SETSCI Conference Indexing System,
Volume 3 (2018), 1499-1502

ISAS 2018-Winter
2nd International Symposium on Innovative Approaches in Scientific Studies
November 30 – December 2, 2018, Samsun, Turkey

Production of 5-FU Drug Loaded Biocomposite Materials : Drug Loading Efficiency and Characterization

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Presentation/Paper Type: Oral / Full Paper

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 affects of toxic drugs used especially in cancer treatments and increase treatment efficiency. In this study, hydroxyapatite-chitosan (HAp-CTS) biocomposites are produced in the presence of simulated body fluid (SBF) as a carrier for 5-Fluorouracil (5-FU). 5-FU, which is widely used for the treatment of colon, rectal, breast, ovary, pancreas, stomach, brain and skin cancer, is selected as drug. Biocomposite materials are produced by wet precipitation method at pH 7.4 and 37°C implementing glutaraldehyde (GA) as a cross-linking agent. Drug loading process is performed during the wet precipitation. In order to observe the effect of GA amount on drug loading efficiency, composites cross-linked with different amounts of GA are released in deinozed water, HCl and phosphate bufffer solution (PBS). Absorbance value of the solution was obtain by Uv-vis (Jenway 6305) spectra and calibration curve was evaluated to calculate the drug concentrations. 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-CTS composites have a potential to be used in drug delivery applications.

Keywords – *Biocomposite material, hydroxyapatite, chitosan, polymer, drug loading*

I. Introduction

Hydroxyapatite (HAp, $Ca_{10}(PO_4)_6(OH)_2$) 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.

Chitosan and gelatin are the two widely used polymers in drug delivery studies alone or as a part of a composite. Chitosan is a natural polymer derived from chitin found in the shells of crustaceans. In addition to its nontoxic, biodegradable and biocompatible characteristics, it has an advantage of being found in the nature in large quantities. Because of its ability to produce gel and being found in the form of copolymers, it is widely preferred in the drug delivery studies [2].

In the current study, hydroxyapatite-chitosan (HAp-CTS) 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 deinozed water, HCl and phosphate bufffer solution (PBS). Composites are analyzed by XRD, TGA, SEM and particle size distribution to observe morphology and structure.

II. Materials and Method

A. Materials

Materials used for HAp-CTS composites preparation including, calcium hydroxide ($Ca(OH)_2$, 96%), phosphoric acid (H_3PO_4 , 85%) and glutaraldehyde are purchased from Merck. Chitosan and 5-Fluorouracil drug are obtained from Sigma-Aldrich. Reactants used for SBF preparation are listed in Table 1.

Sodium chloride (NaCl), sodium hydrogen carbonate ($NaHCO_3$), potassium chloride (KCl), di-potassium hydrogen phosphate trihydrate ($K_2HPO_4.3H_2O$), magnesium chloride hexahydrate ($MgCl_2.6H_2O$), calcium chloride ($CaCl_2$), sodium sulfate (Na_2SO_4) and hydrochloric acid (HCl) are obtained from Merck and tris (hydroxymethyl) aminomethane ($(CH_2OH)_3CNH_2$) is provided from Sigma-Aldrich.

Potassium phosphate dibasic (K_2HPO_4) and potassium phosphate monobasic (KH_2PO_4) which are used for PBS preparation are obtained from Merck and Carlo Erba respectively.

Table 1. Reactants used for SBF preparation.

Reactant	Quantity
NaCl	7.996 g.
$NaHCO_3$	0.350 g.
KCl	0.224 g.
$K_2HPO_4.3H_2O$	0.228 g.
$MgCl_2.6H_2O$	0.305 g.
1M HCl	40 mL
$CaCl_2$	0.278 g.
Na_2SO_4	0.071 g.
$(CH_2OH)_3CNH_2$	6.057 g.

B. *Method*

For SBF preparation, reactants in Table 1 are added to 750 mL deionized water respectively and dissolved under constant stirring at 37°C. $(CH_2OH)_3CNH_2$ 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. 80.2 mL 1 M K_2HPO_4 and 19.8 mL 1 M KH_2PO_4 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-chitosan composites are prepared by mixing 5-FU-$Ca(OH)_2$-SBF and H_3PO_4 (85%)-Chitosan (CTS)-SBF solutions separately for two hours at 37°C and 400 rpm. H_3PO_4 (85%)-CTS-SBF solution is feeded to 5-FU-$Ca(OH)_2$-SBF with peristaltic pump at a feding 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/CTS weight ratio are obtained and crosslinked with 2% (v/v) and 5% (v/v) GA-deionized water solution. Drug loading efficiencies are evaluted according to the Equation 1. in three different medium including deionized water, HCl and PBS.

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

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-CTS composites with different amount of GA are observed by (SEM)(Quanta FEG 250).

III. Results

A. *XRD Analysis of HAp-CTS 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 both samples, confirming the formation of HAp crystals. In addition, no other calcium phosphate phases are detected.

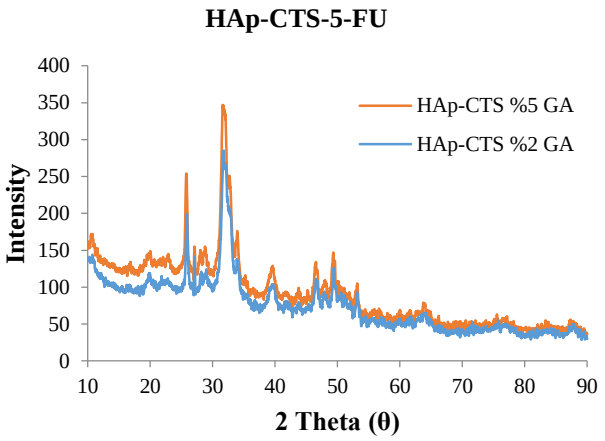


Fig.1 XRD analysis of 5-FU loaded HAp-CTS composites.

B. *TGA Analysis of HAp-CTS composites*

TGA analysis of HAp-CTS composites are given in Fig.2. Analysis are held at a heating rate of 10 °C/minute in a 25-700 °C temperature range.

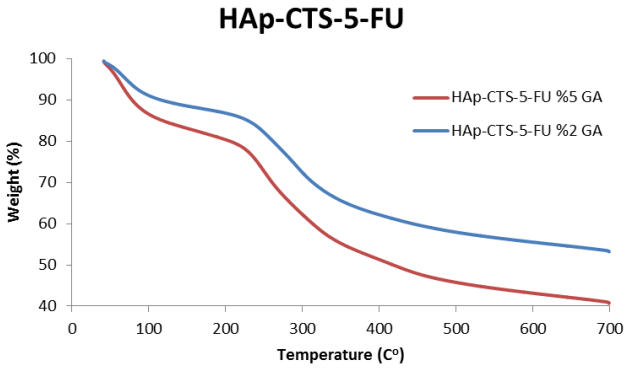


Fig.2 TGA analysis of 5-FU loaded HAp-CTS composites.

C. *Particle size distribution of HAp-CTS composites*

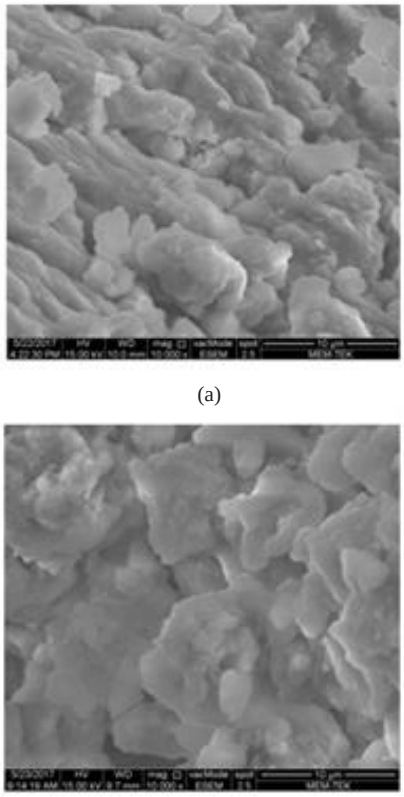
Particle size values of the HAp-CTS composites are given in Table 2.

Table 2. Particle size distribution of HAp-CTS composites.

Sample	d(0.1)	d(0.5)	d(0.9)	Average particle size (µm)
HAp-CTS 2% GA 1:1	68.427	189.573	335.684	195.985
HAp-CTS 5% GA 1:1	56.761	124.776	308.812	150.730

D. *SEM analysis of drug loaded Hap-CTS composites*

SEM analysis of drug loaded HAp-CTS composites are illustrated in Fig.3.



(a)

(b)

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

E. *Drug loading efficiencies of Hap-CTS composites*

Drug loading efficiencies of HAp-CTS composites are given in Table 3.

Table 3. Particle size distribution of HAp-CTS composites.

Composite	**Deionized water (%)**	**HCl (%)**	**PBS (%)**
HAp-CTS 2% GA 1:1	9	31	20
HAp-CTS 5% GA 1:1	7	16	14

IV. DISCUSSION

In Fig.1, for HAp-CTS composites, HAp peaks are confirmed by the peaks at 2 theta of 25º, 31º, 39º, 46º, 49º and 51º. Even though both composites show a similar trend of peaks, it is observable that the intensities of the HAp-CTS composite with 5% GA are higher. This is similar to a previous study [5]. Also, Bera et al. proposed that polimer addition to HAP may supress the growth of the HAp crystals, therefore causing lower intensities in XRD peaks [6]. On the other hand, the peak at 2 theta of 31º is also a characteristic peak of 5-FU and may be overlapped with the characteristic HAp peak [7]. In general, it is concluded that the amount of GA does not affect the intensities distinctively.

As seen in Fig.2, chitosan phase in the composites are degraded gradually. The weight loss between 50-100ºC can be attributed to the evaporation of water molecules in the composites. While the degradation continues with a lowering rate until about 250ºC, a sharp weight loss is observed causing from the pyrolysis of chitosan molecules in the temperature range of 250ºC-400 ºC. At 250ºC, the degradation of chitosan starts and is completed at around 450ºC [6], [8]. At 700 ºC, only inorganic HAp phase is left undegraded in the composites. Chitosan ratios in HAp-CTS 5% GA and HAp-CTS 2% GA are determined approximately 40% and 55% respectively.

As seen in Table 2, average particle sizes vary between 196 and 151 µm.

In Fig.3, HAp-CTS composites show irregular fragmental morphology, mostly generating from the presence of chitosan. When the amount of GA is increased, more crosslinked occurs and agglomeration increases and smoother microspheres are formed. This may be explained by increasing the amount of chitosan retained in the composite material by increasing the amount of crosslinking agent [9]-[12].

According to Table 3, generally, a decrease in drug loading efficiency is observed with increasing GA content. While the sample including HAp-CTS 2% GA, show 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 are more acidic [8], [13], [14]. Overall, drug loading efficiencies of the composites do not exceed 31%.

V. CONCLUSION

Hydroxyapatite-chitosan 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 analysis. TGA analysis provides insight into the organic content amount in composites. SEM analysis reveals the irregular fragmental morphology structure of HAp-CTS. Average partical size of the composites are found to be varied between 196 and 151 µm. In order to observe the effect of GA on drug loading efficiency, release of the composites are observed in deionized water, HCl and PBS medium. A decrease in drug loading efficiency is observed with increasing GA in HAp-CTS composites. Highest drug loading efficiency is found to be 31% 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 [15], [16].

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