Hittite Journal of Science and Engineering, 2017, 4(2) 137-144
ISSN NUMBER: 2149-2123
DOI: 10.17350/HJSE19030000060

Synthesis of Chitosan-Based Hydrogels as a Novel Drug Release Device for Wound Healing

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ABSTRACT

The development of the porous, biocompatible and biodegradable hydrogels has been gaining much attention for wound dressing applications. The hydrogels prepared using freeze-thawing method present important properties of high biocompatibility and non-toxicity. The hydrogels that are able to release drugs for prolonged time are widely used biomaterials for wound healing. In this study chitosan (CS)-based poly-ε-caprolactone (PCL) hydrogels were prepared using poly vinyl alcohol (PVA), poly ethylene glycol (PEG) and poly vinyl pyrrolidone (PVP). PVA-CS-PCL hydrogels only could remain stable at room temperature after synthesis. The properties of the hydrogels were determined with SEM, FTIR, swelling tests and degradation tests. The drugs of ceftazidime (CZ) as an antibiotic and ketoprofen (KP) as an analgesic were loaded onto the hydrogels and the loaded hydrogels were used for the drug release studies at pH 5.5 and pH 7.4. All these results suggest that the developed PVA-CS-PCL hydrogels offer significant potential as a wound dressing material.

Keywords:
Chitosan-based hydrogel, Wound dressing, Drug release, Ceftazidime, Ketoprofen.

Article History:
Received: 2017/03/20
Accepted: 2017/07/19
Online: 2017/11/27

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INTRODUCTION

The ideal wound dressing material should provide a moist healing environment, a physical and chemical barrier to infection, a painless and effective healing process and a comfortable appliance [1, 2]. Also the wound dressings should prevent dehydration of the wound region and be biocompatible and biodegradable [3]. There has been significant increase in the development of wound dressing materials that deliver drugs to the wound site in a controlled manner [4]. The most important advantage of the sustained release of the drugs to the wound is that it prevents the toxic effects of the therapeutic agents, which is a significant problem of the topical treatment [5, 6]. Recently, there has been reported many research articles about the development of the wound dressings that elute drugs [7-9].

In the last decade, the hydrogels prepared via freeze-thawing method have attracted much interest for their use in biomedical applications such as scaffolds [10], drug delivery [11] and wound dressing material [12, 13]. The major advantages of the freeze-thawing method are the absence of the need of any chemical agent for cross-linking and the initiation of the synthesis. Therefore the hydrogels prepared via freeze-thawing have drawn great attention due to their characteristics of high biocompatibility and non-toxicity [14, 15]. Chitosan (CS) is a cationic natural polymer, which is a derivative of chitin has been extensively utilized with its unique properties since it possesses high biocompatibility, biodegradability, non-toxicity and significant antimicrobial activity [16]. Poly-ε-caprolactone (PCL) has important properties such as non-toxic degradation products, good mechanical stability and cost efficiency [17]. Poly vinyl alcohol (PVA) is one of the widely used polymers due to its excellent biocompatibility, non-toxicity and high biodegradability [18]. Poly ethylene glycol (PEG) and poly vinyl pyrrolidone (PVP) that are highly hydrophilic and biocompatible are the important polymers commonly utilized in various biomedical applications [19, 20]. Polymer blending is an attractive approach that is composed of two or more polymers to provide the development of the novel materials with the desired properties [21, 22].

In this study, for the first time to our knowledge, a novel, environmentally-friendly, biocompatible and biodegradable hydrogel via freeze-thawing was developed containing ceftazidime as an antibiotic and ketoprofen as an analgesic drug. Ketoprofen is a routinely used non-steroidal, anti-inflammatory drug having analgesic function and ceftazidime is an antibiotic drug with

broad spectrum. The chitosan-based hydrogels were prepared with/without the presence of PEG, PVP and PVA. The prepared PVA-CS-PCL hydrogels were chabroad spectrum. The chitosan-based hydrogels were prepared with/without the presence of PEG, PVP and PVA. The prepared PVA-CS-PCL hydrogels were characterized with SEM, FTIR, swelling tests and degradation tests. After drug loading onto the hydrogels, they were applied for drug release studies.

MATERIALS AND METHODS

Materials

Chitosan (CS), poly-ε-caprolactone (PCL), poly vinyl alcohol (PVA), poly ethylene glycol (PEG) and poly vinyl pyrrolidone (PVP) were purchased from Sigma Aldrich. Ceftazidime (CZ) and ketoprofen (KP) were purchased from Sigma–Aldrich with 99.99% purity. All chemicals and reagents were used as received without any further purification. These chemicals and the other reagents were chemically pure grade and the water used in all experiments was Millipore Milli-Q grade. Phosphate and acetate buffers (pH 7.4, pH 5.5) were prepared according to standard methods.

Preparation of PVA-CS-PCL hydrogels

PVA-CS-PCL hydrogels were prepared by freeze-thawing method according to a procedure described elsewhere [23]. Firstly, a polymer mixture was prepared, which consisted of CS (2 g, 2% in 0.2 M acetic acid solution), PVA (0.5 g, 1% aqueous solution), PCL (0.5 g, 1% solution dissolved in dichloromethane) and TWEEN-80 (500 µL) was charged in a 250 mL four-neck round-bottom flask equipped with a mechanical stirrer for about 45 min. The mixture was placed on the petri dish. The blend solution was directly kept frozen at -16 °C for 16 hours. Afterwards, the frozen hydrogels were thawed at room temperature for 8 hours. This process of freezing/thawing was repeated for 8 times. The hydrogels were washed with distilled water to remove the unreacted component, and then air-dried at room temperature.

Swelling and degradation tests

To determine the swelling properties, the dried samples were soaked in pH 5.5 and pH 7.4 buffer solutions at 37°C for 24 h. Then, the excess water was removed and dried to a constant weight. The swelling degree was calculated using the following formula:

$$\text{Swelling degree} = \frac{M_s - M_d}{M_d} \quad (1)$$

Where M_d and M_s are the masses of dry and swelled samples, respectively.

To evaluate the degradation amounts of the hydrogels, they were placed at pH 5.5 and pH 7.4 buffer solutions for 2 days at 37°C. Then the samples having weight as W_d were removed from the medium, dried and then weighed (W_f). The weight loss was calculated gravimetrically with the following equation:

$$\text{Degradation \%} = \frac{W_d - W_f}{W_d} x100 \quad (2)$$

Characterization of the PVA-CS-PCL hydrogels

The synthesized hydrogels were characterized by FTIR recorded on Thermo Scientific / Nicolet IS10, within the range of 400–4000 cm^{-1}. To study the morphology of the hydrogels, the scanning electron micrographs were recorded by QUANTA FEG 450 scanning electron microscope.

Adsorption studies

CZ and KP were used as antibiotic and analgesic drugs for investigation for drug release of PVA-CS-PCL hydrogels. Dry hydrogels were loaded with one of drug prepared for 48 hours at 50 rpm with a different dose (250-1000 ppm). The loading capacity (mg/g) was calculated using the following equation:

$$\text{Loading capacity} = \frac{C_i - C_e}{W} xV \quad (3)$$

Where V is the solution volume (L), W is the mass of sample (g), and C_i and C_e are the initial and equilibrium drug concentrations (mg/L) respectively. The drug concentration was determined using UV/VIS spectrometer (Genesys 10S, ThermoFisher Scientific, USA) at λ_{max} of 258 and 255 nm for CZ and KP, respectively.

In-vitro drug release studies

The in-vitro drug release studies, which were immersed in 10 mL of the drug release medium were performed at pH 7.4 and pH 5.5 at 37°C under magnetic stirring at 50 rpm. At appropriate time intervals, 1 mL of the release medium was collected and subsequently same amount of fresh buffer solution was replaced. The drug concentration was determined using UV/VIS spectrometer (Genesys 10S, ThermoFisher Scientific, USA) at λ_{max} of 258 and 255 nm for CZ and KP, respectively.

Table 1. The stability of the polymers at room temperature prepared via freeze-thawing method

Polymer name	*Polymer ratio*	*Result*
PVA-CS-PCL	*1:3:1*	*Stable gels*
PVA-CS-PCL	*2:3:1*	*Stable gels*
PVA-CS-PCL	*3:3:1*	*Stable gels*
CS-PCL	*3:1*	*Unstable gels*
PEG-CS-PCL	*1:3:1*	*Unstable gels*
PVP-CS-PCL	*1:3:1*	*Unstable gels*

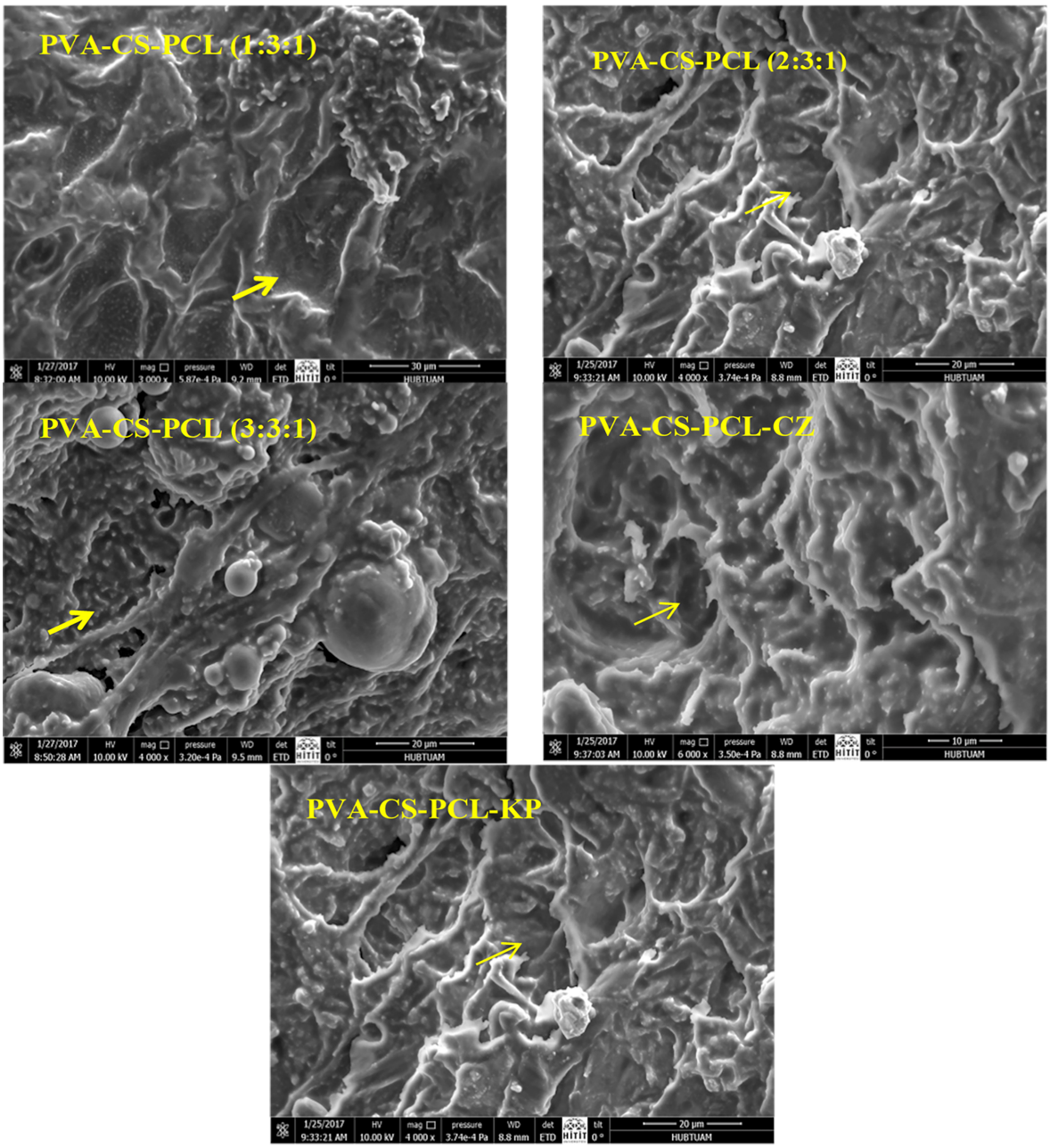

Figure 1. SEM photographs of the PVA-CS-PCL hydrogels with different amounts of PVA, and drug loaded hydrogels.

In-vitro fibroblast response of the hydrogels

MTT assay was performed using L-929 (mouse fibroblast cell line) by direct contact methods as TS-EN 180 10993-5/September 2010 guideline. Briefly, the cells were cultured on 6-well plate at 37°C, 5% CO_2 for 24 h to allow for cell attachment. After incubation, the cultured cells examined by microscopic. The hydrogels were immersed into PBS solution for 48 h according to TS EN ISO 10993-12/April 2013 and the leaching solution was diluted to 0.125 mg/mL. Then, the cells were seeded on 96-well plate at $1x10^4$ cells/well and incubated into the leaching solutions for 24 h. Finally, The absorbance was determined using UV spectrophotometer at 570 nm. The cell viability was calculated as the ratio of the mean absorbance of the sample and the mean absorbance of control.

RESULTS AND DISCUSSION

Characterization studies

As can be seen from Table 1, the hydrogels with different compositions were prepared using the same procedure that was explained in detail above. It was observed that CS-PCL, PEG-CS-PCL and PVP-CS-PCL hydrogels were physically unstable gels. However with the incorporation

Table 2. The swelling degree of the polymers, PVA-CS-PCL, PVA-CS-PCL-CZ and PVA-CS-PCL-KP

pH/Polymer	PVA-CS-PCL	PVA-CS-PCL-CZ	PVA-CS-PCL-KP
5.5	7.10	6.05	6.58
7.4	10.04	8.12	8.90

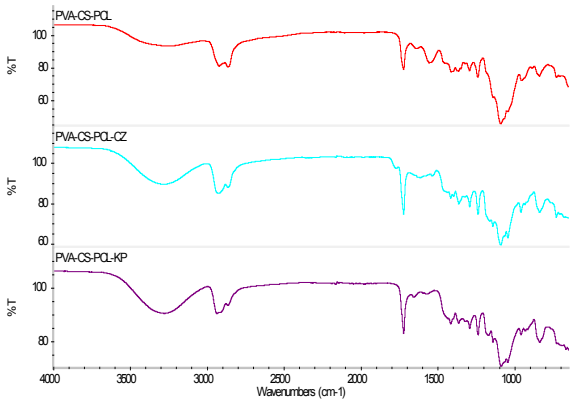

Figure 2. FTIR spectra of PVA-CS-PCL, PVA-CS-PCL-CZ and PVA-CS-PCL-KP hydrogels.

of PVA into the structure of CS-PCL hydrogel, the fabrication of the physically stable gels were achieved. All the hydrogels with the composition of PVA-CS-PCL with different PVA ratio were determined to remain stable at room temperature after synthesis. Therefore the stable gels were selected for further characterization studies.

The physically cross-linked hydrogels prepared with freeze/thawing process exhibit highly porous morphological structure with large interconnected pores [14]. This unique characteristic presents important potential especially for drug delivery applications enabling the diffusion of drug molecules through the vehicle material. The morphology of the prepared PVA-CS-PCL hydrogels having different amounts of PVA was demonstrated in Figure 1. The polymers used in this study of PCL, chitosan and PVA were promoted to form a gel via cross-linking during the subsequent freeze/thawing processes. According to SEM micrographs, all the hydrogels showed 3-D network structure and the pores of the hydrogels were shown with arrows indicating high porosity of the hydrogels. Also the dimensions of the macropores of the hydrogels were calculated using ImageJ Software using at least 20 individual pores and obtained as 13.5±3 μm on an average for all hydrogels since the dimensions were evaluated for PVA-CS-PCL (1:3:1), PVA-CS-PCL (2:3:1), PVA-CS-PCL (3:3:1), PVA-CS-PCL-CZ and PVA-CS-PCL-KP as 14.8±7 μm, 16.6±4 μm, 10.8±3 μm, 9.5±2 μm and 15.5±4 μm respectively. It was clearly determined that PVA-CS-PCL (2:3:1) hydrogel exhibited more regular morphology than that of the other hydrogels. However, PVA-CS-PCL (1:3:1) and PVA-CS-PCL (3:3:1) hydrogels involve spherical particles distributed through matrix resulting a heterogeneous nature. The determined amount of PVA incorporated into the blend hydrogel improves the dispersion of the PCL and CS phases. Also Figure 1 shows the SEM image of PVA-CS-PCL hydrogel with the drugs loaded into the hydrogel namely PVA-CS-PCL-CZ and PVA-CS-PCL-KP. It was determined that no drug crystals were observed onto the hydrogel structure at the SEM images indicating the compatibility of the drug-polymer-solvent system [24].

The FTIR spectrum analysis was obtained to verify the drug loading throughout the PVA-CS-PCL hydrogels. The FTIR spectra of PVA-CS-PCL hydrogels and drug loaded hydrogels namely; PVA-CS-PCL-CZ and PVA-CS-PCL-KP were demonstrated in Figure 2. It was clearly seen that in the spectrum of drug loaded hydrogels, the new peaks were existed and some of the bands were overlapped when compared with those of the non-medicated hydrogels. The significant increase at characteristic band of O-H stretching absorption of the hydroxyl group (3278 cm-1) was observed indicating the physical interactions between the drug molecules and the hydrogel matrix. PVA-CS-PCL hydrogels bear high content of hydroxyl groups that are accessible to form hydrogen bonds with ceftazidime and ketoprofen molecules. The results confirmed the successful incorporation of the drug molecules in the hydrogel structure [25].

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Table 3. Hydrolytic degradation of the polymers PVA-CS-PCL, PVA-CS-PCL-CZ and PVA-CS-PCL-KP at different pH values.

pH 5.5			
	Initial weight, g	Highest weight, g	Weight loss, %
PVA-CS-PCL	0.14	0.50	15.36
PVA-CS-PCL-CZ	0.15	0.39	3.07
PVA-CS-PCL-KP	0.15	0.40	4.27
pH 7.4			
	Initial weight, g	Highest weight, g	Weight loss, %
PVA-CS-PCL	0.15	0.46	13.77
PVA-CS-PCL-CZ	0.16	0.39	2.11
PVA-CS-PCL-KP	0.17	0.38	2.80

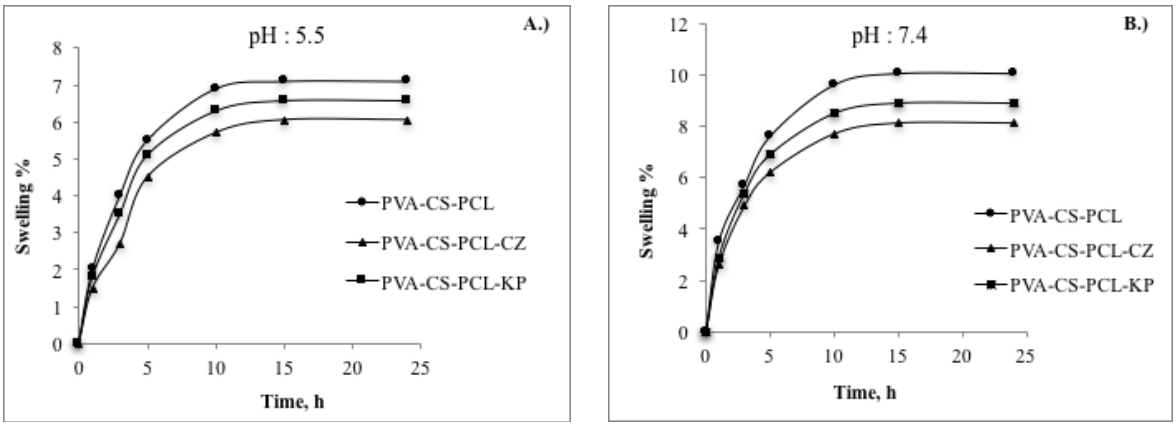

Figure 3. The swelling behavior of PVA-CS-PCL, PVA-CS-PCL-CZ and PVA-CS-PCL-KP hydrogels for A.) pH : 5.5 and B.) pH : 7.4.

The results confirmed the successful incorporation of the drug molecules in the hydrogel structure [25].

The swelling degree of the blank and drug-loaded hydrogels was evaluated at different pH values such as pH 5.5 and pH 7.4 and the results were listed in Table 2. The swelling degree of all the hydrogels at pH 7.4 were obtained higher than that of the swelling degree obtained at pH 5.5. It was also determined that the swelling capacity of the drug-loaded hydrogels was lower than that of the blank hydrogel at both pH values due to the interaction of the drug molecules with the polymer chains, which limits the swelling of the hydrogel matrix [26]. It was also found that the swelling capacity of ceftazidime-loaded hydrogels was less than that of ketoprofen-loaded hydrogels, which indicates the more specific interactions of ceftazidime molecules with the polymeric structure could be formed than that of the ketoprofen

In order to evaluate the swelling behavior of the hydrogels better, the swelling degrees were also calculated with respect to time. Figure 3 shows the swelling kinetics of the hydrogels at pH: 5.5 and at pH: 7.4. It was observed that all the hydrogels had similar swelling tendency and swelled rapidly reaching an equilibrium swelling degree within about 10 h for both pH values.

The hydrolytic degradation properties of the blank and drug-loaded hydrogels at different pH values were examined and given in Table 3. Due to the values of the weight loss, the degradation amount of the blank hydrogels was higher than that of the drug-loaded hydrogels at both pH values indicating the presence of the incorporation of the drug molecules into the polymeric matrix [27]. It was determined that the stability of PVA-CS-PCL-CZ hydrogels were higher than that of PVA-CS-PCL-KP hydrogels according to the presence of stronger bonds between ceftazidime molecules and polymer chains than that of ketoprofen molecules and polymeric structure.

Drug loading

Figure 4 demonstrates the loading capacity of the drugs, ceftazidime (CZ) and ketoprofen (KP), onto the PVA-CS-PCL hydrogels. The drug concentration of the loading medium was utilized in the range of 250-1000 ppm. It was determined that the loading capacity of the hydrogels was increased with increasing drug concentration from 250 ppm to 750 ppm. It was obviously defined that the loading capacity was reached a saturation value after 750 ppm. Therefore, this drug concentration was selected to use for the further drug release studies.

Drug release studies

Figure 5 demonstrates the drug release profiles of ceftazidime-loaded and ketoprofen-loaded PVA-CS-PCL hydrogels at pH 5.5 and pH 7.4 simulating the pH value of the dermis and the blood respectively. It was determined that the cumulative release amount of ceftazidime from PVA-CS-PCL-CZ hydrogels at pH 5.5 (90.9 %) was higher than the release amount of the drug at pH 7.4 (67.8 %) since the dissolution amount of the ceftazidime-loaded hydrogels at pH 5.5 was higher than that of at pH 7.4 (Figure 5.A). Figure 5.B shows the cumulative release profiles of the ketoprofen release from PVA-CS-PCL-KP at pH 5.5 and pH 7.4. The cumulative ketoprofen release from PVA-CS-PCL-KP

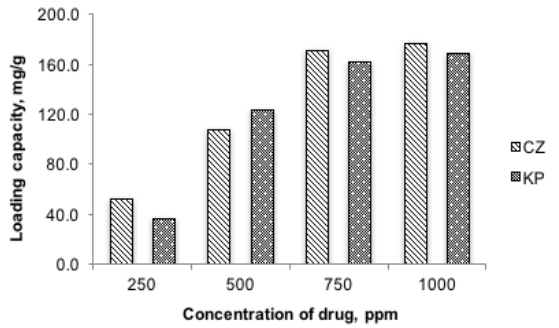

Figure 4. The loading capacity of the drugs, ceftazidime (CZ) and ketoprofen (KP) onto the PVA-CS-PCL hydrogels.

hyrodgels were determined almost same for pH 5.5 and pH 7.4. The in-vitro release of ceftazidime from PVA-CS-PCL-CZ hydrogels presented burst release after 6 h of 63 % and 48 % at pH 5.5 and pH 7.4 respectively. The in-vitro release of ketoprofen from PVA-CS-PCL-KP hydrogels showed burst release of after 6 h of 84 % and 72 % at pH 5.5 and pH 7.4 respectively. The initial burst releases from the drug-loaded hydrogels for the first 6 h may be because of the fast release of the drug molecules that were bound onto the hydrogel matrix by weak interactions [28]. It was also defined that the release profiles of ceftazidime and ketoprofen from drug-loaded hydrogels reached a plateau value after 2 days period. The ceftazidime release properties from PVA-CS-PCL-CZ hydrogels compromise with the degradation chracteristics of the hydrogels since the degradation percentage of these hydrogels was higher at pH 5.5 than that of at pH 7.4. Thus these results indicate that the release of ceftazidime from PVA-CS-PCL-CZ hydrogel matrix was controlled dominantly by the dissolution of the polymeric structure and diffusion of the drug molecules [29, 30]. Shafaghi et al. prepared poly(vinyl acetate-co-maleic anhydride) hydrogels modified with melamine as a drug delivery system using ceftazidime as a model drug. The in-vitro drug release studies showed that after approximately 24 h, the release profile established an equilibrium at pH 3 and 6 with cumulateive release amount of 75% and 80% respectively. It was determined that at pH 8, after 10 h 90% of the drug was delivered indicating a significant burst release [31]. Shefy-Peleg et al. developed gelatin-alginate hydrogels for wound healing with ceftazidime eluting. The cumulative drug release percent form the prepared hydrogels was determined as 50% after 24 h release period indicating a partial release from the matrix [32]. Josef et al. synthesized a novel gelatin hydrogel for ketoprofen delivery. The complete delivery of the drug was occured within 24 h with a cumulative release of 80% [33]. Huang et al. prepared pH-sensitive cationic guar gum/poly acrylic acid hydrogels with different acrylic acid content. The equilibrium release amount of ketoprofen was obtained with the hydrogels having the highest release amount as 99.8% after 10 h [34]. It was clearly seen that the percentage of cumulative release and release time obtained in this study are comparable with the results reported in the literature. The results also show that prolonged release of the drugs could be achieved using the resultant hydrogels since they serve as an appropriate carrier indicating their stable interactions with drug molecules for wound healing.

In-vitro fibroblast response of PVA-CS-PCL and drug loaded PVA-CS-PCL hydrogels

In order to demonstrate the cytotoxicity of PVA-CS-PCL and drug loaded PVA-CS-PCL hydrogels, L-929 mouse fibroblast cells were utilized to evaluate the biocompatibility of the hydrogels via the incubation of the cells with the leaching solutions extracted from the hydrogels for 24 h. When compared to the control group, the cell viability values of the PVA-CS-PCL and drug loaded PVA-CS-PCL hydrogels were found as 83.1 and 71.0 respectively. The viability value of 70 % is determined as threshold between cytotoxicity and non-cytotoxicity [35]. It was shown that the plain hydrogel namely PVA-CS-PCL has no cytotoxic effects. However the drug loaded PVA-CS-PCL hydrogel that was prepared via the loading of ketoprofen and ceftazidime to the hydrogel matrix has low toxicity to L-929 cells. This may be caused because of presence of the antibiotic drug since it was reported that human fibroblasts were more susceptible to ceftazidime [36]. Due to the results, it was determined that the loading amount of the drugs should be defined to provide safety margin for efficient utilization of the wound dressings.

CONCLUSION

The novel biocompatible and biodegradable hydrogels with high porosity were developed for wound dressing material. PVA-CS-PCL hydrogels could successfully prepared via freeze-thawing method without using any toxic chemical agent and applied for the first time as a drug release system for wound healing. The hydrogels were characterized with FTIR, SEM, swelling and degradation tests. Ceftazidime as an antibiotic and ketoprofen as an analgesic was selected to enhance the wound healing process for controlled drug release from the hydrogel

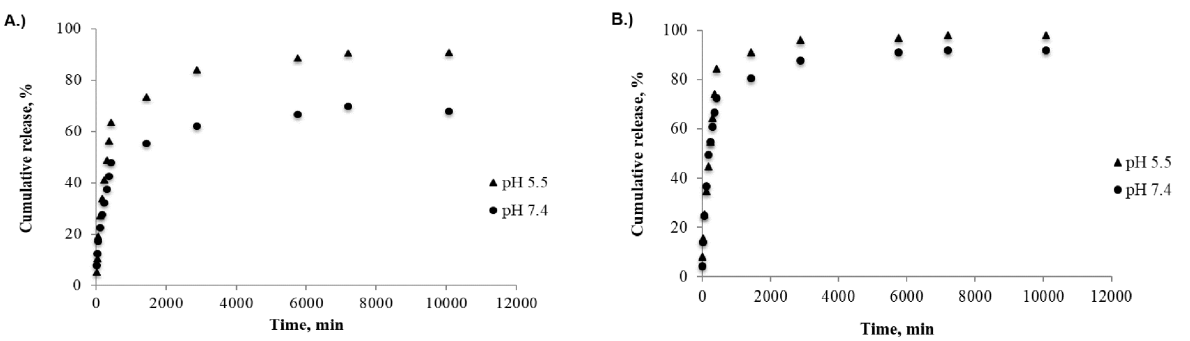

Figure 5. Cumulative release profiles of A.) Ceftazidime, B.) Ketoprofen from the drug-loaded hydrogel matrix.

wound dressing. The in-vitro release of the drugs was exhibited prolonged release for 2 days period. All the results suggest that the prepared drug-loaded PVA-CS-PCL hydrogels present a significant potential as a wound dressing material.

ACKNOWLEDGMENT

This work was financially supported by Hitit University Scientific Research Project namely, 'The development of Chitosan-Based PVA/PEG Hydrogel Wound Dressing Materials' No. MUH19002.17.001.

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