

Research on Drug Loading Performance of Hydroxyapatite-Gelatin Composites by Co-precipitation Method

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Abstract: Hydroxyapatite/polymer composites are promising materials for drug delivery applications. Studies focusing on the development of such composites are available in recent years, as using these materials as a carrier allows us to overcome the side effects of toxic drugs used especially in cancer treatments and increase treatment efficiency. In this study, hydroxyapatite-gelatin (HAp-GEL) composites are produced in the presence of simulated body fluid (SBF) as a carrier for 5-Fluorouracil (5-FU). Composites are produced by wet precipitation method at pH 7.4 and 37 °C and crosslinked with glutaraldehyde (GA). In order to observe the effect of GA amount on drug loading efficiency, composites cross-linked with different amounts of GA are released in deionized water, HCl and phosphate buffer solution (PBS). Composites are analyzed by X-Ray Diffraction (XRD), Thermogravimetric Analyses (TGA), Scanning Electron Microscopy (SEM) and particle size distribution to observe morphology and structure. It is concluded that drug loaded HAp-GEL composites have a potential to be used in drug delivery applications.

Key words: Hydroxyapatite, gelatin, drug loading, composite.

1. Introduction

Hydroxyapatite (HAp, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) is one of the major constituents of human body which builds up the 60% of bones, 97% of tooth enamel and 70% tooth dentin. Due to its bioactive, biocompatible and nontoxic characteristics, it has been widely used as a bone-graft substitute material, coating material, bone and teeth implant material and drug delivery agent [1]. In some cases, highly fragile and hard structure of hydroxyapatite can limit its usage. Polymer incorporation is a way of overcoming these mechanical disadvantages and enhancing its properties. Gelatin is the widely used polymer in drug delivery studies alone or as a part of a composite. Gelatin is a biocompatible, biodegradable and nontoxic polymer used widely in food and medicine industries obtained

by partial hydrolysis of collagen. As gelatin has an efficient swelling-releasing characteristic, does not produce antigen and does not have a negative effect on cell viability, it is a typical polymer used in drug delivery studies [2].

In the current study, hydroxyapatite-gelatin (HAp-GEL) composites are produced in the presence of simulated body fluid (SBF). 5-FU, which is widely used for the treatment of colon, rectal, breast, ovary, pancreas, stomach, brain and skin cancer, is selected as drug [3, 4]. Composites are produced by wet precipitation method implementing glutaraldehyde (GA) as a cross-linking agent. In order to observe the effect of GA amount on drug loading efficiency, composites cross-linked with different amounts of GA are released in deionized water, HCl and phosphate buffer solution (PBS). Composites are analyzed by XRD, TGA, SEM and particle size distribution to observe morphology and structure.

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2. Experimental Procedure

2.1 Materials

Materials used for HAp-GEL composites preparation including gelatin, calcium hydroxide (Ca(OH)₂, 96%), phosphoric acid (H₃PO₄, 85%) and glutaraldehyde are purchased from Merck. 5-Fluorouracil drug is obtained from Sigma-Aldrich. Reactants used for SBF preparation are listed in Table 1.

Sodium chloride (NaCl), sodium hydrogen carbonate (NaHCO₃), potassium chloride (KCl), di-potassium hydrogen phosphate trihydrate (K₂HPO₄ 3H₂O), magnesium chloride hexahydrate (MgCl₂ 6H₂O), calcium chloride (CaCl₂), sodium sulfate (Na₂SO₄) and hydrochloric acid (HCl) are obtained from Merck and tris (hydroxymethyl) aminomethane ((CH₂OH)₃CNH₂) is provided from Sigma-Aldrich. Potassium phosphate dibasic (K₂HPO₄) and potassium phosphate monobasic (KH₂PO₄) which are used for PBS preparation are obtained from Merck and Carlo Erba respectively.

2.2 Methods

For SBF preparation, reactants in Table 1 are added to 750 mL deionized water respectively and dissolved under constant stirring at 37 °C. (CH₂OH)₃CNH₂ is dissolved slowly to prevent instant pH increase. Afterwards, pH of the solution is adjusted to 7.4 with 1 M HCl. SBF solution left rested for 1 day and

completed to 1 L with deionized water. And 80.2 mL 1 M K₂HPO₄ and 19.8 mL 1 M KH₂PO₄ are prepared and mixed to obtain PBS solution. The mixture is completed to 1 L with deionized water and adjusted to pH 7.4 with 1 M HCl solution.

5-FU loaded hydroxyapatite-gelatin composites are prepared by mixing 5-FU-Ca(OH)₂-SBF and H₃PO₄ (85%)-GEL-SBF solutions separately for two hours at 37 °C and 400 rpm. H₃PO₄ (85%)-GEL-SBF solution is feeded to 5-FU-Ca(OH)₂-SBF with peristaltic pump at a feeding rate of 5 mL/min resulting in the formation of HAp crystals in the solution. pH is adjusted to 7.4 with 1 M NaOH or HCl depending on the final pH value of the solution. Obtained solution is mixed at 37 °C and 400 rpm for another 2 hours and left rested.

After 24 hours of aging for the completion of HAp crystals growth, GA-deionized water solution is added to solution for crosslinking. At the end of 3 hours of stirring, solution is filtered and precipitated composites are washed with sodium bisulfate and de-ionized water several times.

Finally, obtained precipitates are dried in incubator at 40 °C for 24 hours and collected afterwards. Composites with 1:1 HAp/GEL weight ratio are obtained and crosslinked with 2% (v/v) and 5% (v/v) GA-deionized water solution. Drug loading efficiencies are evaluated according to Eq. (1) in three different medium including deionized water, HCl and PBS.

$$\text{Drug loading efficiency} = \frac{\text{Theoretical drug amount}}{\text{Drug amount in composites}} \times 100 \quad (1)$$

Table 1 Reactants used for SBF preparation.

Reactant	Quantity
NaCl	7.996 g
NaHCO ₃	0.350 g
KCl	0.224 g
K ₂ HPO ₄ 3H ₂ O	0.228 g
MgCl ₂ 6H ₂ O	0.305 g
1 M HCl	40 mL
CaCl ₂	0.278 g
Na ₂ SO ₄	0.071 g
(CH ₂ OH) ₃ CNH ₂	6.057 g

5-FU concentration in the release medium is evaluated by UV spectrophotometer at the wavelength of 266 nm in triplicate essays. The crystallinities of the composites are analyzed by XRD (Bruker D8 Advance X-Ray Diffractometer) in the 2 Theta (θ) range at 10-90 °C. TGA analyses are held by TA SDT Q600 in the temperature range of 25-700 °C. Particle size of the composites are measured with Mastersizer. The morphologies of the HAp-GEL composites with different amount of GA are observed by (SEM) (Quanta FEG 250).

3. Results and Discussions

3.1 XRD Analysis of HAp-GEL Composites

XRD patterns of composites crosslinked with different amount of GA (2% and 5% GA solution) are given in Fig. 1. Characteristic HAp peaks are observed in all samples, confirming the formation of HAp crystals. In addition, no other calcium phosphate phases are detected.

In HAp-GEL composites, characteristic HAp peaks are confirmed by the peaks at 2 theta of 25°, 31°, 39°, 45°, 46° and 50°. Even though both composites show a similar trend of peaks, it is observable that the intensities of the HAp-GEL composite with 2% GA

are higher. In a previous study, it is shown that gelatin amount of the composite with more GA can be higher [4]. Also, Bera et al. [5] proposed that gelatin addition to HAP may suppress the growth of the HAp crystals, therefore causing lower intensities in XRD peaks.

Similarly, HAp-GEL composites with 5% GA may have higher amount of gelatin than HAp-GEL 2% GA composite, and higher amount of gelatin may cause higher suppression of HAp crystals, leading to lower intensities observed in Fig. 1. On the other hand, the peak at 2 theta of 31° is also a characteristic peak of 5-FU and may be overlapped with the characteristic HAp peak [6]. It can be proposed that higher intensity in HAp-GEL 2% GA sample may be caused by containing higher amount of 5-FU than HAp-GEL 5% GA. In general, it is concluded that the amount of GA does not affect the intensities distinctively [7, 8].

3.2 TGA Analysis of HAp-GEL Composites

TGA analysis of HAp-GEL composites is given in Fig. 2. Analysis is held at a heating rate of 10 °C/min in a 25-700 °C temperature range. As seen in Fig. 2, gelatin phases in the composites are degraded gradually. The weight loss between 50-90 °C can be attributed to the evaporation of water molecules in the

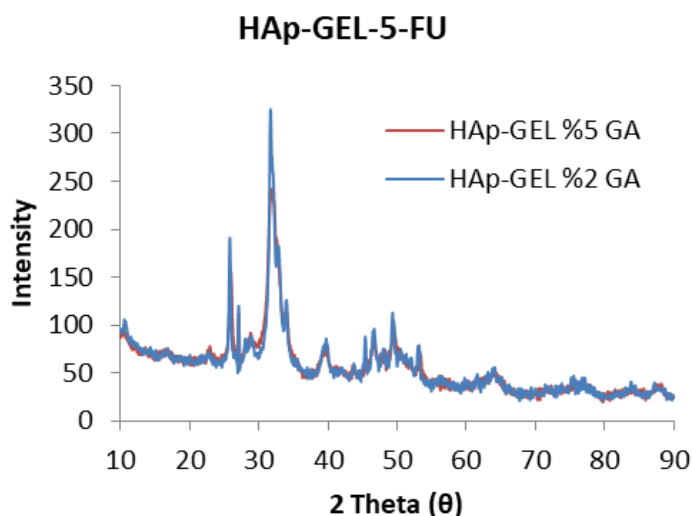


Fig. 1 XRD analysis of 5-FU loaded HAp-GEL composites.

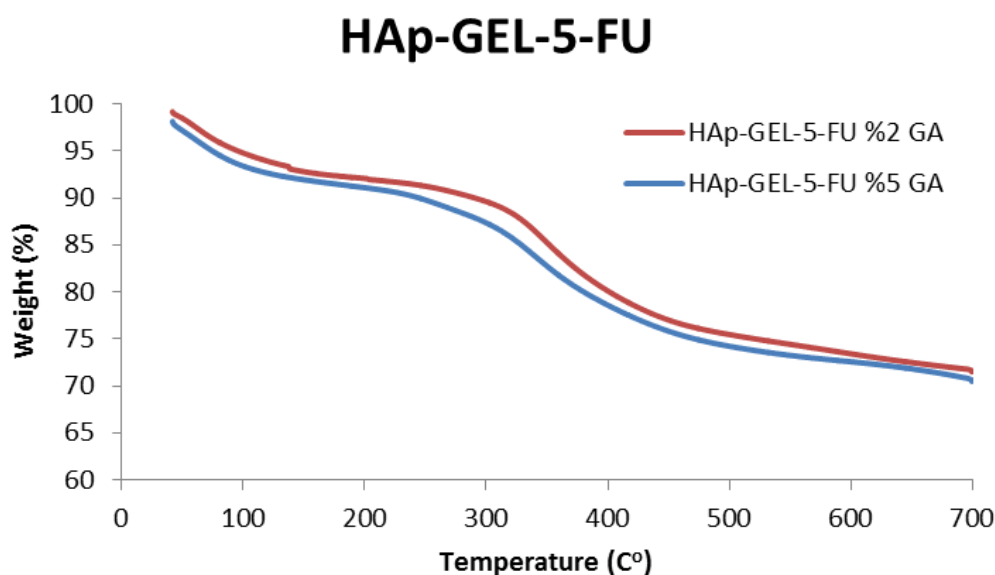


Fig. 2 TGA analysis of HAp-GEL and HAp-CTS composites.

Table 2 Particle size distribution of HAp-GEL composites.

Sample	<i>d</i> (0.1)	<i>d</i> (0.5)	<i>d</i> (0.9)	Average particle size (µm)
HAp-GEL 2% GA 1:1	50	203	349	204
HAp-GEL 5% GA 1:1	65	209	874	312

composites. While the degradation continues with a lowering rate until about 300 °C, a sharp weight loss is observed causing from the pyrolysis of gelatin molecules in the temperature range of 300-400 °C. At 700 °C, only inorganic HAp phase is left un-degraded in the composites. The gelatin ratio in both HAp-GEL composites is determined as 30% [4].

3.3 Particle Size Distribution of HAp-GEL Composites

Particle size values of the HAp-GEL composites are given in Table 2. As seen in Table 2, average particle sizes vary between 204 and 312 µm.

3.4 SEM Analysis of Drug Loaded HAp-GEL Composites

SEM analysis of drug loaded HAp-GEL composites is illustrated in Fig. 3. Each composite shows a laminated morphology which is expected because of the used wet precipitation method. HAp-GEL 5% GA sample shows a higher agglomerated structure, which

can be attributed to the higher gelatin amount causing from higher GA.

3.5 Drug Loading Efficiencies of HAp-GEL Composites

Drug loading efficiencies of HAp-GEL composites are given in Table 3. Generally, a decrease in drug loading efficiency is observed with increasing GA content while the sample including HAp-GEL 2% GA, shows the highest drug loading efficiency in acidic HCl medium which is consistent with our previous study [4]. It is a known fact that the pH of the environment around the cancer cells is more acidic [8-10]. Overall, drug loading efficiencies of the composites do not exceed 36.5%.

4. Conclusions

Hydroxyapatite-gelatin composites crosslinked with different amount of GA are produced and loaded with 5-FU cancer drug by a wet precipitation method. Formation of hydroxyapatite phase is confirmed by XRD

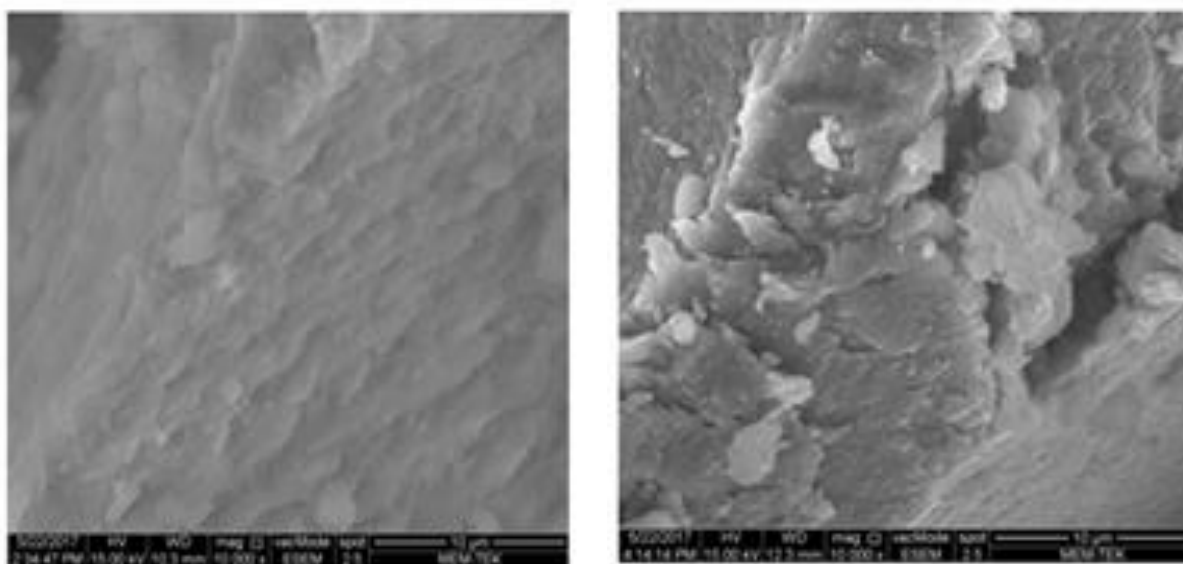


Fig. 3 SEM Analysis of HAp-GEL composites. (a) HAp-GEL 2% GA, (b) HAp-GEL 5% GA.

Table 3 Drug loading efficiencies of HAp-GEL composites.

Composite	Deionized water (%)	HCl (%)	PBS (%)
HAp-GEL 2% GA 1:1	13.6	36.5	17.6
HAp-GEL 5% GA 1:1	1.8	17.0	12.0

analysis. TGA analysis provides insight into the organic content amount in composites. SEM analysis reveals the laminated structure of HAp-GEL. Average partical sizes of the composites are found to be varied between 204 and 312 μm . In order to observe the effect of GA on drug loading efficiency, release of the composites is observed in deionized water, HCl and PBS medium. A decrease in drug loading efficiency is observed with increasing GA in HAp-GEL composites. Highest drug loading efficiency is found to be 36.5% in HCl medium. Mostly, the released drug percentage increases in acidic HCl medium which is beneficial in drug release applications because of the low pH values around the cancer cells [10, 11].

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