Arabian Journal of Chemistry (2015) 8, 38–47

King Saud University

Arabian Journal of Chemistry

www.ksu.edu.sa
www.sciencedirect.com

ORIGINAL ARTICLE

Poly (vinyl alcohol)-alginate physically crosslinked hydrogel membranes for wound dressing applications: Characterization and bio-evaluation

Elbadawy A. Kamoun [a,*], El-Refaie S. Kenawy [b], Tamer M. Tamer [c], Mahmoud A. El-Meligy [b], Mohamed S. Mohy Eldin [a,c]

[a] *Polymer Materials Research Department, Advanced Technology & New Materials Research Institute (ATNMRI), City of Scientific Research and Technological Applications (SRTA-City), New Borg Al-Arab City, P.O. Box 21934, Alexandria, Egypt*
[b] *Polymer Research Group, Department of Chemistry, Faculty of Science, University of Tanta, Tanta 31527, Egypt*
[c] *Biotechnology Applications Research Group, Polymer Materials Research Department, Advanced Technology & New Materials Research Institute (ATNMRI), City of Scientific Research and Technological Applications (SRTA-City), New Borg Al-Arab City, P.O. Box 21934, Alexandria, Egypt*

Received 2 July 2013; accepted 5 December 2013
Available online 13 December 2013

KEYWORDS

Poly(vinyl alcohol);
Alginate;
Freeze–thawing method;
Hydrogel membranes;
Wound dressing;
Bio-evaluation

Abstract PVA-sodium alginate (SA) hydrogel membranes containing sodium ampicillin as a topical antibiotic were developed using the freeze–thawing method for wound dressing application. Aqueous solution of sodium alginate has been blended in a certain ratio with PVA, followed by the crosslinking method has been conducted by freeze–thawing method as physical crosslinking instead of the use of traditional chemical crosslinking to avoid riskiness of chemical reagents and crosslinkers. The physicochemical properties of PVA-SA membranes e.g. gel fraction and water uptake % have been performed. Increased SA content with PVA decreased gel fraction, elasticity, and elongation to break of PVA-SA membranes. However, it resulted in an increase in swelling degree, protein adsorption, and roughness of membrane surface. High SA content in PVA membranes had apparently an impact on surface morphology structure of hydrogel membranes. Pore size and pore area distribution have been observed with addition of high SA concentration. However, high SA content had an insignificant effect on the release of ampicillin. The hydrolytic degradation of PVA-SA membranes has prominently increased with increasing SA content. Furthermore, hemolysis (%) and *in vitro* inhibition (%) for both Gram positive and negative bacteria have been sharply affected by addition of SA into PVA, indicating the improved blood hemocompatibility.

* Corresponding author. Tel.: +20 1283320302; fax: +20 34593414.
E-mail address: badawykamoun@yahoo.com (E.A. Kamoun).

Peer review under responsibility of King Saud University.

ELSEVIER Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.arabjc.2013.12.003

Thus, PVA-SA hydrogel membrane based wound dressing system containing ampicillin could be a good polymeric membrane candidate in wound care.

1. Introduction

The typical idea of wound dressing maintenance is based on the presence of a moist environment around the wound which absorbs the exudates from the wound surface. Therefore, complete and partial wounds showed an apparent increase in re-epithelialization rates when they were maintained in a moist local environment (Hinman and Maibach, 1963; Winter, 1962). Accordingly, hydrogels are three-dimensional crosslinked hydrophilic polymers with a very high intrinsic content of water, which can provide a moist or wetted environment to the wound area and absorb the exudates. From this principle, hydrogels have been chosen as good candidate for wound dressing materials. Many hydrogels are prepared by physical or chemical crosslinking methods, (Hassan and Stewart, 2000; Razzak et al., 2001; Yang et al., 2004) while the physical crosslinking method such as repeated freeze–thawing cycles is regarded the proper crosslinking method and has been explored for biomedical and pharmaceutical applications due to its non-toxicity, solvent free, and biocompatibility. Furthermore, the obtained gel from this method has feasible physical properties e.g. rubbery nature and high water uptake compared to that obtained by chemical crosslinking (Peppas and Stauffer, 1991). Polyvinyl alcohol hydrogels have been prepared with a freeze–thawing method for wound dressing application because of their derisible clinical features. Additionally, PVA possesses desirable properties such as non-toxicity, biocompatibility, high hydrophilicity, easier film forming ability, chemical resistance, and mechanical resistance (Kim et al., 2008). Alginate is derived from brown algae, is an anionic linear polysaccharide composed of 1,4-linked β-D-mannuronic acid residue and 1,4-linked α-L-guluronic acid residue varying properties (Ress and Welsh, 1997). Alginate polymer has high hydrophilicity, biocompatible and relatively economical use, it has been widely used in biomedical applications e.g. wound dressing, (Kim et al., 2008) scaffolds, (Zmora et al., 2002) and dental or surgical impression materials (Nandini et al., 2008). The importance of the polymer blending with PVA, has been increased due to the blended polymeric materials which have desired properties, improve physical properties, low cost of basic polymer materials, improved process ability of film formation, and biologically acceptable. Accordingly, some blended polymers have been utilized with PVA to improve the clinical properties of obtained polymeric membranes for wound dressing applications, such as alginate (Kim et al., 2008; Levic et al., 2011), dextran (Fathi et al., 2011; Hwang et al., 2010), and chitosan (Kanatt et al., 2012; Yang et al., 2008). The obtained hydrogel film can be used to cleanse a big area from secreting lesions depending on the idea of high absorption of strong hydrophilicity of PVA-SA hydrogels, which limit wound secretions and reduce bacterial contamination (Kim et al., 2008). Meanwhile, PVA-SA hydrogel blend combines advantages of both; PVA hydrogels alone possess high mechanical properties, while alginates advantage sufficient physical and biological properties. Thus, sodium alginate has been chosen and is suitable for this purpose due to its high hydrophilicity, high protein adsorption ability, and good biological properties. Additionally, alginate fiber film can catch in a wound and is an easily biodegradable material without unexpected behavior (Gilchrist and Martin, 1983).

In this study, crosslinked PVA-alginate (PVA-SA) hydrogel films, loaded with sodium ampicillin as an antibiotic model, were prepared by the freeze–thawing cycle method to avoid the common harms which are arising from the traditional chemical crosslinking. PVA-SA hydrogels formed a matrix of physically crosslinked polymeric chains containing uncrosslinked polymers, water, and sodium ampicillin. Properties of hydrogel films such as gel fraction, swelling behavior, mechanical, morphology, and roughness, in addition, release studies of ampicillin from the prepared films were investigated. Finally, bio-evaluation studies of essential wound dressing characters like protein adsorption, hemocompatibility, hydrolytic degradation and antibacterial activity behavior were investigated under *in vitro* conditions.

2. Experimental

2.1. Materials

PVA (typically average M_w = 72,000 g/mol; 98.9% hydrolyzed) was obtained from Biochemica, Germany. SA (medium viscosity, average M_w = 130,000 g/mol as determined by GPC), albumin from bovine serum (BSA, fraction V, minimum 96% electrophoresis, nitrogen content 16.2%), and ampicillin sodium salt were purchased from Sigma–Aldrich Chemie GmbH, Steinheim, Germany. Folin and Ciocalteu's phenol reagent (FC, 2 N with respect to acid), were purchased from Park Scientific Limited, Northampton, UK. Distilled water was used throughout this research. All other chemicals were used without any further purification.

2.2. Preparation of PVA-SA hydrogels

PVA-SA hydrogel membranes were prepared by freezing–thawing (F–T) cycle according to the reported procedure of (Peppas and Stauffer, 1991). Briefly, aqueous solution containing 10% (w/v) PVA and 1.5% (w/v) SA and 20 mg of sodium ampicillin sodium salt were carefully dissolved in de-ionized water. Different proportions of PVA and SA content (0%, 25%, 33%, 50%, 65%, and 75%) solutions were mixed, sonicating, and vortexing for three hours. Proper amounts of this mixture were poured in Petri dishes, followed by freezing at −20 °C for 18 h and thawing for 6 h at 25 °C for three continuous cycles, to provide mechanically acceptable hydrogels for further experiments. The obtained PVA-SA hydrogel membranes were frozen in liquid nitrogen for 10 min before being lyophilized fractures for SEM investigations. All samples were left in deionized water for 72 h to extract leachable sol fraction or unconnected HES from polymer matrix for further characterizations.

2.3. Determination of gel fraction

The obtained PVA-SA hydrogel membranes were dried in a vacuum oven at 50 °C for 24 h and weighted (W_0), then soaked in distilled water for 24 h up to an equilibrium swelling weight (W_s) for removing the leachable or soluble SA parts from membrane. The gel membrane then dried again at 50 °C in a vacuum oven and weighted again (W_e). The gel fraction (GF%) was calculated by the following Eq. (1) Yang et al., 2008.

$$\text{Gel fraction (GF\%)} = (W_e/W_0) \times 100 \quad (1)$$

2.4. Determination of swelling behavior

In order to measuring the swelling degree of PVA-SA hydrogel membranes, membrane samples were cut into 2 cm × 2 cm pieces and dried at 50 °C in a vacuum oven for 6 h, the weight of dried sample was determined (W_e). The dried samples were soaked in distilled water, maintained and incubated at 37 °C, then weighted (W_s) at specific interval times. The water uptake of PVA-SA hydrogel membranes was determined using the following Eq. (2) Yan et al., 2008.

$$\text{Water uptake or swelling ratio (SR) \%} = [(W_s - W_e)/W_e] \times 100 \quad (2)$$

2.5. Study of protein adsorption onto hydrogel surface

The amount of adsorbed bovine serum albumin (BSA) was detected by UV–visible spectrophotometer (type: Ultrospec 2000, Pharmacia Biotech, Cambridge, England). In order to establish the relationship between the visible absorbance of BSA at 630 nm and the concentration of BSA, a calibration curve was drawn for standard solution of BSA ranging from 3–60 mg/ml. All standard solutions were prepared with distilled water. From the calibration curve a study was made restricting the curve to the linear part that followed Beer's law in Eq. (3),

$$A = acL \quad (3)$$

where A is the absorbance, c is the concentration, a is a proportionality constant, and L is the path-length which is constant (Queiroz et al., 2001).

Pieces of PVA hydrogel membranes cut into 1 cm × 1 cm were immersed in 10 ml phosphate buffer saline (pH 7.4), and incubated at 37 °C for 24 h until reaching equilibrium swelling weight. The swollen hydrogel pieces were transferred to buffer solution containing BSA (30 mg/ml) and shaken for 4 h at 37 °C. After protein adsorption, the hydrogel pieces were gently removed. The protein adsorption of the each sample was calculated by the difference between protein concentrations before and after immersing hydrogel pieces in protein/phosphate buffer solution using albumin reagent kit (absorbance range at 630 nm), this procedure has been adapted and modified from the procedure of Lin et al. (2006).

2.6. Determination of hydrolytic degradation

Dried membrane samples with size of 15 × 8 mm, were weighted and immersed in 3 ml phosphate buffer saline (0.1 M, pH 7.4, at 37 °C). The samples were removed at timed intervals and then wiped gently with soft paper to remove surface water. The samples were dried under vacuum at room temperature and finally weighted again. All experiments were done in duplicate.

2.7. Hemocompatibility test

Hemolysis experiments were carried out on the surface of the obtained PVA-SA xerogel membranes as described elsewhere (Chhatri et al., 2011; Singh and Ray, 1994). Typically, xerogel membranes were first equilibrated with saline solution (NaCl, 0.9 w/v at 37 °C for 24 h). Before conducting the test, anticoagulated blood was used for this test, where 1 ml of anticoagulated acid citrate dextrose solution (ACD) was added to 9 ml of fresh human blood. The equilibrated PVA-SA xerogel membranes (8 cm^2) were transferred into polypropylene test tubes and 7 ml of phosphate buffer saline (PBS, pH 7.4) was added and incubated for 72 h. The PBS was removed from the last test tubes and 1 ml of ACD solution was added to each sample and incubated for 37 °C for 3 h. Positive and negative controls were prepared by adding the same amount of ACD to 7 ml of distilled water and PBS, respectively. Each tube was incubated and gently inverted twice each 30 min to guarantee the continuous contacting between membrane and ACD blood. The fluids were transferred to a proper tube then centrifuged at 3000 rpm for 20 min. The hemoglobin released by hemolysis calculation by determination the optical densities (OD) of the supernatant. The supernatant was taken and its absorbance was monitored at 540 nm using a spectrophotometer (model: Ultrospec 2000). The percentage hemolysis was calculated by the following equation: (Chhatri et al., 2011)

$$\text{Hemolysis (\%)} = [(A_{(\text{sample of membrane})} - A_{(-)\text{control}})/(A_{(+)\text{control}} - A_{(-)\text{control}})] \times 100 \quad (4)$$

where, A is absorbance spectro-photometric value. $A_{(\text{sample of membrane})}$ is absorbance of tested membrane sample, $A_{(-)\text{control}}$ is absorbance of tube without membrane sample which contains ACD solution and 7 ml PBS, and $A_{(+)\text{ control}}$ is absorbance of tube without membrane sample which contains ACD solution and 7 ml distilled water. As results of hemolysis test, the membrane materials were classified into three types according to their hemolytic index as follows: (a) hemolytic materials have hemolysis (%) > 5%, (b) slightly hemolytic materials have hemolysis (%) between 2% and 5%, and (c) non-hemolytic materials have hemolysis (%) < 2% (American Society for Testing and Materials, 2000).

2.8. Antibacterial studies

Antibacterial assay of crosslinked PVA-SA xerogel membranes containing sodium ampicillin has been measured according to the methods which are discussed elsewhere (Jeon and Kim, 2000; Jumaa et al., 2002). The antibacterial study was carried out depending on the determination of the zone of inhibition method. Typically, the bacteria were inoculated in a Luria–Bertani medium (LB medium) (1% peptone, 0.5% yeast beef extract, and 1% NaCl). The inoculation was carried out at 37 °C for 24 h with continuous shaking. The obtained bacteria suspension was diluted by the same peptone medium solution for 100 times. 0.1 ml of diluted bacteria

suspension was cultured in a 10 ml liquid sterilized peptone medium (sterilization at 120 °C for 20 min.). This peptone medium contains 1 cm × 5 cm PVA-SA membrane pieces using the direct contact method between cultured bacteria and membrane surface. The inoculation medium was maintained at 37 °C for 18 h with continuous shaking. The numbers of inhibited bacteria were counted by using the ultraviolet–visible light spectrophotometry absorbance or optical density value (A) of culture medium at 620 nm. The indicative inhibition ratio (%) results are shown in Table 1, the inhibition ratios (%) of PVA-SA hydrogel membranes were calculated using Eq. (5):

$$\text{Inhibition ratio } (\%) = 100 - [(A_t - A_0)/(A_{con} - A_0)] \times 100 \quad (5)$$

where A_0 is absorbance of the bacterial broth medium prior to incubation, A_t and A_{con} are the absorbance of the bacterial solutions after incubating PVA-SA membranes and control sample for the desired interval, respectively.

2.9. Release profile study

15 ml of FC reagent was added into a series of 50 ml conical flasks containing sodium ampicillin (0.2–0.8 mg). Sodium ampicillin was chosen as a topical long acting antibiotic which also has good water solubility in an aqueous solution and it has broad-spectrum antibiotic which means that it kills more kinds of bacteria than other penicillin family and gentamicin antibiotics. Sodium ampicillin was utilized as an antibiotic instead of gentamicin sulfate salt which is loaded with PVA-dextran membranes and was discussed elsewhere (Hwang et al., 2010). The contents were completely mixed and kept into a thermostated water bath at 95 °C for 30 min. The flasks were taken out cooled at room temperature at ~25 °C, then transferred into a 25 ml standard volumetric flask and diluted up to the mark with distilled water. The absorbance of the resulting blue color of dye was measured against a blank reagent at 750 nm using spectrophotometer as previously discussed (Queiroz et al., 2001; Lin et al., 2006; Ahmad et al., 2004).

Two pieces of PVA-SA hydrogel membranes containing sodium ampicillin (each piece size is almost 55 cm^2), were immersed in phosphate buffer (pH 8, at 37 °C) and kept in continuous shaking. 200 μL of last solution was withdrawn at timed intervals each 15 min and was added to 3 ml FC reagent. The last mixture was heated at 95 °C for 30 min, and then taken out for cooling at room temperature; 1.8 ml of FC reagent was added to the cooled mixture and carefully vortexed before measuring. The absorbance of sodium ampicillin released from prepared samples was detected by spectrophotometer at 750 nm. All experiments were done in triplicate.

2.10. Characterizations

- FT-IR

Vacuum dried samples of freeze–thawed PVA-SA xerogels were analyzed by FT-IR on an EQUINOX 55 instrument (BRUKER, Germany). Translucent KBr-disks were prepared by grinding the dried sample materials together with infrared grade KBr and then pressing. The FTIR spectrums were obtained by recording 64 scans between 4000 and 400 cm^{-1} with a resolution of 2 cm^{-1}. All samples were freeze-dried using liquid nitrogen, crushed to a fine powder (KBr: sample = 140 mg: 2 mg) respectively, and pressed by applying a force 105 N into transparent disk (maximum disk weight = 150 mg) with a diameter 13 mm. All samples were measured in absorbance mode.

- Mechanical property measurements

The maximum tensile strength and the elongation degree to break of PVA-SA blend hydrogel membranes have been conducted using a tensile test machine (model: AG-I/ 50 N-10KN, Japan). PVA-SA membranes were cut into a specific dog-bone shape (6 cm long, 2 cm wide at the ends, and 1 cm at the middle). The analysis was performed at stretching rate 20 mm/min with pre-load of 0.5 N to determine load for each sample (Alencar et al., 2003). The thickness of membrane samples was measured with a digimatic caliper before examination.

- Scanning electron microscope

The surface and internal structure of the xerogel membrane samples were investigated by Analytical-SEM (type: JEOL, JSM-6360LA, Japan) with 15 kV voltage for secondary electron imaging. The xerogel membranes were dehydrated by freeze-dryer and coated with Au using an ion sputter coater (model: 11430, USA, combined with vacuum base unit or SPi module control, model: 11425, USA).

- Surface roughness

The surface roughness of the substrate of PVA-SA hydrogel membranes which are designed for wound dressing is a very important parameter. The surface upper-side of PVA-SA hydrogel membranes was measured by surface roughness tester (model: Mattito SJ-201P, Japan). The samples were mounted onto a glass slide with double-sides. The minimum sample dimensions were 25 mm × 25 mm. All results are measured in triplicate.

Table 1 Effect of SA contents in PVA hydrogel membranes on bacterial inhibition (%) and hemolysis (%) values.

Inhibition (%) of (G + ve) bacterial			Inhibition (%) of (G −ve) bacterial		Hemolysis (%)
SA content (%, w/w)	*Staphylococcus pyogenes*	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Proteus vulgaris*	
0	100	99.6	100	99.2	1.22
25	99.5	99.1	99.8	99	1.04
33	99.5	99.4	99.2	98.8	1.01
50	99.3	99.2	99.2	98.6	0.84
65	99.2	99.1	99	98.5	0.56
75	99	98.7	98.9	98.2	0.06

3. Results and discussion

3.1. FTIR spectroscopy

Poly (vinyl alcohol)-alginate (PVA-SA) blend hydrogel membranes were synthesized using the freeze–thawing technique, while the crosslinking was accomplished physically by crystallization step. In Fig. 1, the IR spectra of freeze–thawed PVA-SA blend xerogel membranes are shown, while the IR spectra of crosslinked PVA and virgin SA were discussed elsewhere, respectively (Kim et al., 2008; Chhatri et al., 2011). The IR spectrum of SA exhibited characteristic absorption bands for hydroxyl groups (–OH) at 3445 cm^{-1} and carboxylic groups (–COOH) at 1419 cm^{-1}. It clearly reveals the main peaks associated with freeze–thawed PVA. For example, it can be easily observed C−H broad alkyl stretching band (v = 2925 cm^{-1}) and the typical strong –OH group bands for free unreacted alcohol (non-bonded –OH stretching band at v = 3650–3590 cm^{-1}) and hydrogen bonded bands (bonded –OH stretching bands at v = 3590–3200 cm^{-1}). The hydrogen bonding between –OH groups at (v = 3445 cm^{-1}) can occur among PVA chains due to high hydrophilic forces Mansur et al., 2004. Also, the presence of sharp absorption peak was noted at v = 1150–1050 cm^{-1}. This stretching band has been used as an indicator for PVA structure, because it is a semi-crystalline synthetic polymer able to form some domains depending on several process parameters such as the F–T cycle number, the molecular weight and concentration of used PVA (Mansur et al., 2004). Additionally, it was found that a notable stretching band at v = 1549–1453 cm^{-1} of $–CH_2$ groups which are regarded as feature groups for chemical structure of PVA and PVA-SA blend polymer. All last mentioned stretching peaks, have been detected in structure of both PVA and PVA-SA blend polymer.

3.2. Study of physicochemical properties of PVA-SA hydrogel membranes

The consecutive F–T cycles produced entangled PVA-SA polymer hydrogel membranes. The influence of blending of sodium alginate (SA) contents (0%, 25%, 33%, 50%, 65%, and 75%) and sodium ampicillin introduction as a drug model on the gel fraction percentage (GF%), is calculated by Eq. (1) and shown in Fig. 2. Generally, the lower gel fraction was observed and accompanied to the less flexibility of obtained gel. In the absence of SA and drug (0% SA content and without drug), the gel fraction increased apparently to the maximum value, which was about 80%, suggesting that the PVA was almost crystallized in the highest degree and consequently entangled. This result is consistent with the obtained results by Yokoyama et al. (1986) While, GF% monotonically decreased with increasing SA content or addition of ampicillin in PVA hydrogel and decreased drastically to less than 39% at 75% of SA content. This behavior can be attributed to SA content and additions of ampicillin in PVA hydrogel might reduce the entanglement reaction and consequently the gelation process is reduced clearly. The obtained results have provided the principle of wound dressing materials in terms of saving a moist local environment with blending SA in varied ratios with PVA hydrogel membranes.

Fig. 3 presents the water uptake percent or swelling behavior of PVA-SA hydrogel membranes versus SA contents using Eq. (2). In the light of our swelling study, when PVA-SA hydrogel membrane was immersed in distilled water for 20 min, a small amount of blended SA was dissolved in swelling medium. The dissolved amount of SA is sharply dependent on the initial blended SA in PVA hydrogel. Moreover, the dissolved amount of SA significantly affected the swelling test. As shown in Fig. 3, the maximum swelling ability increases with an increase in the SA content in PVA hydrogel up to a certain

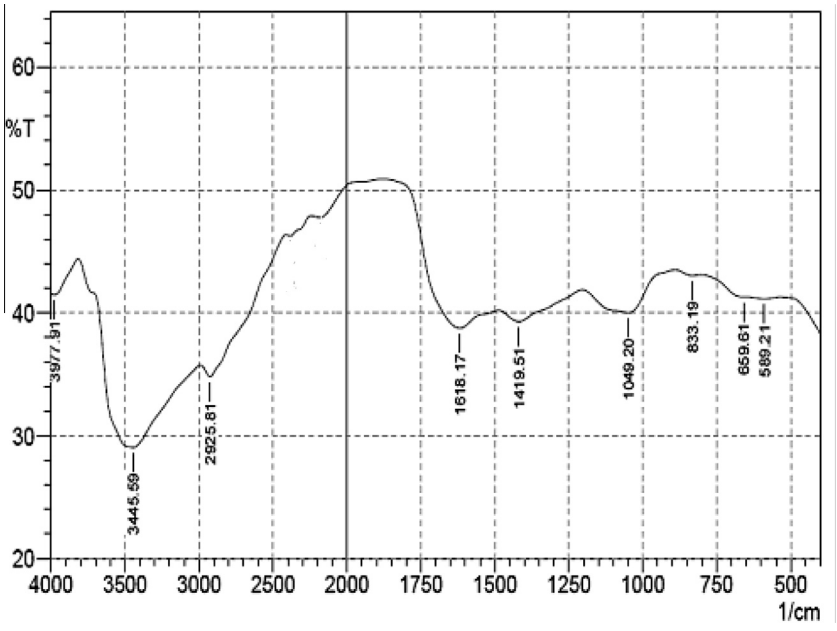

Figure 1 The IR spectra of PVA-SA xerogel membranes without ampicillin.

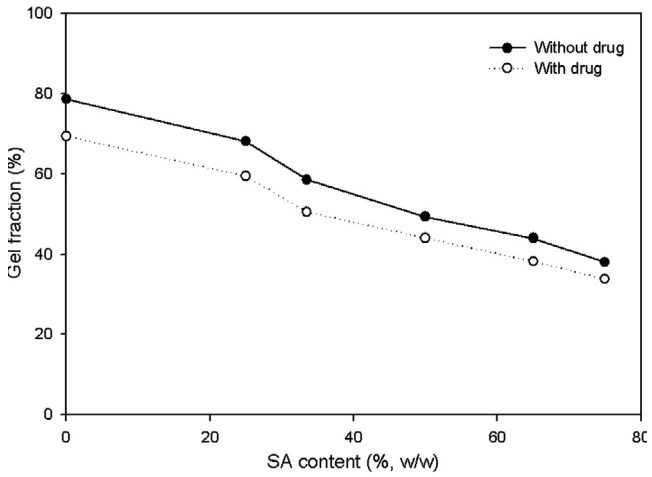

Figure 2 Effect of SA content in PVA hydrogel membranes on gel fraction (GF%).

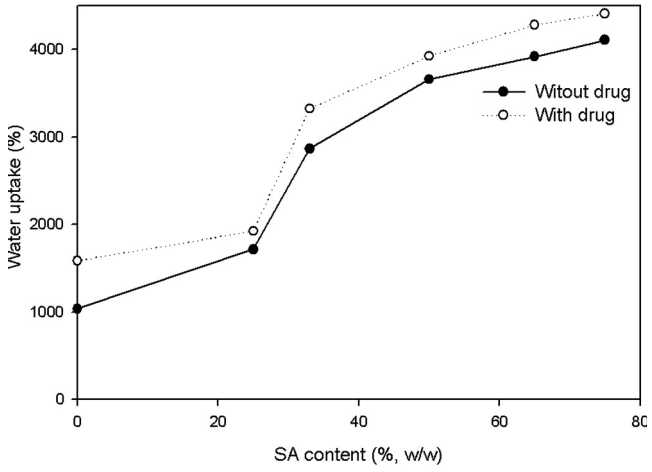

Figure 3 Effect of SA content in PVA hydrogel membranes on swelling degree or water uptake (%).

limit of huge swelling, the hydrogel structure was then destructed. This is due to SA as a blended material does not crosslink and has high ability to be soluble in water of swelling medium. While, in the absence of SA (0%, SA content); high crosslinked structure for PVA hydrogel has been obtained and this structure could not retain water amount within which result in low swelling ability, is about 1500% with a drug. After increasing SA content to 75%, the water uptake % progressively increases to 4200%. This is due to the high content of SA in PVA film increases the wettability and hydrophilicity characters of hydrogel which sometimes result in partial or complete destruction of hydrogel with much higher SA contents. These results are compatible with the reported results by Balakrishnan et al. (2005) and Choi et al. (1999).

3.3. Mechanical properties of PVA-SA hydrogel membranes

To investigate the blending influence of SA on the mechanical properties of PVA hydrogel membranes, the maximum tensile strength and elongation at break have been carried out and presented in Fig. 4. As shown, the maximum tensile strength and elongation at break of PVA-SA hydrogel membranes progressively decreased with increasing SA contents. Proportionally, the maximum tensile strength at break possessed the same pattern of behavior to elongation at break of hydrogel membranes. These results can be ascribed to the addition of SA into PVA hydrogels that might accelerate and destabilize the break elongation of hydrogel, which result in decreasing and deconstructing the maximum tensile strength. Furthermore, the obtained humble mechanical properties of PVA-SA due to the addition of high SA content might increase the hydrophilic susceptibility and decrease the entanglement degree which results in very weak mechanical resistance. These results are consistent with the obtained results by Rosiak et al. (2001). They have revealed that the maximum tensile strength of PVA hydrogel decreased with increasing the blended materials due to decreased crosslinking density. Similarly, our results are completely consistent with the reported results by Hwang et al. (2010). They have demonstrated that the maximum tensile strength of PVA hydrogel has sharply decreased with increasing dextran portions in the hydrogel.

3.4. Morphological investigation of PVA-SA hydrogel membranes

The surface morphology of PVA-SA hydrogel membranes was investigated by SEM. The SEM micrographs of the surface of PVA hydrogel membranes versus SA portions are shown in Fig. 5. According to SEM micrographs, the absence of SA presents a very smooth, uniform, and non pores shape surface structure. However, addition of SA into PVA hydrogel in different potions 25% and 65% provides very tiny pores at surface; these pores notably increase with increasing SA contents. Furthermore, the pore diameters, the number of pores, and pore size distribution are significantly noticed at the surface of PVA hydrogel, when high SA contents were incorporated. This morphological change can be attributed to the extraction of SA particles in different agglomeration numbers and this explanation has been previously verified in swelling study (Fig. 3). Although, excess amount of SA in PVA hydrogel can perturb the formation of PVA crystallites because of partial miscibility with PVA, these parts of excess SA can be

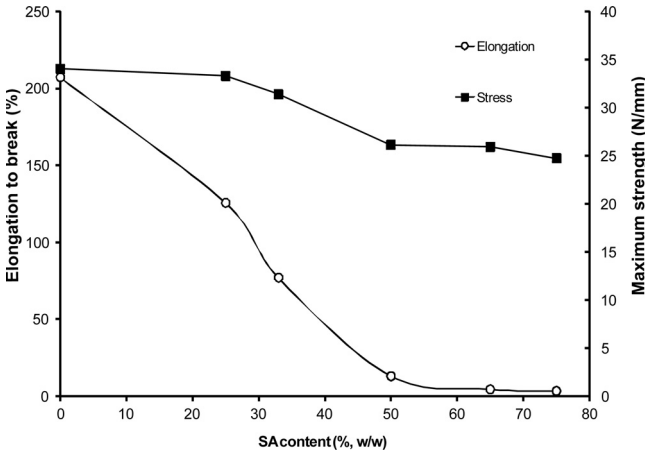

Figure 4 Effect of SA content in PVA hydrogel membranes on maximum strength and elongation to break.

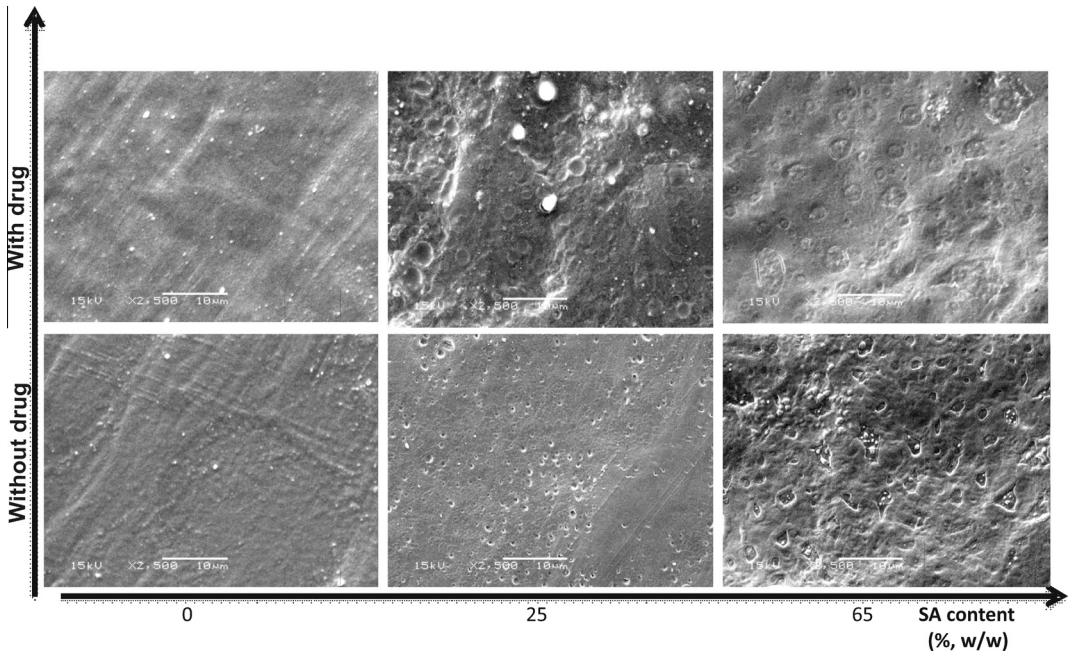

Figure 5 SEM micrographs showing the surface morphology distinctions of different SA contents in PVA hydrogel membranes, (original magnification was ×2500).

dissolved again and transfer from the hydrogel network into swelling solution. Also, it can be speculated that morphological changes are due to the presence of a big difference in the homogeneity, hydrophilicity, or miscibility degrees between two components of membrane (i.e. PVA and SA), which resulted in ordered-crystalline phase and uniform shape structure in case of 0% of SA, due to high entanglement of PVA and disordered-crystalline phase for PVA hydrogel blended with high portions of SA. The morphological results and our speculation are compatible with obtained results by Fathi et al. (2011) and Cascone et al. (1999).

The distinctions of the surface roughness of PVA hydrogel membranes versus SA portions are drawn in Fig. 6. It was easily noticed that the surface roughness drastically increased with increasing the blended SA contents regardless of the presence or absence of the loaded ampicillin as a drug model. This behavior is explained as the addition of high SA contents, might result in the formation of high amorphous like structure and formation of more structural pores due to the big difference in the miscibility degree between PVA and SA which produced significant surface roughness differences.

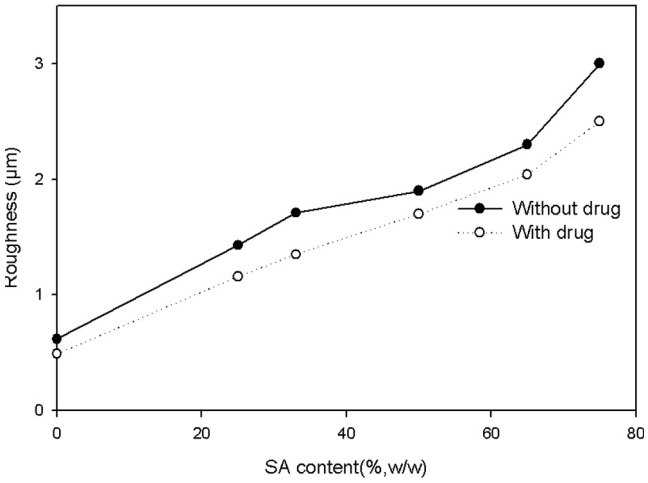

Figure 6 Effect of SA contents in PVA hydrogel membranes on roughness (µm) of membrane's surface.

3.5. Drug release profile study

The cumulative ampicillin percentage release profile from PVA-SA hydrogel membranes was conducted in phosphate buffer solution (pH 8.0 at 37 °C) and the obtained data are drawn in Fig. 7. The initial release profile of ampicillin exhibited rapid release behavior in the first 15 min as known the fact of burst effect of drug release (Huang and Brazel, 2001). However, the release behavior increased slightly after several hours. The burst release of ampicillin varies in the range 38–45%, can be attributed to the rapid diffusion of ampicillin which was loaded close to the membranes' surface. After the first 15 min, the increase of ampicillin release profile from PVA-SA hydrogel membranes can be ignored and no significant distinctions in the total release percentage were observed after 6 h of release. Furthermore, addition of SA in different portions to PVA hydrogel membranes has adverse influence on the release behavior of ampicillin. It is clearly noticed that an increase in SA content hindered the diffusion of ampicillin into phosphate buffer solution. It is likely that a bonding might occur between negatively charged COO^- groups of sodium alginate and positively charged NH_3^+, Na^+, or H^+ groups of sodium ampicillin. This potential bridging agent can decrease and impede the release behavior of ampicillin. The current explanation is consistent with the obtained results of Takamura et al. (1992). They found that incorporation of SA in different portions decreased the released drug percent.

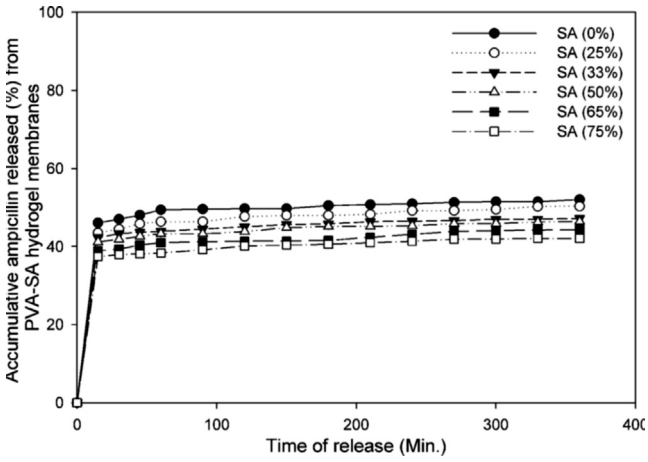

Figure 7 Effect of SA contents on accumulative release percentage of ampicillin from PVA-SA hydrogel membranes in phosphate buffer solution (at 37 °C, pH 8.0).

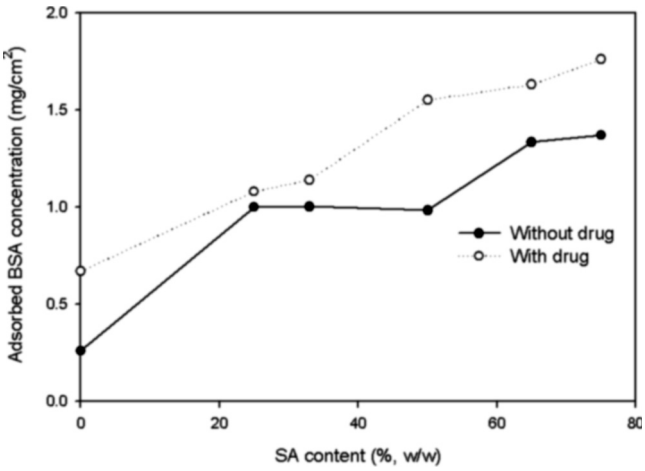

Figure 8 Effect of SA content in PVA hydrogel membranes on protein adsorption values.

3.6. Wound dressing evaluation (in-vitro studies)

Essential characters for wound dressing films, such as protein adsorption, hydrolytic degradation, hemocompatibility, and antibacterial activity behavior were investigated under *in vitro* conditions to evaluate using our membranes as wound dressing materials. The obtained data are discussed in the following.

3.7. Protein adsorption

The protein adsorption onto PVA-SA blend hydrogel membranes has been conducted via *in vitro* experiments and calculated using Eq. (3), the protein adsorption results have been shown in Fig. 8. As shown in Fig. 8, the adsorption of BSA increased from 0.7–1.8 mg/cm^2 as the amounts of SA increased in the PVA hydrogels from 0–75 (%, w/w). Interestingly, the PVA hydrogels with loaded ampicillin showed a feasible adsorption of BSA as compared to free-ampicillin hydrogels, indicating that SA content affected the protein adsorption behavior onto the surface of PVA hydrogel. These results are completely consistent with the reported results by Kim et al. (2008) they have revealed that adsorption of protein increased with increasing blended alginate in PVA hydrogels. Although the hydrophilicity of membranes increased with SA content which exhibited a decrease of non-specific adsorption of proteins, the obtained results may be revealed to the incorporation of BSA molecules into the resulted pores in the membrane structure.

3.8. Hydrolytic degradation

Gravimetric determination was used to study the *in vitro* hydrolytic degradation of PVA-SA hydrogel membranes (Gan et al., 1999). Due to the hydrolytic cleavage of hydrogen bonding among –OH groups of PVA chains, apparently weight loss values can be observed within the depicted results in Fig. 9. Also, this weight loss was previously noticed and expected during experiments in Fig. 3, due to the high water solubility of SA as a blend material and high hydrophilic forces among PVA chains. As shown in Fig. 9, it was found that the weight loss of pure freeze–thawed PVA membrane (0% SA) is relatively limited; almost 18%. While for PVA-SA membrane 75% SA content reaches 60%. These results refer to the weight loss of PVA-SA hydrogels that is dramatically increased with increasing SA contents. This phenomenon can be ascribed to the degradation of PVA-SA hydrogel membranes are predominantly the cleavage of entanglement segments of PVA and is consistent with the fact that the degradation of PVA is quite limited, whereas the degradation of PVA-SA is quite high. In addition, as PVA and SA are nontoxic materials, (Kamoun and Menzel, 2012; Xiao and Zhou, 2003) the PVA-SA hydrogel membranes and their degraded by-products might be expected to be nontoxic too.

3.9. Hemocompatibility

The *in vitro* blood compatibility of the prepared PVA-SA xerogel membranes was determined here by the method of hemocompatibility test using compensation in Eq. (4). Table 1 represents the obtained hemolysis (%) values from PVA-SA xerogel membranes. According to the classification of hemolytic tendency of polymeric materials, (American Society for Testing and Materials, 2000) it is clear from the depicted data of hemolysis (%) in Table 1 that the hemolysis values vary in the range 0.06–1.22% with SA contents increasing, which indicate for a good compatibility of the xerogel membranes and non-hemolysis materials were found. These results can be explained by the fact that both PVA and SA are highly hydrophilic and biocompatible polymeric materials. Furthermore, no chemical crosslinking was used; the xerogel membranes thus showed excellent hemocompatibility property too. The hemocompatibility results are consistent with the obtained results by Chhatri et al. (2011).

3.10. Antibacterial activity

The *in vitro* antibacterial activity of PVA-SA hydrogel membranes containing sodium ampicillin was conducted and the data of the zone inhibition of bacteria are summarized in Table 1. The Gram positive bacteria (e.g. *Staphylococcus*

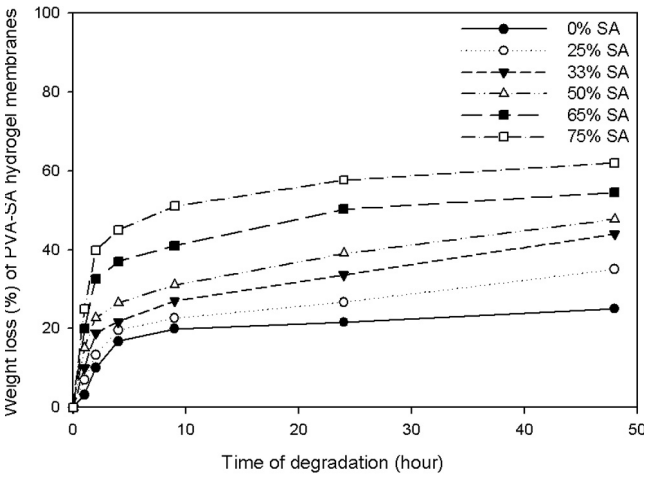

Figure 9 Effect of SA contents on weight loss of the PVA-SA hydrogel membranes versus different degrading times in phosphate buffer saline (PBS) (0.1 M, pH 7.4, at 37 °C).

pyogenes and *Staphylococcus aureus*) and the Gram negative bacteria (e.g. *Pseudomonas aeruginosa* and *Proteus vulgaris*) were utilized in this assay. Using antimicrobial drugs or antibiotic like ampicillin is necessary that play a vital role to stop microbes from growing or killing them outright (Chhatri et al., 2011). Moreover, these disinfectants can control of infectious disease spreading and inhibit the microbe activity till dead. Therefore, the wound dressing applications adapted from polymeric materials should be loaded with antimicrobial drugs, even the used polymeric material possesses antimicrobial activity itself e.g. chitosan (Kanatt et al., 2012; Yang et al., 2008). In the current investigation, the depicted data in Table 1 indicate that the loaded disinfectants (i.e. ampicillin) have broad antibacterial activity against pathogenic organisms such as Gram positive and negative bacteria, however addition of SA in different portions has no significant impact on antibacterial activity. These results clearly demonstrate that the prepared PVA-SA hydrogel membranes possess sufficient antibacterial activity.

4. Conclusions

In conclusion, PVA-SA hydrogel wound dressing membranes loaded with sodium ampicillin have been developed using the physical crosslinking method. The physical entanglement between PVA and SA has been verified by IR analysis. The results showed that incorporation of SA in the physically crosslinked PVA network notably affected its molecular structure and morphological properties. SEM and roughness results referred that, surface morphology of PVA membranes was strongly dependent on SA content. The release profile of ampicillin released from PVA-SA hydrogel membranes exhibited an adverse effect due to potential bonding occurred between SA and ampicillin moieties, therefore SA had feasible effect on ampicillin release behavior. Furthermore, the significant antibacterial activity for PVA-SA hydrogel membranes has been detected similar to that with only PVA membranes. The PVA-SA hydrogel membranes exhibited apparent swellable, flexible, elastic, perforated surface shape structure, high adsorbed protein, high hydrolytic degradation, non hemolytic materials, and active antimicrobial materials, compared to PVA ones. Therefore, PVA-SA containing ampicillin could be applied as a potential wound dressing material with accepted blood compatibility through hemocompatibility results.

References

Ahmad, A.S., Rahman, N., Islam, F., 2004. Spectrophotometric determination of ampicillin, amoxycillin, and carbenicillin using Folin-Ciocalteu phenol reagent. J. Anal. Chem. 59 (2), 119–123.

Alencar, d.Q.H., Humberto, G.F., Gustavo, A.A., Maria, M.F., Antonio, L.B., Julio, S.R., 2003. Development of new hydroactive dressings based on chitosan membranes: characterizations and in vivo behavior. J. Biomed. Mater. Res. 64A, 147–154.

Balakrishnan, B., Mohanty, M., Umashankar, P.R., Jayakrishnan, A., 2005. Evaluation of an in situ forming hydrogel wound dressing based on oxidized alginate and gelatin. Biomaterials 26, 6335–6342.

Cascone, M.G., Maltinti, S., Barbani, N., 1999. Effect of chitosan and dextran on the properties of poly(vinyl alcohol) hydrogels. J. Mater. Sci. Mater. Med. 10, 431–435.

Chhatri, A., Bajpai, A.K., Shandhu, S.S., Jain, N., Biswas, J., 2011. Cryogenic fabrication of savlon loaded macroporous blends of alginate and polyvinyl alcohol (PVA). Swelling, deswelling and antibacterial behaviors. Carbohydr. Polym. 83, 876–882.

Choi, Y.S., Hong, S.R., Lee, Y.M., Song, K.W., Park, M.H., Nam, Y.S., 1999. Study on gelatin -containing artificial skin. Part I. Preparation and characteristics of novel gelatin-alginate sponge. Biomaterials 20, 409–417.

Fathi, E., Atyabi, N., Imani, M., Alinejad, Z., 2011. Physically crosslinked polyvinyl alcohol–dextran blend xerogels: morphology and thermal behavior. Carbohydr. Polym. 84, 145–152.

Gan, Z., Yu, D., Zhong, Z., Liang, Q., Jing, X., 1999. Enzymatic degradation of poly(ε-caprolactone)/poly(dl-lactide) blends in phosphate buffer solution. Polymer 40 (10), 2859–2862.

Gilchrist, T., Martin, A.M., 1983. Wound treatment with Sorbsan-an alginate fiber dressing. Biomaterials 4, 317–320.

Hassan, C.M., Stewart, J.E., 2000. Diffusional characteristics of freeze/thawed poly(vinyl alcohol) hydrogels: applications to protein controlled release from multiaminate devices. Europ. J. Pharm. Biopharm. 49, 161–165.

Hinman, C.D., Maibach, H., 1963. Effect of air exposure and occlusion on experimental human skin wounds. Nature 200, 377–378.

Huang, X., Brazel, C.S., 2001. Review On the importance and mechanisms of burst release in matrix-controlled drug delivery systems. J. Control. Release 73, 121–136.

Hwang, M.R., Kim, J.O., Lee, J.H., Kim, Y., Kim, J.H., Chang, S.W., Jin, S.G., Kim, J.-A., Lyoo, W.S., Han, S.S., Ku, S.K., Young, C.S., Choi, H.G., 2010. Gentamicin-loaded wound dressing with polyvinyl alcohol/dextran hydrogel: gel characterization and in vivo healing evaluation. AAPS Pharm. Sci. Technol. 11 (3), 1092–1103.

Jeon, Y.J., Kim, S.K., 2000. Production of chitooligosaccharides using an ultrafilteration membrane reactor and their antibacterial activity. Carbohydr. Polym. 41, 133–141.

Jumaa, M., Furkert, F.H., Muller, B.W., 2002. A new lipid emulsion formulation with high antimicrobial efficacy using chitosan. Eur. J. Pharm. Biopharm. 53, 115–123.

Kamoun, E.A., Menzel, H., 2012. HES-HEMA nanocomposite polymer hydrogel: swelling behavior and characterization. J. Polym. Res. 19, 9851–9865.

Kanatt, S.R., Rao, M.S., Chawla, S.P., Sharma, A., 2012. Active chitosan-polyvinyl alcohol films with natural extracts. Food Hydrocolloids 29, 290–297.

Kim, J.O., Park, J.K., Kim, J.H., Jin, S.G., Yonga, C.S., Li, D.X., Choi, J.Y., Woo, J.S., Yoo, B.K., Lyoo, W.S., Kim, J.-A., Choi, H.G., 2008. Development of polyvinyl alcohol–sodium alginate

gel-matrix-based wound dressing system containing nitrofurazone. Int. J. Pharm. 359, 79–86.

Levic, S., Rac, V., Manojlovic, V., Rakic, V., Bugarski, B., Flock, T., Krzyczmonik, K.E., 2011. Limonene encapsulation in alginate-poly(vinyl alcohol). Procedia Food Sci. 1, 1816–1820, V., N.,.

Lin, W.C., Yu, D.G., Yang, M.C., 2006. Blood compatibility of novel PGA (poly glutamic acid)/poly vinyl alcohol hydrogels. Colloids Surf. B Biointerf. 47, 43–49.

Mansur, H.S., Oréfice, R.L., Mansur, A.A.P., 2004. Characterization of poly(vinyl alcohol)/poly(ethylene glycol) hydrogels and PVA-derived hybrids by small-angle X-ray scattering and FTIR spectroscopy. Polymer 45 (21), 7193–7202.

Nandini, V.V., Venkatesh, K.V., Nair, K.C., 2008. Alginate impressions: a practical perspective. J. Conserv. Dent. 11 (1), 37–41.

Peppas, N.A., Stauffer, S.R., 1991. Reinforced uncrosslinked poly(-vinyl alcohol) gels produced by cyclic freezing–thawing processes: a short review. J. Control. Release 16, 305–310.

Queiroz, A.C., Santos, J.D., Monteiro, F.J., Gibson, I.R., Knowles, J.C., 2001. Adsorption and release studies of sodium ampicillin from hydroxyapatite and glass-reinforced hydroxyapatite composites. Biomaterials 22 (11), 1393–1400.

Razzak, M.T., Darmawan, D., Zainuddin, S., 2001. Irradiation of polyvinyl alcohol and polyvinyl pyrrolidone blended hydrogel for wound dressing. Radiat. Phys. Chem. 62, 107–113.

Ress, D.A., Welsh, E.J., 1997. Secondary and tertiary structure of polysaccharides in solutions and gels. Angew. Chem. Int. Ed. Engl. 16, 214–224.

Rosiak, M.T., Darmawan, D., Zainuddin, S., 2001. Irradiation of polyvinyl alcohol and polyvinyl pyrrolidone mixed hydrogel for wound dressing. Radiat. Phys. Chem. 62, 107–113.

Singh, D.K., Ray, A.K., 1994. Graft copolymerization of 2-hydroxyethyl methacrylate onto chitosan films and their blood compatibility. J. Appl. Polym. Sci. 53, 1115–1121.

American Society for Testing and Materials: Standard Practices for Assessment of Haemolytic Properties of Materials, 2000. ASTM F 756–00, Philadelphia.

Takamura, A., Ishii, F., Hidaka, H., 1992. Drug release from poly(vinyl alcohol) gel prepared by freeze–thaw procedure. J. Control. Release 20 (1), 21–27.

Winter, G.D., 1962. Formation of the scab and the rate of epithelization of superficial wounds in the skin of the young domestic pig. Nature 193, 293–294.

Xiao, C., Zhou, G., 2003. Synthesis and properties of degradable poly(vinyl alcohol) hydrogel. Polym. Degrad. Stab. 81, 297–301.

Yang, J.M., Su, W.Y., Leu, T.L., Yang, M.C., 2004. Evaluation of chitosan/PVA blended hydrogel membranes. J. Membr. Sci. 236, 39–51.

Yang, X., Liu, Q., Chen, X., Yu, F., Zhu, Z., 2008. Investigation of PVA/ws-chitosan hydrogels prepared by combined gama-irradiation and freeze–thawing. Carbohydr. Polym. 73, 401–408.

Yokoyama, F., Masada, I., Shimamura, K., Ikawa, T., Monobe, K., 1986. Morphology and structure of highly elastic poly vinyl alcohol) hydrogel prepared by repeated freezing-and melting. Colloid. Polym. Sci. 264, 595–601.

Zmora, S., Glicklis, R., Cohen, S., 2002. Tailoring the pore architecture in 3-D alginate scaffolds by controlling the freezing regime during fabrication. Biomaterials 23, 4087–4094.