VIETNAM JOURNAL OF CHEMISTRY VOL. 52(2) 240-245 APRIL 2014

ANTIMICROBIAL ACTIVITY AND PHOSPHORUS RELEASE BEHAVIOR OF STARCH/CHITOSAN HYDROGEL

Le Thi Phuong[1], Tran Ngoc Quyen[1], Duong Thi Be Thi[2], Nguyen Cuu Khoa[1*]

[1]Institute of Applied Materials Science, VAST, 1 Mac Dinh Chi, Ho Chi Minh City

[2]Can Tho University, Can Tho City

[3]Institute of Chemical Technology, VAST, 1 Mac Dinh Chi, HoChiMinh City

Received 23 January 2014

Abstract

Use of slow release fertilizer has become a new trend to improve fertilizer use efficiency and minimize environmental pollution. In this paper, the phosphorus release behavior of controlled-release fertilizer (CRF) hydrogels, which were prepared from starch/chitosan and formaldehyde crosslinker, was investicated. The antimicrobial activities of these membranes were also investigated. The results showed, that these membranes have high activity against *E. coli*, *Aspergillus niger* and *F. oxysporum*. The membranes exhibited capability of encapsulation and slow release of phosphorus fertilizer. Therefore, such membranes can be used to prolong release of fertilizer for long-term plants.

Keywords: Slow release fertilizer, hydrogel, chitosan, *E. coli*.

1. INTRODUCTION

The growth of plants and their quality is mainly a function of the quantity of fertilizer and water. So it is very important to improve the utilization of water resources and fertilizer nutrients. However, about 40-70 % of nitrogen, 80-90 % of phosphorus, and 50-70 % of potassium of the applied normal fertilizers is lost to the environment and cannot be absorbed by plants, which causes not only large economic and resource losses but also very serious environmental pollution [1-5]. Controlled release is a method used to solve this problem.

Chitosan (poly-β(1,4)-d-glucosamine), a cationic polysaccharide, is obtained by alkaline deacetylation of chitin, the principal exoskeleton component in crustaceans. The natural polymer exhibits biodegradability, biocompatibility, and antifungal activity. Therefore, chitosan and its modified analogs have shown many applications in controlled release systems for biomedical materials and agriculture [6-12]. Chitosan has a lower swelling ability when it forms hydrogel due to theslower relaxation rate of polymer chains [13]. Therefore, blending chitosan with other hydrophilic polymers improving its water absorbency has been extensively studied for several applications.

Starch is a polysaccharide derived from plants that can be produced at low cost and large scale. It is abundant, edible, fully biodegradable, easily renewable, a low cost and a promising candidate for developing sustainable materials. Recently, many researchers have extensively explored the development of starch composite films with other polymers such as collagen, poly (vinyl alcohol), carrageenan, gelatin, lignin, chitosan.

In this study, starch/chitosan blended hydrogel membranes were prepared using formaldehyde as chemical crosslinking agent. The influence of CS on the antibacterial activity of these membranes and the phosphorus release behavior of CRF hydrogels was investigated.

2. EXPERIMENTAL

2.1. Materials

The biopolymers used in the experiments are commercial starch and chitosan. Chitosan (M_w = 100,000-300,000) and a degree of deacetylation of 80 % was obtained from Acros, Soluble starch was purchased from sigma USA. Formaldehyde 37 %, Calcium dihydrophosphate ($Ca(H_2PO_4)_2$) was purchased from Guangdong, China.

2.2. Preparation of starch/CS blended hydrogel membranes

The starch/CS blended hydrogel membranes were prepared by mixing 10 % w/v starch solution with different amount of formaldehyde from 5-30 % wt. formaldehyde, based on the total dry weight of polymer at 60-65 ^oC for 40 minutes, the pH was raised to 8-9 by 10 % (w/v) NaOH. The temperature of solution was got down 40 ^oC and the pH was adjusted to 5 by 10 % HCl. Then the mixture was added with 2.67 % w/v chitosan solution at a ratio 1:1 by weight and stirred constantly until homogeneous. After mixing, the gel was formed within 30 minutes. The product was dried at 60 ^oC in a vacuum oven overnight.

2.3. Preparation of CRF hydrogels

The CRF starch/CS hydrogel was prepared by the following method. Starch solution was mixed with chitosan solution at a ratio 1:1 by weight, treated with formaldehyde. The mixture was stirred constantly until homogeneous and the appropriate amount of $Ca(H_2PO_4)_2$ fertilizer was added into the mixture under constant stirring. After mixing, the gel was formed within 30 minutes. The CRF hydrogel product was dried at 60 ^oC in a vacuum oven overnight. Amount of starch, CS, formaldehyde, fertilizer used for preparing the CRF hydrogels as shown in table 1.

2.4. Characterizations

Structure of starch/CS blended hydrogel was analyzed using Fourier-transform infrared (FTIR) spectrophotometer (Equinox 55 Bruker). The release behaviors of phosphorus from the CRF hydrogels were evaluated by UV-visible spectrophotometry (UV-1800 Shimadzu).

Table 1: Formulation of CRF hydrogels

CRF hydrogels	10 %w/v Starch, ml	2.67 %w/v CS, ml	Fertilizer, g	37 % formaldehyde solution, ml
CS1	15	15	0,5	0.34
CS2	15	15	1	0.34
CS3	15	15	2	0.34
CS4	15	15	3	0.34

2.5. Water Absorbency of CRF Hydrogels

A preweighed dry hydrogel sample was immersed into a certain amount of deionized water. At certain time intervals the hydrogel was taken out of the water. Excessive surface water of the swollen hydrogel was removed with a filter paper, and the weight of the swollen sample was measured. Swelling ratio (% SR) of the hydrogel was calculated using the equation:

$$\%SR = \frac{W_s - W_d}{W_d} \times 100$$

with W_s and W_d refer to the weight of swollen and dry hydrogels, respectively.

2.6. Antimicrobial assessment

Eight strains of microorganisms were used to test the antimicrobial activity of membranes, including: *Escherichia coli*, *Pseudomonas aeruginosa* (Gram-negative bacteria); *Staphylococcus aureus, Bacillus subtillis* (Gram-negative bacteria); *Aspergillus niger*, *Fusarium oxysporum* (fungus) ; *Candida albicans, Saccharomyces cerevisiae* (yeast). Antimicrobial activity of prepared membranes was assayed by Vander Bergher and Vlietlinck method (1991), performed using a sterile 96 well-microplate. The bacteria were cultured in Trypcase Soya Broth (TSB), while yeast/fungus was cultured in Saboraud Dextrose Broth (SDB) and incubated at 37 ^oC for 24 hours. Then, the active cultures were inoculated into 10 ml of TSB for bacteria and SDB for yeast/fungus and incubated at 37 ^oC/24 hours (bacteria) or 37 ^oC/48 hours (yeast/fungus). Antimicrobial activity of hydrogel was recorded in terms of MIC, which was defined as the lowest concentration of sample required to completely inhibit microbial growth.

2.7. Encapsulation efficiency analysis

To study encapsulation efficiency of fertilizer in the CRF hydrogels, a CRF hydrogel sample was immersed into a certain amount of deionized water for 1 min and then kept aliquot solution was sampled for P determination, assayed to determine the concentration of the unencapsulated fertilizer.

Encapsulation efficiency (%) was calculated by following formula:

%Encapsulation efficiency = [1-Unencapsulated fertilizer/Total fertilizer]×100.

2.8. Release behavior in water

The release behaviors of phosphorus from the CRF hydrogels in deionized water were investigated by UV-visible spectrophotometry (UV-1800 Shimadzu). A 5.00 mL fertilizer sample solution was pipetted into a 25.00 mL volumetric flask. Then, 5.00 mL of molybdovanadate reagent was added. Deionized water was also added to make a 25.00 mL solution. After 30 minutes, at the room temperature. The absorbance of the sample solution was measured at a wavelength of 420 nm by UV spectrophotometer. The amount of phosphorrus in the sample solution was calculated using the calibration curve [15].

3. RESULTS AND DISCUSSION

3.1. Structure and characterization of hydrogels

There are various kinds of crosslinking agents that can crosslink chitosan. Among them aldehydes and anhydrides e.g. formaldehyde, acetaldehyde, glutaraldehyde, etc are commonly used as crosslinking agents because they are cheap and easily available. In the study, hydrogel membrane was formed via formaldehyde crosslinker as shown in figure 1.

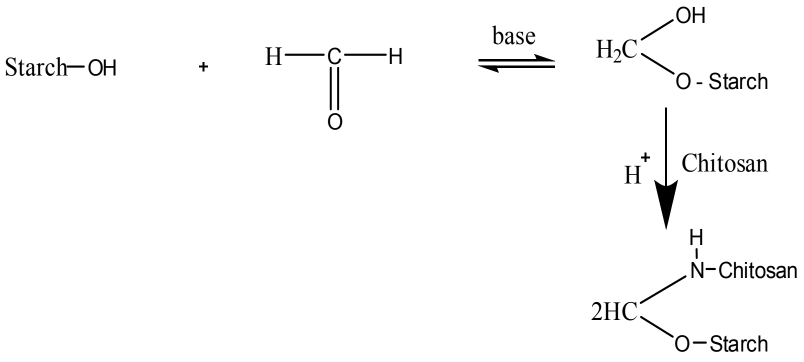

Figure 1: Crosslinking reaction of chitosan and starch with formaldehyde

The IR spectra of starch/CS hydrogel (Fig. 1) shows two picks around 1664 and 1648 cm^{-1}, indicating Carbonyl groups of chitosan and some formed imine bond (C=N) via Schiff's base structure by the reactions between amino groups of chitosan and aldehyde groups of formaldehyde. Besides formation of imine bond, some secondary amine could occured formation of methylol between amine-chitosan and formaldehyde resulting in rosslinking chitosan and starch chains. And a strong absorption at peak 1160 cm^{-1} was found, relating to O–CH$_2$–O groups, indicating a formation of acetal bridges.

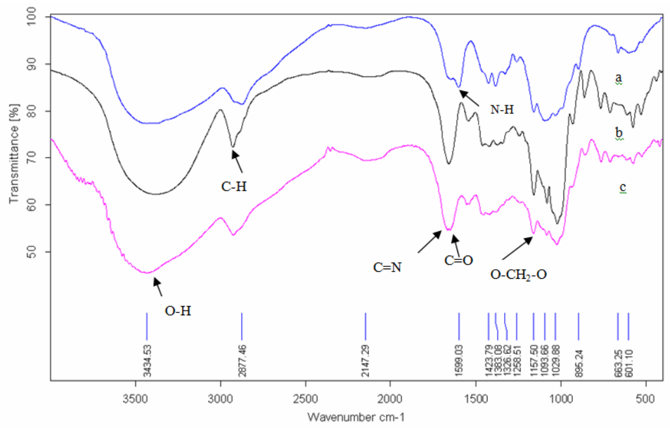

Figure 2: FT–IR spectra of (a) chitosan, (b) starch and (c) starch/chitosan hydrogel crosslinked with formaldehyde

3.2. Antimicrobial activity of starch/CS hydrogel membranes

As shown in table 2, only starch/CS hydrogel membranes exhibited inhibition against test microorganisms, such as *E. coli*, *A. niger* and *F. oxysporum*. It's due to the strong antifungal and antibacterial activities of chitosan. Among these microorganisms, *E. coli* appeared to be most susceptible to hydrogels, which showed the lowest MIC value or highest inhibitory effect. These results will be significant to prolong stability of the membrane for several applications.

Table 2: Antimicrobial activity of hydrogel membranes

Hydrogel membranes	MIC (µg/ml)							
	Gram-negative bacteria		Gram-positive bacteria		Fungus		Yeast	
	E.coli	*P.aeruginosa*	*B.subtiliis*	*S.aureus*	*A.niger*	*F.oxysporum*	*S.cerevisiae*	*C.albicans*
Starch hydrogel	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Starch/CS hydrogel	**25**	(-)	(-)	(-)	**50**	**50**	(-)	(-)

3.3. Swelling behavior of hydrogels

The strength and water preservation efficiency of hydrogel is greatly affected by the amount of crosslinking agent. The linear structure of chitosan molecule can be transformed into network structure through crosslinking and water molecule can be preserved in this structure. With the same ratio of starch and CS, the hydrogels exhibited different swelling ratio with different amount of formaldehyde. As shown in figure 2 the swelling ratio of hydrogel was highest when the amount of formaldehyde was 0.34 ml (equal to 20 %wt. formaldehyde, based on the total dry weight of polymer). But in case of excessive amount of crosslinking agent, lower swelling ratio appeared. It could be explained that the degree of crosslinking was higher, resulting in the decrease of network volume for water preservation efficiency of the hydrogel. Similar results have been reported in literature (Wu et al. 2001; Lin-Gibson et al. 2003). In case of 20 %wt. formaldehyde, amount of crosslinking agent was neither low nor high; therefore, it had highest water preservation efficiency.

The swelling ratio of hydrogel after 60 days is shown in Fig. 3. The hydrogel exhibited highly initial swelling rates and then the rate was constant after 5 days. It can also be seen from the figure that, at equilibrium, hydrogel showed the highest water absorbency (≈ 310 %) on the 30th day. With this high swelling ratio, phosphorus would diffuse out of the CRF hydrogels more easily. Therefore, we could control the phosphorus release behaviors of the CRF hydrogels superiorly.

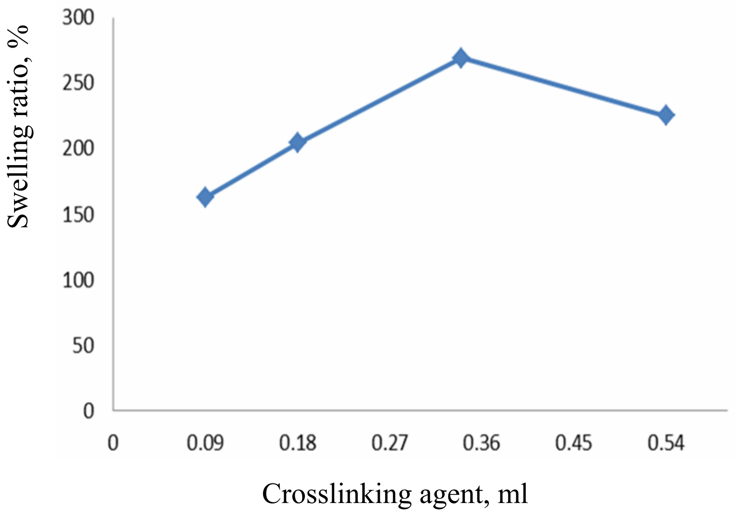

Figure 3: Swelling ratio (%) of hydrogels with varying amounts of crosslinking agent

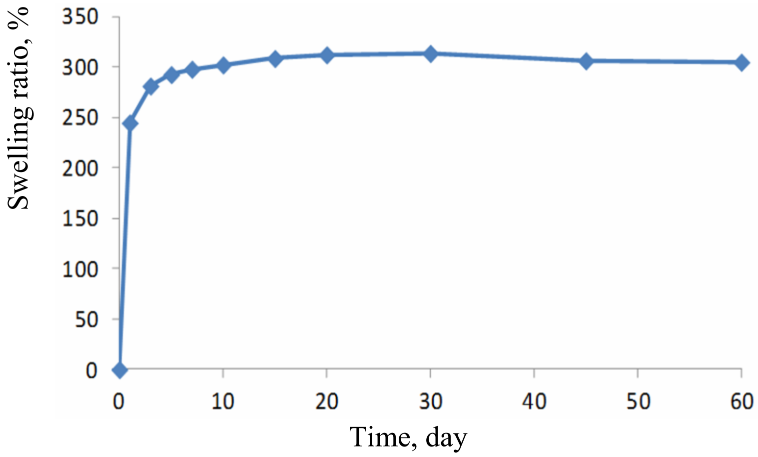

Figure 4: Swelling ratio (%) of starch/CS hydrogel (treated with 0.34 ml formaldehyde)

3.4. Encapsulation efficiency analysis and release behavior in water

It was found that the CS1, CS2, CS3, CS4 hydrogels show the highest encapsulation efficiency values of 76.58 %; 75.3 %; 72.2 % and 70.07 %, respectively.

The phosphorus release behavior of the CRF hydrogel in water was shown in figure 3. The release rate of the CRF hydrogel was high initially and constant after 3-6 days. It was due to the high concentration difference between the inside structure of the CRF hydrogel and the outer solution at the beginning of the release period. Then, the phosphorus release rate decreased as the concentration difference decreased. The result was in good agreement with the results reported by Rui et al. [16].

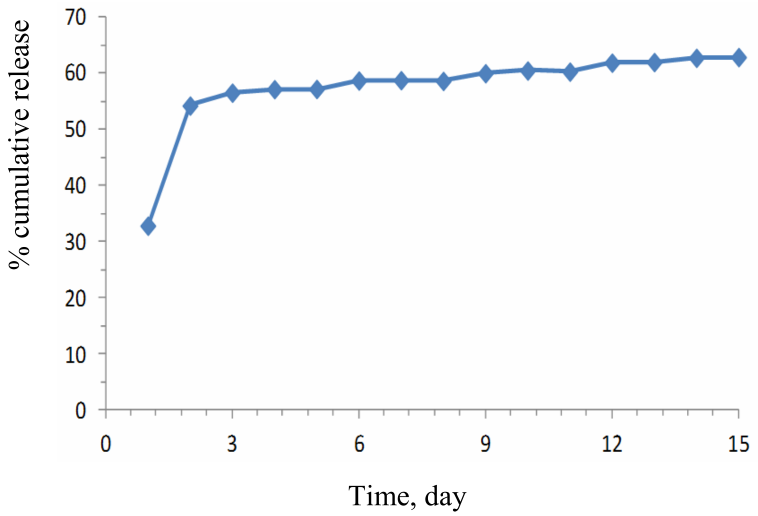

Figure 5: Release behaviors of phosphorus in water of hydrogel

4. CONCLUSIONS

Controlled release fertilizer (CRF) hydrogels prepared from starch/CS exhibited high swelling ratio (≈ 300 % after 10 days) and a strongly antimicrobial activity. The phosphorus fertilizer was sustainably released from the CRF hydrogels membrane (62.85 % amount of phosphorus over 15 days). This is one of the most important properties of the CRF hydrogels for their agricultural applications. Different fertilizers encapsulating the hydrogel membranes are ongoing evaluate and apply for plants.

REFERENCES

1. S. K. Behera, R. K. Panda. *Integrated management of irrigation water and fertilizers for wheat crop using field experiments and simulation modeling*, Agricultural Water Management, **96**, 1532-1540 (2009).
2. K. G. Mandal, K. M. Hati, A. K. Misra. *Biomass yield and energy analysis of soybean production in*

relation to fertilizer-NPK and organic manure, Biomass and Bioenergy, **33**, 1670-1679 (2009).

3. Y. F. Gu, Z. P. Zhang, S. H. Tu, K. Lindström. *Soil microbial biomass, crop yields, and bacterial community structure as affected by long-term fertilizer treatments under wheat-rice cropping*, European Journal of Soil Biology, **45**, 239-246 (2009).
4. M. Saigusa. *Broadcast application versus band application of polyolefin-coated fertilizer on green peppers grown on andisol*, Journal of Plant Nutrition **23**, 1485-1493 (2000).
5. J. Ge, Y. Cui, Y. Yan, W. Jiang. *The effect of structure on pervaporation of chitosan membrane*, J. Membr. Sci., **165**, 75-81 (2000).
6. D. Knorr. *Dye binding properties of chitin and chitosan*, J. Food Sci., **48**, 36-41 (1983).
7. D. K. Kweon, S. B. Song, Y. Y. Park. *Preparation of water-soluble chitosan/heparin complex and its application as wound healing accelerator*, Biomaterials, **24**, 1595-1601 (2003).
8. Y. Hu, X. Jiang, Y. Ding, H. Ge, Y. Yuan, C. Yang. *Synthesis and characterization of chitosan-poly(acrylic acid) nanoparticles*, Biomaterials, **23**, 3193-3201 (2002).
9. M. Ishihara, K. Nakanishi, K. Ono, M. Sato, M. Kikuchi, Y. Saito, H. Yura, T. Matsui, H. Hattori, M. Uenoyama, A. Kurita. *Photocrosslinkable chitosan as dressing for wound occlusion and accelerator in healing process*, Biomaterials, **23**, 833-840 (2002).
10. X. Y. Shi, T. W. Tan. *Preparation of chitosan/ethylcellulose complex microcapsule and its application in controlled release of vitamin D2*, Biomaterials, **23**, 4469-4473 (2002).
11. S. J. K. Francis, H. W. T. Matthew. *Application of chitosan-based polysaccharide biomaterials in cartilage tissue engineering: a review*, Biomaterials **21**, 2589-2598 (2000).
12. F. W. Mi, C. Y. Kuan, S. S. Shyu, S. T. Lee, S. F. Chang. *The study of gelation kinetics and chain-relaxation properties of glutaraldehyde-cross-linked chitosan gel and their effects on microspheres preparation and drug release*, Carbohydrate Polymer, **41**, 389 (2000).
13. AOAC - Official Methods of Analysis (15th ed.), Association of Analytical Chemistry, Washington, DC, USA (1990).
14. L. Rui, L. Mingzhu, W. Lan. *Controlled release NPK compound fertilizer with the function of water retention*, React. Funct. Polym., **67**, 769 (2007).

Corresponding author: **Nguyen Cuu Khoa**
Institute of Applied Materials Science, VAST,
1 Mac Dinh Chi, Ho Chi Minh City
Email: nckhoavnn@yahoo.com.