CHITOSAN/POLY(VINYL ALCOHOL) HYDROGELS AS CONTROLLED DRUG DELIVERY SYSTEMS

Aleksandra Kocemba*, Maria Mucha

Lodz University of Technology,
Faculty of Process and Environmental Engineering,
ul. Wolczanska 213, 90-924, Lodz, Poland
e-mial: aleksandra.kocemba@p.lodz.pl,

Abstract

In order to achieve hydrogel and drug release profiles, a comprehensive knowledge of the types, properties and syntheses of hydrogel polymer networks are needed. For this reason, a natural biopolymer hydrogel based on chitosan was described. Chitosan has many advantages, which meet the requirements necessary for the preparation of medical materials; for example, wound dressings. This article focused on the biomedical use of a chitosan hydrogel: chitosan–poly(vinyl alcohol) (PVA). The method of preparation of hydrogels containing a drug as an active wound dressing was described. To obtain a hydrogel dressing to be applied in patients with burns or difficult curative wounds, gentamicin (an aminoglycoside antibiotic) was used as a medicament. The effect of the PVA concentration in hydrogels on the release rate of the antibiotic was examined. For this, the crosslinking agent of the hydrogel, glutaraldehyde, was used. The release process of gentamicin was described by using an equation of first order kinetics.

Keywords: chitosan/PVA hydrogel, gentamicin, glutaraldehyde, release kinetics, swelling

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1. Introduction

Hydrogels are in a three-dimensional network structure and are generated through crosslinking from linear polymer materials. They have a wide range of applications, especially in medical and pharmaceutical areas.

The moist environment provided by a hydrogel can promote the healing of wounds [1–3]. There are many kinds of preparation methods of hydrogels, and these can be divided into three main methods: physical, chemical and by radiation [4,5]. X-ray irradiation is a suitable method for the formation of hydrogels without crosslinking agents.

In the method of chemical crosslinking, crosslinking agents can reduce structural bio-compatibility due to their inherent cytotoxicity [5].

Chitosan is known as the best hemostatic material. It is a non-toxic, biodegradable polycationic polymer with low immunogenicity. This natural polysaccharide has been widely used in a variety of biomedical applications, such as bioactive dressings and films. In order to optimise the mechanical properties, a crosslinked mixture of chitosan with polyvinyl alcohol leading to hydrogels has been investigated [6–14].

In the present study, glycerin is combined with polyvinyl alcohol/chitosan aqueous blends, which were crosslinked by glutaraldehyde, leading to good mechanical properties of the resulting hydrogel. This hydrogel can be also used as an effective controlled drug delivery system.

2. Materials and methods

2.1. Materials

Chitosan (CH) was prepared from shrimp shells (gray-yellow powder, DD = 85%, Mw = 200,000 Da) and purchased from Heppe Biomaterials GmbH (Chitosan 85/120/A1). Two percent acetic acid was used as a solvent for CH. PVA with a molecular weight of 72,000 (g/mol) was purchased from POCH S.A. (Gliwice, Poland) and glutaraldehyde (GA – pentane-1,5-dial) $C_5H_8O_2$ from Sigma-Aldrich GmbH, Germany. Gentamicin (GEN – $C_{21}H_{43}N_5O_7$) was used as an active substance. It has a characteristic band absorbance at a wavelength of λ = 255 nm. GEN was purchased from KRKA, Poland.

2.2. Methods

2.2.1. Chitosan, PVA and blend films preparation

Thin films were made for FTIR studies. Chitosan solution was prepared by dissolving CH in 2% acetic acid with constant stirring for 12 hours at room temperature. The PVA solution in distilled water at a concentration of 5 g/l was prepared. The solutions were used to obtain blend films and hydrogels. To obtain the CH/PVA blend, 0.09 weight fraction of GA, 0.09 weight fraction of GEN and 0.2 PVA in relation to CH were mixed together. The solutions were casted on glass plates. After evaporation of solvent, films of 20 μm approximate thickness were obtained.

2.2.2. Hydrogel formation

Chitosan and PVA solution were mixed in different contents to obtain various compositions (Table 1). The mixture was incubated under stirring for 30 minutes at room temperature until a clear solution was formed. Then, the 0.09 weight fraction of GEN and 1% of glycerin (as plasticiser) were added. Subsequently, the GA was slowly added under constant mixing. The final weight fractions of GA in relation to the CH precursors were 0.09, 0.17, 0.2. The solution was poured into a container to stand for 24 hours. Table 1 presents the composition characteristics of the hydrogel samples.

Table 1. Composition characteristics of the CH/PVA hydrogel samples.

Sample number	ωPVA	ωGA
1	0	0.09
2	0.09	0.09
3	0.2	0.09
4	0.33	0.09
5	0.5	0.09
6	0.33	0.17
7	0.33	0.27

ω_{PVA} – weight fraction of PVA in relation to CH, ω_{GA} – weight fraction of GA in relation to CH,

ω_{GEN} – weight fraction of GEN (in each sample is the same) in relation to CH was 0.09, ω_g – weight fraction of glycerin was 0.01. Weight fraction of chitosan was derived with the following formula: $\omega_{Ch} = 1 - \omega_{PVA} - \omega_{GA} - \omega_{GEN} - \omega_g$

2.2.3. Swelling process

The purpose of the swelling experiment was to determine the effects of the GA content. Thus, the chosen hydrogel sample (about 10 g) with various weight fractions of GA, equal to 0.09 and 0.17, was subjected to the swelling process. These hydrogels contained the same fraction of PVA and GEN (samples 4 and 6: Table 1). The test of the hydrogel swelling was carried out in glass beakers containing 100 cm^3 of distilled water at pH 7, stirred for 27 hours at 36°C. The results are presented as the dependence of the swelling degree α on time. The parameter α is determined from formula (1):

$$\alpha = \frac{m_k - m_p}{m_k} \cdot 100 \qquad (1)$$

where:
α – degree of swelling in %, m_p – initial weight of the hydrogel sample in g, m_k – mass of hydrogel sample after a certain time t in g.

2.2.4. Spectroscopic methods – FTIR

FTIR spectra were recorded in the frequency ranges from 400 to 4000 cm^{-1} and from 400 to 2000 cm^{-1}. The study was performed using a Mattson Instruments Genesis II FTIR Spectrometer System.

2.2.5. Methodology of drug delivery

The release of GEN was carried out in a glass vessel containing 100 cm^3 of distilled water at pH 7. The experiments were carried out at the temperature of 36 ± 0.5°C. The

hydrogel containing the active substance was introduced into distilled water and stirred (by magnetic stirrer). A water sample containing the released drug was removed at specified intervals for analysis by UV-Vis spectrophotometer (UV-Vis Jasco 630). The concentration of the active substance was measured taking into account the characteristic absorbance band of GEN at λ = 255 nm (the calibration curve of GEN was determined at the start of the study). Release kinetics was described by using the equation of first order kinetics (2):

$$f_t = A_1 \cdot (1 - exp(-k_1 t)) \quad (2)$$

where:

f_t – fraction of released drug in time t (-), A_1 – the fraction of GEN released during the process (-), k_1 – the constant of first order release kinetics (1/h) [8].

3. Results and discussion

3.1. Swelling kinetics

Swelling kinetics of the two hydrogels of differing GA content was compared. The experimental data were presented on the graph depicting dependence of swelling degree (α) as a function of time (Figure 1).

The hydrogel with a crosslinking agent (GA) content of 0.09 achieved a degree of swelling (α) of 311%. Increasing the amount of GA reduced the degree of swelling to 153%. In both cases, after 22.5 hours, the hydrogel disintegrated (weight loss). Increasing crosslinking is related to a reduced mobility of the polymer chains, decreased pores size and thus a reduction of the swelling degree of the hydrogel.

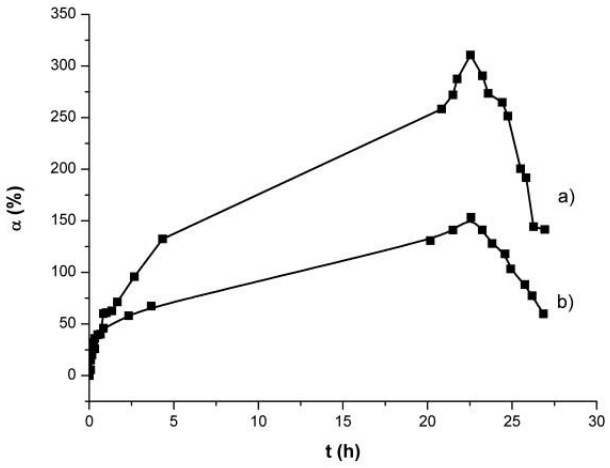

Figure 1. The swelling of hydrogels containing different GA contents a) 0.09 b) 0.17; the other components were 0.33 PVA and 0.09 GEN. All contents are the fraction in relation to CH.

3.2. FTIR spectroscopy

Figure 2a shows theFTIR spectrum of CH. Peaks around 893 cm^{-1} correspond to the saccharin structure. The absorption peaks at 1415 cm^{-1} are characteristic of bending

vibration in the C-H group. The absorption peaks at 1633 and 1322 cm^{-1} have been reported as –C=O in the amine group. The peak from 1526 to 1592 cm^{-1} represents amine II, $–NH_2$ bending vibration in the amino group. The broad peak at 1030 and 1080 cm^{-1} indicates the C=O stretching vibration in CH. The peak at 1157 cm^{-1} is characteristic of the–C-O-C group in glycosidic linkage [15–17].

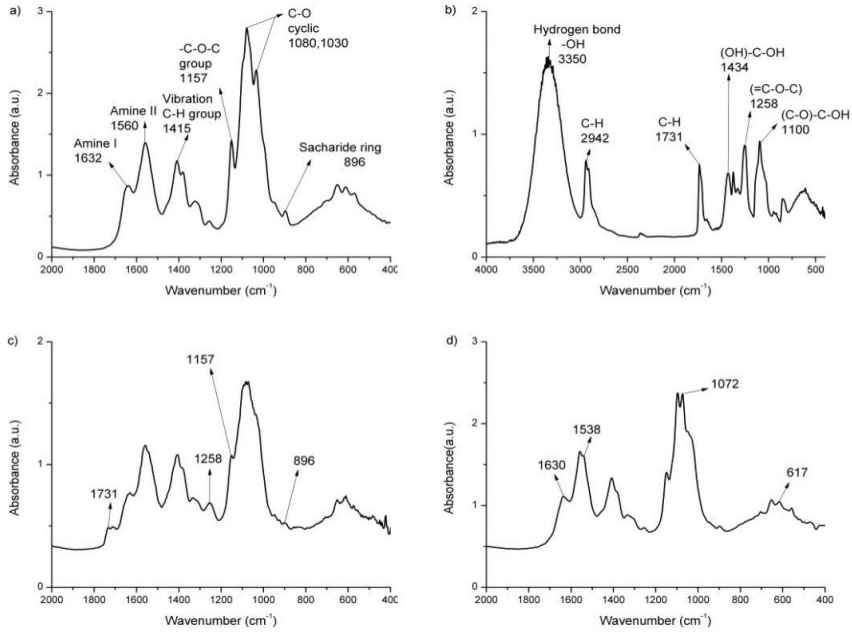

Figure 2. FTIR spectra of a) CH, b) PVA, c) CH with PVA, GEN, GA, d) CH with GEN.

In Figure 2b, the FTIR spectrum of the PVA sample is shown. Here, a C–H broad alkyl stretching band (ν = 2850-3000 cm^{-1}) can be observed along with typical strong hydroxyl bands for free alcohol (unbonded –OH stretching band at ν = 3600–3650 cm^{-1}) and hydrogen bonded band (ν = 3200–3570 cm^{-1}). An important absorption peak was verified at ν = 1142 cm^{-1}. This band has been used as an assessment tool of PVA structure. The semicrystalline synthetic polymer is able to form some domains depending on several process parameters. The peak at 1731 cm^{-1} may be related to the vibration of the –C=O group, and it was consequently associated with the degree of hydrolysis of PVA, since the acetate groups show such a –C=O functional ending [18]. Reis at al. [19] conducted research on crosslinking PVA with GA. They showed that the C-O stretching at approximately 1100 cm^{-1} in pure PVA is replaced by a broader absorption band (ν = 1000–1140 cm^{-1}), which can be attributed to the ether (CO) and the acetal ring (C-O-C) bands formed by the crosslinking reaction of PVA with GA [1,9].

Comparative IR spectra of CH and GA crosslinked CH was discussed in previous work [20]. Shoulders at 1562 cm^{-1} due to ethylene can be observed relatively to the intensity of other peaks. An aliphatic amino group was present at 1100 cm^{-1}, where the

intensity decreases. It indicates that the crosslinking with GA turns the membrane more hydrophobic as amino groups are blocked with aliphatic chains (indicated in spectra with arrow).

Comparative IR spectra of CH and CH with GEN are shown in Figure 2d. As expected, the intensities of absorption bands at 1000–1200 cm^{-1} and about 615 cm^{-1} are attributed to GEN sulphate characteristics [21]. On the basis of a study by Lakdawala et al., the peak at 1072 cm^{-1} was caused the HSO_4^{-1} group of GEN. The peak at 617 cm^{-1} was due to the SO_2 band in GEN [22]

In Figure 2c, the FTIR spectrum of CH with PVA, GEN and GA is shown, which is different compared to the IR spectra in Figure 2a, b, d. Comparing this spectrum with the spectrum of CH, the changes of the peak absorbance of the saccharide ring at 896 cm^{-1} can be observed. The absorbance decrease was also observed at 1157 cm^{-1}. This FTIR spectra showed a new peak at 1731 cm^{-1} due to PVA. A decrease in the absorbance in the peak at 1258 cm^{-1}, due to changes in the –C-O-C group, is observed. However, the largest decrease in absorbance was observed from 1000 to 1126 cm^{-1}.

3.3. Release study

Studies of the release rate of GEN from hydrogel samples 1–6 are shown on Figures 3a and 3b in the form of graphs $f_t = f(t)$. The parameters determined by fitting of first order kinetics (equation 2) are presented in Table 2.

The rate of gentamicin release from pure chitosan hydrogel was the highest. The PVA additive modifies the constant of first order release kinetics k_1 (Figure 4a). With increasing PVA content, the release process became slower, but the release efficiency A_1 was also lower (Figure 4b).

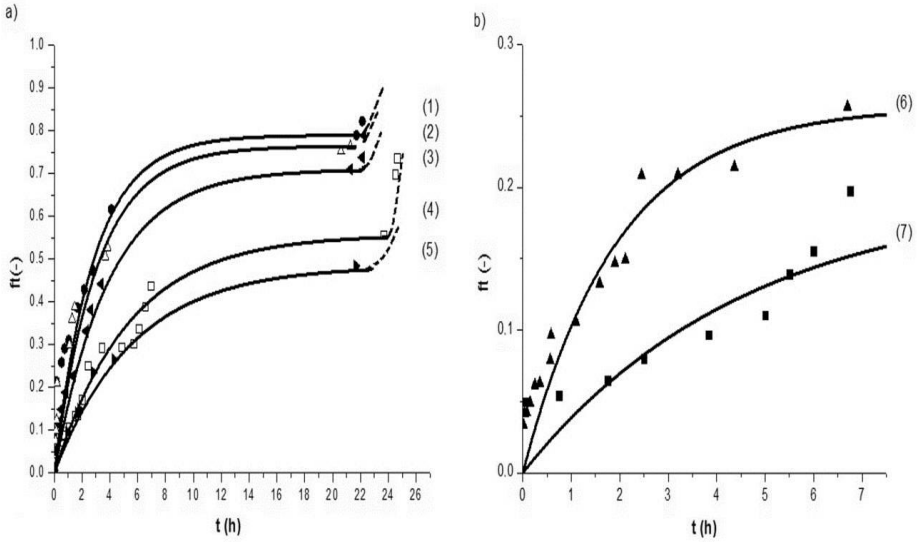

Figure 3. The rate of GEN release from modified chitosan hydrogels. The numbers of the curves correspond to samples a) 1–5 b) 6,7, as described in Table 1 The dashed line --- shows the predicted later release of the drug resulting from the destructive effect of hydrolysis

Table 2. Kinetics parameters of the release process. The numbers correspond to the samples as presented in Table 1.

	Release (first order kinetics)						
The result (research)	**Sample (see Table 1)**						
	Nr 1	Nr 2	Nr 3	Nr 4	Nr 5	Nr 6	Nr 7
k_1	0.393	0.332	0.256	0.186	0.176	0.512	0.217
A_1	0.789	0.752	0.701	0.562	0.473	0.256	0.197
R^2	0.987	0.946	0.963	0.956	0.977	0.951	0.941

A_1 – release efficiency: the amount of gentamicin released during the process under study; k_1 – the rate constant of first order release kinetics (1/h); R^2 – coefficient of determination.

The rate of GA release was studied with differing contents of the crosslinking agent. Figure 3b shows two curves of GA release for different contents of GA: 0.17 and 0.27. The study was conducted for only 7 hours due to the overlapping UV spectra for high ω_{GA}.

Increasing the content of the crosslinking agent GA results in a significant reduction in the amount of active substance release and its release efficiency (A_1). A three-dimensional network of hydrogel forms a spatial structure filled with liquid in which the transport of active substances occurs. The increase of the crosslinker content causes the creation of a more rigid network of lower porosity and a decrease in the rate of drug release (fraction f_t).

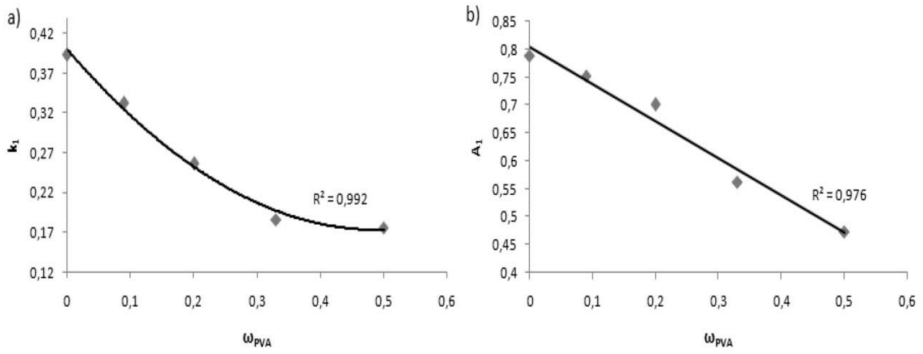

Figure 4 a) Rate constant k_1 as a function of ω_{PVA}, b) Release efficiency A_1 as a function of ω_{PVA}.

Also, the PVA content in CH/PVA hydrogels was found to be an important parameter in controlling the drug release profiles. With increasing PVA content, the rate constant of the release process decreased. However, the release efficiency A_1 of gentamicin delivered in the first stage of the process (up to 22 hours) became lower (Figure 4). The crosslinking agent influenced the porosity of the hydrogel structure. The denser the network structure of hydrogel matrices (leading to smaller pores), the slower the process of swelling and drug release.

4. Conclusions

The hydrogels based on crosslinked CH/PVA blends can be potentially used in biomedical applications, such as biomaterials for wound dressings. However, in the presented case, the hydrogels contained the glutaraldehyde (GA) crosslinking agent, which (when accidentally not reacted) can be released into the environment and may result in undesirable toxic effects [2]. Recently, our interest has been developing on the use of a tripolyphosphate compound for CH/PVA crosslinking. This hydrogel would be more acceptable as biomaterial.

It seems that the presented results are valid from the point of view of the observed effects. The efficiency and rate of drug release from the CH/PVA hydrogel depended on the content of the crosslinking agent GA and the PVA concentration. Both parameters (GA and PVA contents) influenced the porosity of the hydrogel. Lower porosity and a more rigid structure of the material led to a decrease in the rate of drug release.

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