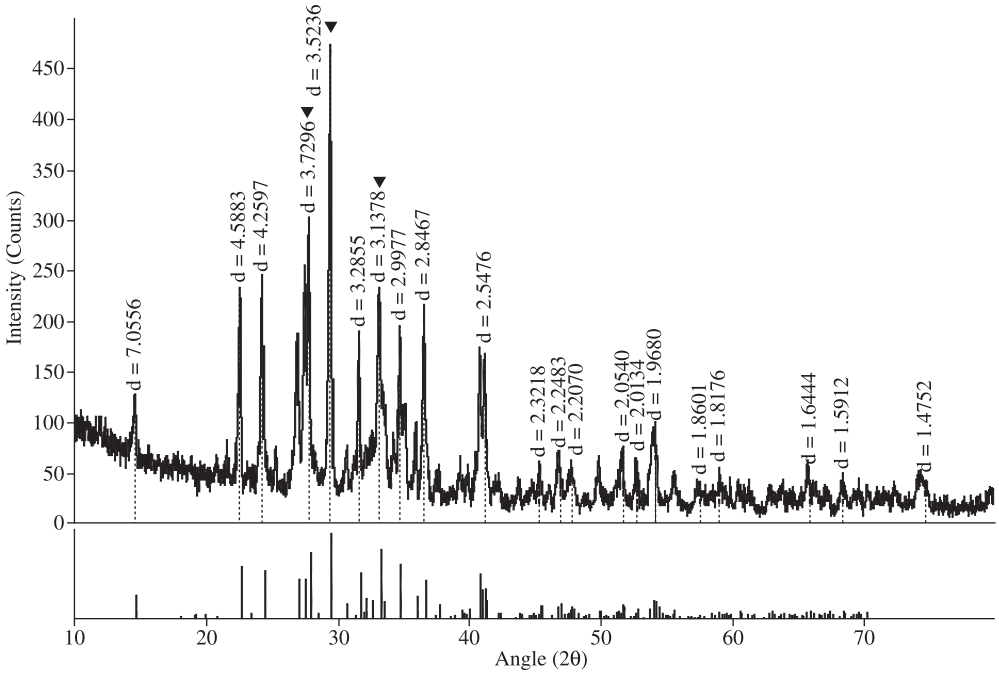

Figure 2. XRD pattern of calcium polyphosphate.

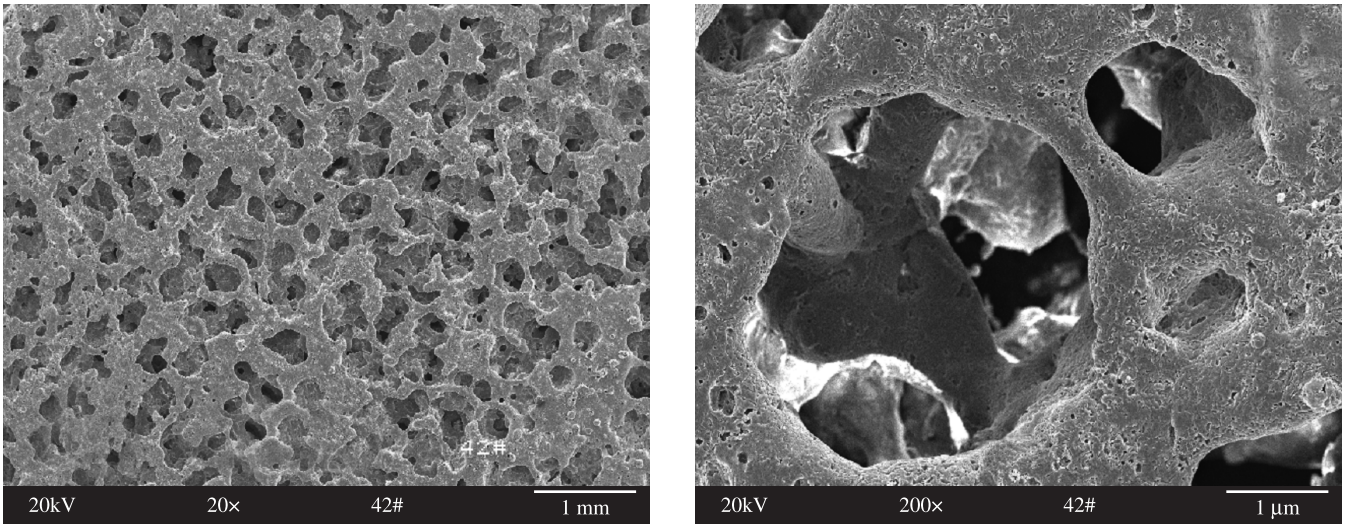

Figure 3. SEM pictures of calcium polyphosphate.

metabolic waste. The optimal pore size required for bone ingrowth has been suggested in the range of 100-800 µm[17].

And for in vitro degradation testing, the porosity can obviously affect the degradability of biomaterials. In this study, stearic acid was used as a pore former. The porosity of CPP scaffold exhibited (52.87 ± 3.82)% porosity. It was noted that the percent of porosity was higher than theoretical result, which indicated that sintering procedure would cause some pore.

3.2. Degradation behavior

3.2.1. Weight loss

As shown in Figure 4, the weight loss of scaffold in both degradation mediums were constantly increased during immersion period. After 48 hours of immersion, the weight of scaffold lost 2.25% for Tris-HCl buffer solution and 3.38% for citric acid buffer solution. The weight loss showed a steady increase with the increase of the immersion period, which presented a similar trend for both conditions.

3.2.2. Calcium ion and phosphate ion analyse

As showed in Figure 5 and 6, for both ions measured and for both testing solution, the ion concentration of the testing solution increased with the increase of the soaking time, which supports the relative weight loss values presented in Figure 4. Compare the Figure 5 and 6, it was showed that the degradability of calcium polyphosphate increased with the decrease of pH value. For example, the phosphate ion concentration lost 870.3 $mg.L^{-1}$ after immersion in Tris-HCl buffer solution over 48 hours, and lost 1006.8 $mg.L^{-1}$ for the citric acid buffer solution over the same time

of immersion. Both conditions presented a similar trend, with a steady increase in phosphate ion concentration as the immersion period increased. The CPP showed different degradation rate for the two testing solutions.

3.2.3. pH value

Figure 7 depicted the variation of pH of the two testing solutions with immersion time. It was observed a significant decrease in the pH of both testing solutions. The pH was decreased from 7.4 to approximately 7.13 after 48 hours of immersion in Tris-HCl buffer solution, and for the citric acid buffer solution the pH from 3.0 to 2.67 was observed up to 48 hours.

3.2.4. SEM analyse

Figure 8 was the surface topography of CPP scaffold before and after degradation. From the SEM patterns, it showed significant differences in the progress of surface degradation in both testing solutions. A much more crack was observed on the CPP surface after 48 hours immersion in the citric acid buffer solution (Figure 8d) compared with the experiment performed at pH 7.4 (Figure 8b), which supports the relative changes presented in Figures 4-6.

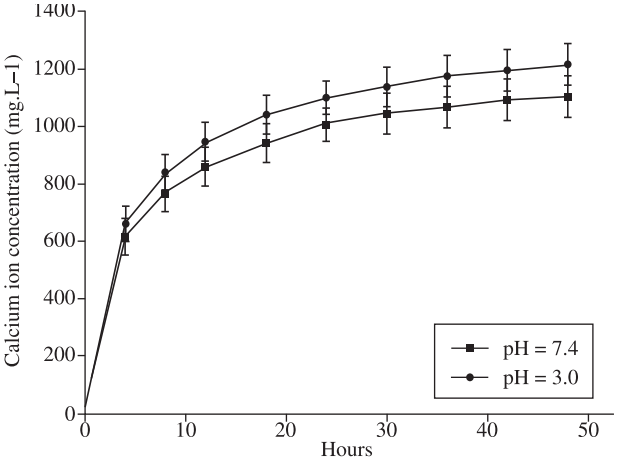

Figure 6. The calcium ion concentration in different degradation medium.

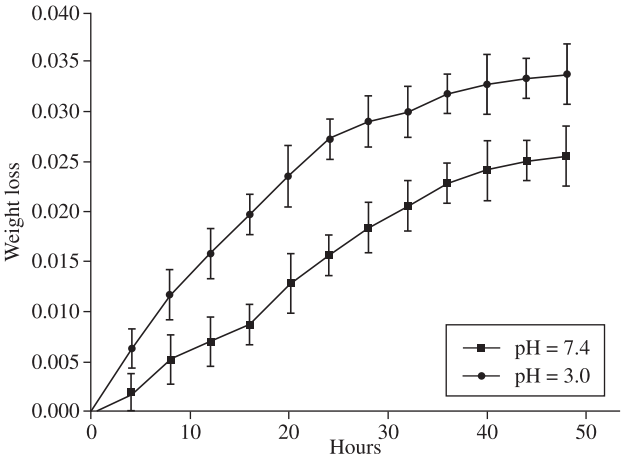

Figure 4. Weight loss of calcium polyphosphate in different testing solution.

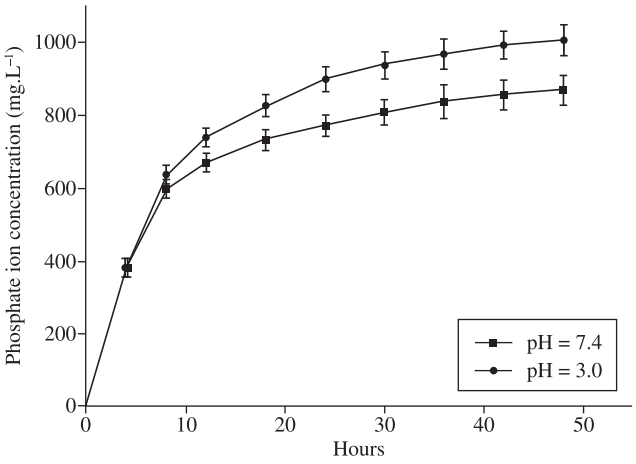

Figure 5. The phosphate ion concentration in different degradation medium.

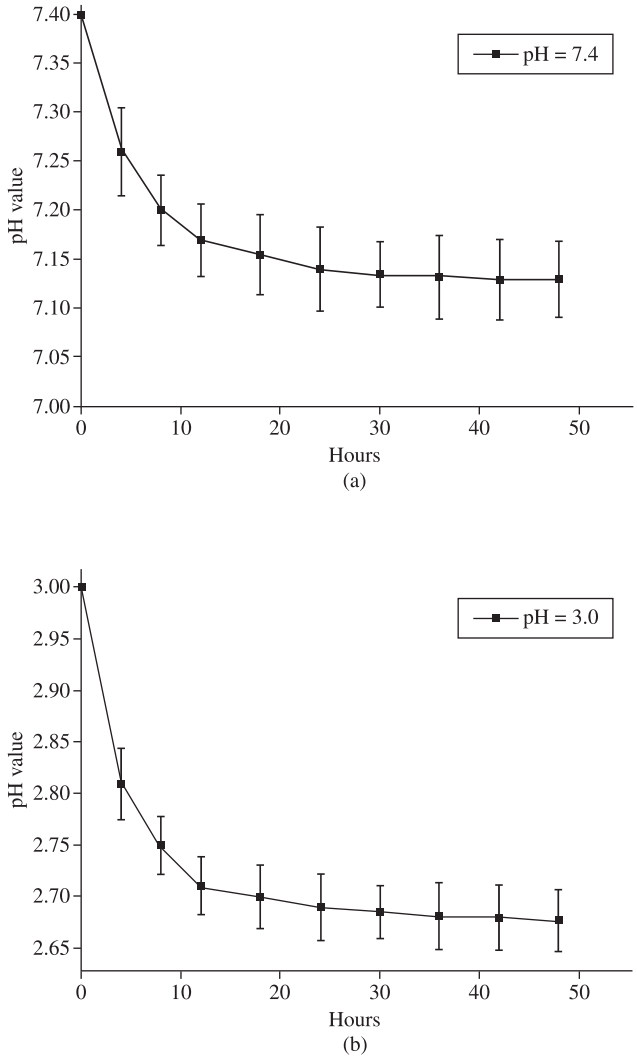

Figure 7. pH change in the different degradation solution: a) Tris-HCl solution, b) citric acid solution

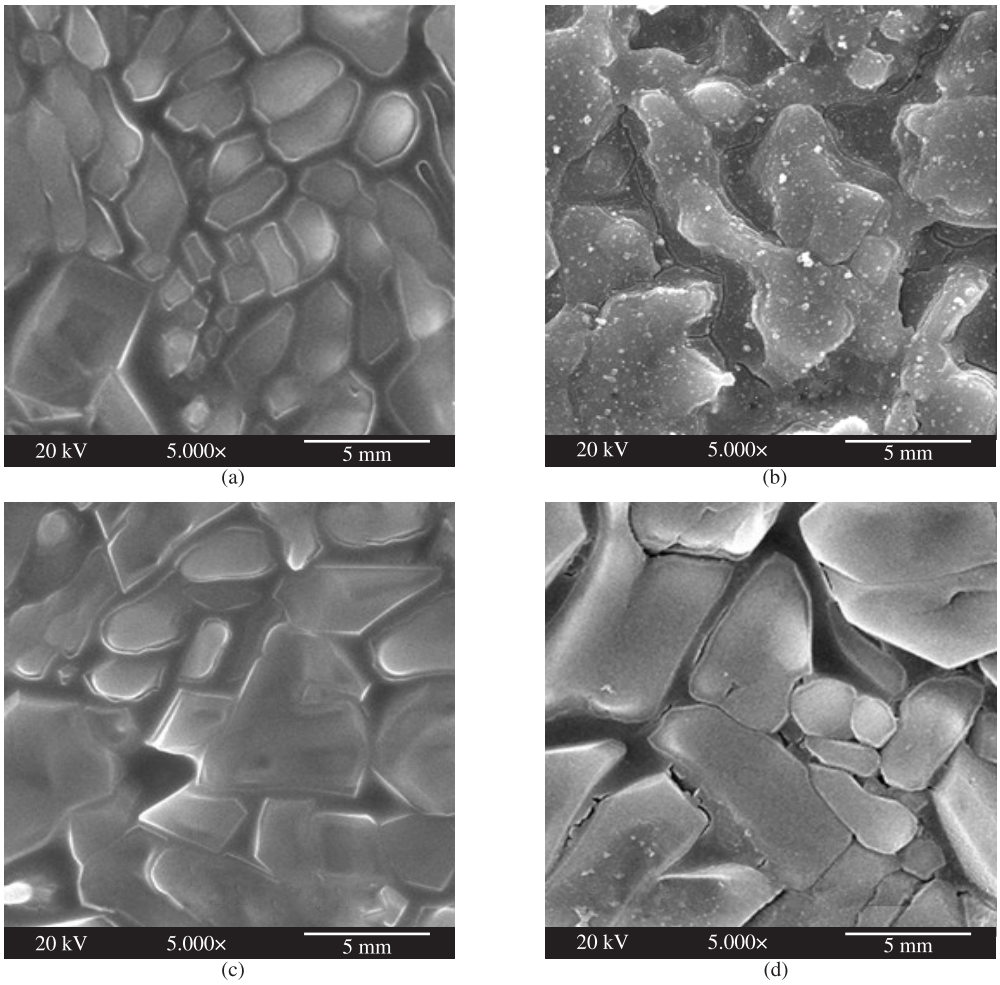

Figure 8. SEM pictures of calcium polyphosphate surface before and after degradation in different testing solution: a) before degradation in Tris-HCl solution, b) after degradation in Tris-HCl solution, c) before degradation in citric acid solution, and d) after degradation in citric acid solution.

3.3. Dissolution mechanism and degradation kinetics of calcium polyphosphate

3.3.1. Degradation kinetics model of polyphosphate

Polyphosphate degradation is probably a very complex reaction involving numerous breakdown products and intermediates. Polyphosphate, with many phosphates in a chain, probably breaks down into smaller chains as the reaction progresses.

$$
\begin{aligned}
&P_n \xrightarrow{k_n} P_{n-1} + Pi \\
&P_{n-1} \xrightarrow{k_{n-1}} P_{n-2} + Pi \\
&P_{n-2} \xrightarrow{k_{n-2}} P_{n-3} + Pi \\
&\quad . \quad . \quad . \\
&P_3 \xrightarrow{k_3} P_2 + Pi \\
&P_2 \xrightarrow{k_2} Pi + Pi
\end{aligned} \tag{5}
$$

where "P" will be used as an abbreviation polyphosphate and "n" is the number of phosphates in the chain. If the degradations of polyphosphates are first order process,

$$
\begin{bmatrix} \frac{d[P_n]}{dt} \\ \frac{d[P_{n-1}]}{dt} \\ \frac{d[P_{n-2}]}{dt} \\ \cdots \\ \frac{d[P_2]}{dt} \\ \frac{d[Pi]}{dt} \end{bmatrix} = \begin{bmatrix} -1 & 0 & 0 & 0 & \ldots & 0 \\ 1 & -1 & 0 & 0 & \ldots & 0 \\ 0 & 1 & -1 & 0 & \ldots & 0 \\ . & . & . & . & . & . \\ 0 & 0 & \ldots & 0 & 1 & -1 \\ 1 & 1 & 1 & \ldots & 1 & 2 \end{bmatrix} \begin{bmatrix} v_n \\ v_{n-1} \\ v_{n-2} \\ \ldots \\ v_3 \\ v_2 \end{bmatrix} = \begin{bmatrix} -1 & 0 & 0 & 0 & \ldots & 0 \\ 1 & -1 & 0 & 0 & \ldots & 0 \\ 0 & 1 & -1 & 0 & \ldots & 0 \\ . & . & . & . & . & . \\ 0 & 0 & \ldots & 0 & 1 & -1 \\ 1 & 1 & 1 & \ldots & 1 & 2 \end{bmatrix} \begin{bmatrix} k_n[P_n] \\ k_{n-1}[P_{n-1}] \\ k_{n-2}[P_{n-2}] \\ \ldots \\ k_3[P_3] \\ k_2[P_2] \end{bmatrix} \tag{6}
$$

$$
\Rightarrow \begin{cases} \frac{d[P_n]}{dt} = -k_n[P_n] \\ \frac{d[P_{n-1}]}{dt} = k_n[P_n] - k_{n-1}[P_{n-1}] \\ \frac{d[P_{n-2}]}{dt} = k_{n-1}[P_{n-1}] - k_{n-2}[P_{n-2}] \\ \quad . \quad . \quad . \\ \frac{d[P_2]}{dt} = k_3[P_3] - k_2[P_2] \\ \frac{d[Pi]}{dt} = k_n[P_n] + k_{n-1}[P_{n-1}] + k_{n-2}[P_{n-2}] + \ldots + k_3[P_3] + 2k_2[P_2] \end{cases} \tag{7}
$$

$$\frac{d[Pi]}{dt} = k_n[P_n] + k_{n-1}[P_{n-1}] + k_{n-2}[P_{n-2}] + \ldots + k_3[P_3] + 2k_2[P_2] \quad (8)$$

$$\frac{d[P_n]}{dt} = -k_n[P_n] \quad (9)$$

$$[P_n] = [P_n]_0 e^{-k_n t} \quad (10)$$

In this degradation mechanism, P_{n-1}, P_{n-2} … P_2 are intermediates production. If the steady-state approximation is assumed to simplify the kinetic analysis, the concentrations of these intermediates would stay on the same level throughout the reaction except at the beginning and end. Therefore,

$$\frac{d[P_{n-1}]}{dt} = 0 = k_n[P_n] - k_{n-1}[P_{n-1}] \quad (11)$$

$$[P_{n-1}] = \frac{k_n}{k_{n-1}}[P_n] \quad (12)$$

In a similar way, $[P_{n-2}] = \frac{k_{n-1}}{k_{n-2}}[P_{n-1}]$ (13)

…….

$$[P_2] = \frac{k_3}{k_2}[P_3] \quad (14)$$

Substituting Equations 9, 11, 12 and 13 into Equation 7,

$$\frac{d[Pi]}{dt} = nk_n[P_n]_0 e^{-k_n t} \quad (15)$$

$$[Pi] = n[P_n]_0 (1 - e^{-k_n t}) \quad (16)$$

The Equation (16) is used to model phosphate concentration vs. time. The general form:

$$[Pi] = a(1 - e^{-bt}) \quad (17)$$

where, "*a*" and "*b*" had something to do with the different synthesizing procedures and degradation medium. The general form, BoxLucas model, was used to model any polyphosphate degradation.

3.3.2. Estimation of the theoretical model

As shown above, the Equation 17 (BoxLucas model) was used to model any polyphosphate multi-step first order reaction process. The non-linear fitting curves of the degradation data were drawn by Origin7.0 program (Figure 9). The reliability-square of the fitting curves was about 0.99. The result indicated that the

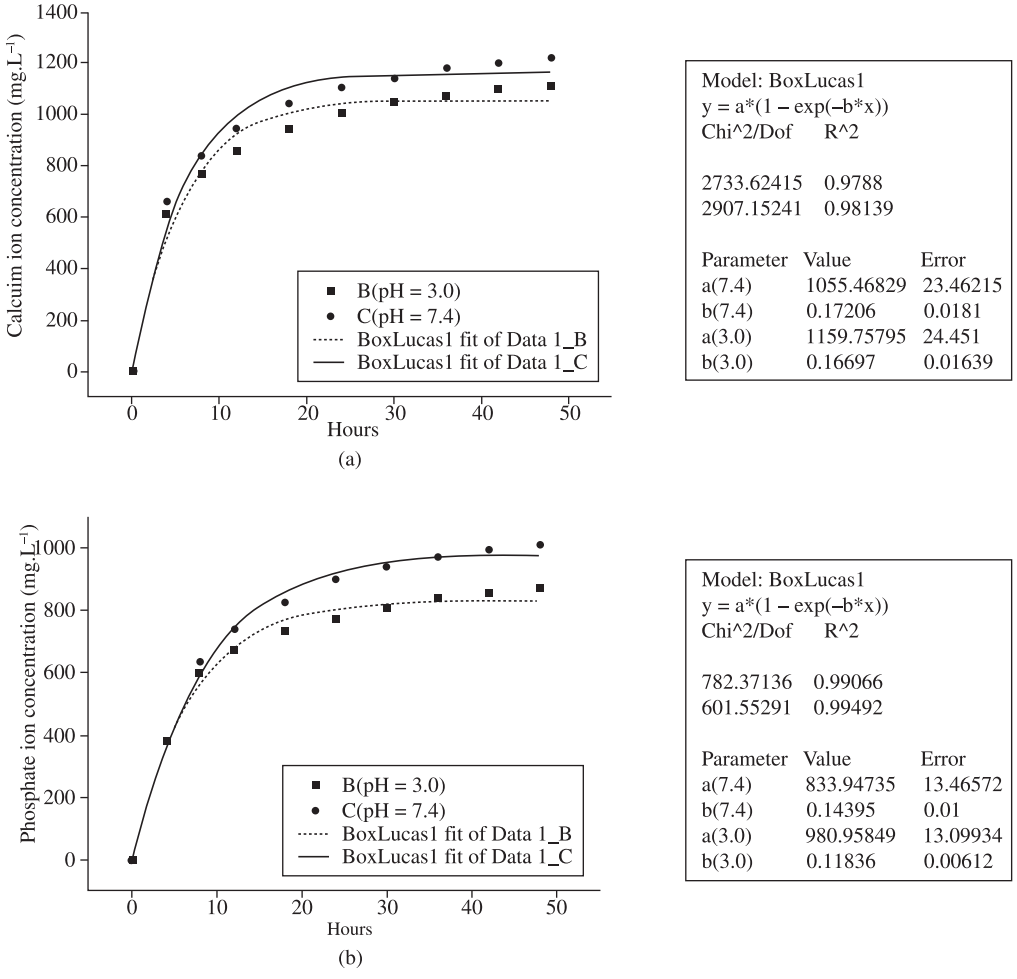

Figure 9. The non-linear fitting curves of the degradation data: a) calcium ion, b) phosphate ion.

theoretical degradation kinetics model is accurate for polyphosphate degradation.

3.3.3. Calcium polyphosphate dissolution mechanism

Calcium polyphosphate degradation is probably a very complex reaction. According to the generally accepted theory of glass dissolution[18-20], polyphosphate glasses dissolve in aqueous medium in the following two interdependent steps:

1) Hydration reaction: when CPP immersed in degradation medium, calcium ions in the sample first exchanged with hydrogen in the solution leading to the formation of a hydrated layer on the glass surface at the glass–water interface; and
2) Network breakage: under the attack of hydrogen ions and water molecules, the P–O–P bonds broke up and resulted in the destruction of polyphosphate chains.

Trauss et al.[21] studied hydrolysis of branched sodium polyphosphate solutions. They found a first-order dependence based on viscosity measurements. Ray[22] also mentioned first-order hydrolytic degradation with an activation energy of 104 ± 8 kJ.mol^{-1} determined by viscosity measurements in a temperature range of 25-90 °C. In this paper, we established a polyphosphate degradation model basing on first order process. And a theoretical model was used to curve-fit the ion concentrations from the results of the degradation experiment.

4. Conclusion

In this paper, we investigated the dissolution behavior of calcium polyphosphate, and a polyphosphate degradation kinetics model was established basing on the chemical reaction kinetics. The results of in vitro degradation indicated that calcium polyphosphate showed a rapid degradation rate in the extreme solution compared with the simulation solution. The relationship between ion concentration and the degradation time correspond with Boxlucas model function ($y = a\,(1–e^{–bx})$).

Acknowledgements

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