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Gelation of photocrosslinkable carboxymethyl chitosan and its application in controlled release of pesticide

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ABSTRACT

The gelation of a photocrosslinkable carboxymethyl chitosan (Az-CMCS), which was synthesized by mixing azidobenzaldehyde (Az) and an aqueous solution of carboxymethyl chitosan (CMCS), has been studied. The photocross-linking reaction kinetics were rapid, and the time required for completely converting into the insoluble hydrogels was less than 60 s for 10% aqueous solution of Az-CMCS samples upon exposure to 273.5 nm irradiation of UV lamp (4 W or 20 W). A relatively high photoirradiation intensity and Az content incorporated in AZ-CMCS could obviously shorten the time. The obtained hydrogels showed good pH sensitivity and the lysozymatic degradability. Diuron-loaded hydrogel membranes could also be formed rapidly by solar-simulated UV irradiation of a mixed aqueous solution of AZ-CMCS and diuron. Release profiles of diuron from the diuron-loaded hydrogel membranes in a buffer of pH 6.0 were shown to be diffusion controlled. The formation of a porous structure in the hydrogels on swelling was assumed to be the reason for this diffusion behavior.

1. Introduction

Hydrogel controlled release systems have attracted increasing attention during the past decades. In addition to their inertness and good biocompatibility, the ability of hydrogels to release entrapped drug in an aqueous medium and the ease of regulating such drug release by controlling water swelling and cross-linking density, make hydrogels particularly suitable as drug carriers in the controlled release systems (Singh, McCarron, Woolfson, & Donn, 2009). A variety of natural biopolymeric hydrogels have been employed as the controlled release systems due to their good bioproperties such as biocompatibility, biodegradability, and non-toxicity (Fini & Orienti, 2003; Ravi Kumar, Muzzarelli, Muzzarelli, Sashiwa, & Domb, 2004). Among them, chitosan is one of the most commonly used.

To prepare a controlled release hydrogel, chitosan needs to be cross-linked due to its hydrophilic property. Various cross-linking agents such as formaldehyde and glutaraldehyde have been used to cross-link chitosan (Chen, Tian, & Du, 2004; Illum, 1998; Risbud, Hardikar, Bhat, & Bhonde, 2000). However, this chemically cross-linking method has two drawbacks: firstly, the cross-linking reaction of these cross-linking agents with the amino groups of chitosan is extremely rapid. As a result, the cross-linking agents and the aqueous solution of chitosan cannot be mixed uniformly. Secondly, due to the high viscosity of the aqueous solution of chitosan, it is difficult to remove the large number of bubbles that are present in the solution before the cross-linking reaction, which in turn leads to the generation of substantial apertures inside the hydrogels. A convenient alternative is to crosslink linear photocrosslinkable polymer chains through a photochemical reaction. This reaction is readily controlled by adjusting the irradiation condition.

Recently, a photocrosslinkable chitosan or one of its derivatives (Fukuda et al., 2006; Karp et al., 2006; Ling et al., 2008; Obara et al., 2003) has been of particular interest in the biomedical material field due to the following reasons: the cross-linking reaction kinetics are rapid, the photoreactive functional groups eliminate the use of photoinitiators, the cross-linking reaction does not leave toxic by-products (the only by-product is N_2) and the cross-linked chitosan or its derivative is biocompatible and biodegradable (Ishihara et al., 2002). However, while there are many reports of photocrosslinking chitosan hydrogels, these hydrogels are not pH sensitive, so they have not been used for controlled release systems.

Pesticides are widely used in agriculture although their usage may bring hazards to both humans and the environment (Li et al., 2006). In some developing countries, farmers even apply pesticides in more than recommended dosage. In addition, as much as 90% of the applied conventional pesticide is lost or decomposed, which not only increases the cost but also leads to environmental pollution (Margni, Rossier, Crettaz, & Jolliet, 2002; Peppas & Langer, 1994). Controlled release of pesticides can remarkably reduce the amount of active agent by maintaining an effective concentration in the target for longer periods of time.

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In our recent study (Yin et al., 2010), we reported a photocrosslinkable, water-soluble carboxymethyl chitosan (Az-CMCS) precursor, based on a simple one-step mixing of azidobenzaldehyde (Az) and carboxymethyl chitosan (CMCS) in water solution at room temperature. The aim of this study was, using the Az-CMCS, to develop a pH sensitive photocrosslinked hydrogel system for controlled-release pesticide. It was expected that aqueous solution of Az-CMCS could be photocrosslinked via solar UV radiation to form pH sensitive hydrogel membrane at the target by being sprayed on leaves, the membrane would lead to a control release of pesticide by swelling in response to the pH of dew.

2. Materials and methods

2.1. Materials

Chitosan (CS, MW = 5.6×10^5 Da) with a deacetylation degree of 91.13% was purchased from the Zhejiang Yuhuan Biotechnology Company. Lysozyme was purchased from Sigma. 4-Aminobenzaldehyde was provided by 3B Scientific Corporation, USA. Sodium azide (analytical grade) was purchased from Sinopharm Group Chemical Reagent Co. Ltd. Diuron was purchased form Zhejiang Anji Bang Chemical Co., Ltd.

2.2. Preparation of 4-azidobenzaldehyde

4-Aminobenzaldehyde (3.5 g, 0.03 mol) was added to glacial acetic acid (13 ml) containing concentrated sulfuric acid (3.2 ml), which yielded a tarry black solid. Maintaining the reaction mixture below 5 °C, a solution of sodium nitrite (2.07 g, 0.03 mol) in a minimal amount of cold water was then added dropwise with stirring, giving a yellow solution. After 15 min, a solution of sodium azide (1.95 g, 0.03 mol) in a minimal amount of cold water was added dropwise, during which copious frothing occurred. After 1 h, the reaction product was extracted with diethyl ether, and the combined ether layers were washed with saturated aqueous $NaHCO_3$. The organic layer was dried over anhydrous sodium sulfate and concentrated under minimal laboratory light using a rotary evaporator at less than 40 °C to yield the product as a dark orange oil (Walton & Lahti, 1998).

2.3. Synthesis of carboxymethyl chitosan (CMCS)

CMCS was synthesized according to our recent publication (Tu et al., 2010). Briefly, 3 g of chitosan (MW = 5.6×10^5 Da, deacetylation degree = 91.1%) was stirred with isopropanol (35 ml) for 6 h and then filtered. NaOH solution (50 wt%) was added to the beaker with the filter residue, and this solution was fully stirred to mix evenly and then put in the refrigerator, where it froze overnight. Chloroacetic acid (4.5 g) dissolved in isopropanol (10 ml) was then dropped onto the frozen chitosan mixture, and the mixture was stirred at a temperature below 15 °C for 20 h. This solution was then filtered and the filter residue was washed with absolute ethanol. The crude product was then removed by centrifugation and subsequent dialysis against deionized water, and then freeze-dried to obtain CMCS. The elemental analyses results indicate that the N,O-substitution degree of CMCS is about 0.70, and the degree of N-substitution is about 0.17. Analytical data: C 38.84; N 5.98; H 6.74; O 48.44; C/N = 7.58:1 (molar ratio).

2.4. Preparation of photocrosslinkabe carboxymethyl chitosan (Az-CMCS)

Az-CMCS were synthesized according to our recently reported method (Yin et al., 2010). Briefly, azidobenzaldehyde dissolved in ethanol was dropped into CMCS water solution, and this mixture was stirred overnight at room temperature. A photocrosslinkable carboxymethyl chitosan solution was then obtained. The solution was divided into two parts. One part was directly used for the preparation of controlled-release pesticide formulation; the other part was added with cold alcohol. The resulting precipitate was collected by filtration, then washed thrice with 95% alcohol (v/v), redissolved in deionized water, dialyzed against deionized water, and then freeze-dried to obtain Az-CMCS. By changing the amount of Az, Az-CMCS samples with various substitution degrees of Az were prepared, and then named. Their elemental analytical data: C 44.56, N 7.15, H 6.08, O 42.21, C/N = 7.27:1 (for Az-CMCS-1); C 44.70, N 7.33, H 6.05, O 41.92, C/N, = 7.11:1 (for Az-CMCS-2); C 44.75, N 7.39, H 6.04, O 41.82, C/N = 7.07:1 (for Az-CMCS-3), respectively. The data for the substitution degrees to the C-2 amino groups estimated by elemental analysis were calculated to be 2.1%, 3.2%, and 3.6%. The $COOH/NH_2$ molar ratios were 0.786:1, 0.796:1, and 0.800:1.

2.5. Gelation of photocrosslinkabe carboxymethyl chitosan (Az-CMCS)

Gelation of aqueous solutions of Az-CMCS was performed as follows: Az-CMCS was weighed (Ws), and then dissolved in distilled water to form a solution (10.0%). The bubbles in the solution were removed under vacuum, and the solution was poured into a shallow silanized glass dish (with a diameter of 8.5 cm), and then cross-linked to form smooth, transparent hydrogel by irradiating it for a predetermined time at a lamp distance of 2 cm with an ultraviolet lamp (4 W or 20 W, 253.7 nm). The gelled sample obtained was immersed in distilled water at room temperature for 48 h to remove unreacted Az-CMCS, and then dried under vacuum. The dried hydrogel membrane (about 0.12 mm in thickness) was weighed (Wd). The gelation efficiency (GE) was calculated using the following equation:

$$\mathrm{GE}(\%) = \frac{\mathrm{Wd}}{\mathrm{Ws}} \times 100 \quad (1)$$

After the Az-CMCS aqueous solution was completely gelled, each of the resulting hydrogels with the different degrees of crosslinking were cut into circular disks (φ = 10 mm), used to investigate the swelling behavior and enzymatic degradation of the hydrogel. The hydrogel membranes prepared using Az-CMCS samples with various substitution degrees of Az (Az-CMCS-1, Az-CMCS-2 or Az-CMCS-3) were named as G-1, G-2 and G-3, respectively.

2.6. Swelling of the hydrogels

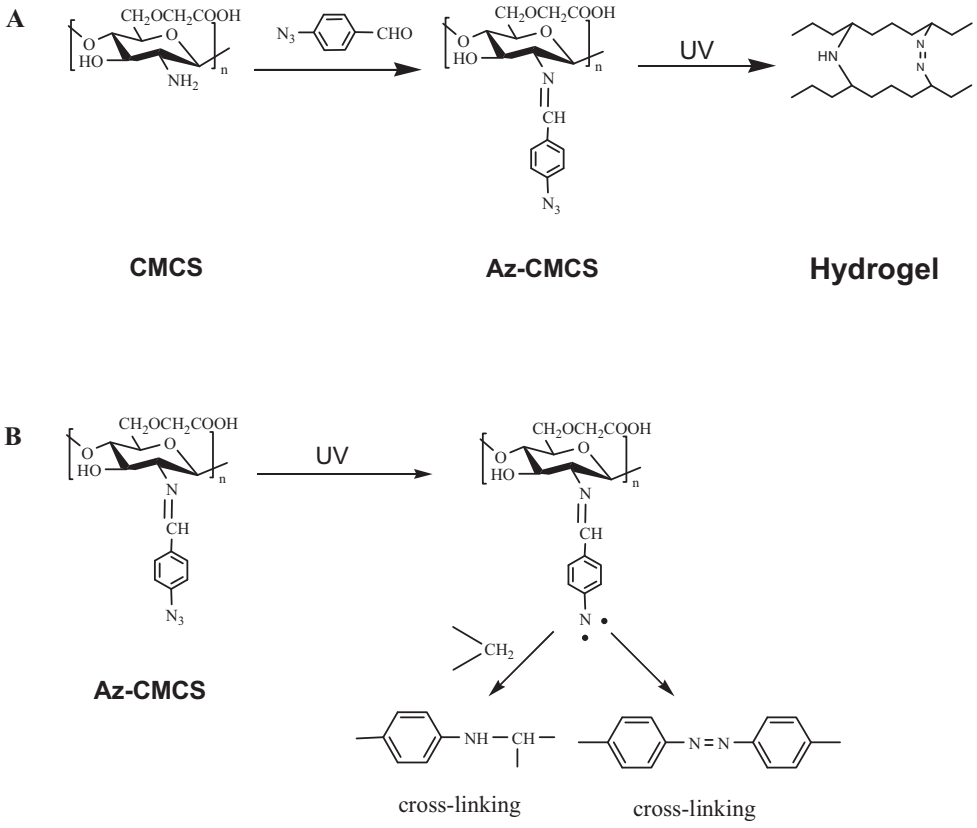
The swelling characteristics of the hydrogels were analyzed in a phosphate–citrate buffer solution system at 25 °C. The dried gel disks were soaked in buffer solutions with different pH values. The swollen gels were removed from the buffer solutions at regular time intervals and weighed after removing excess surface water with a filter paper, and then placed back into the same buffer solution. This procedure was repeated until equilibrium swelling was reached. The normalized degree of swelling Q_t was calculated using the following expression:

$$Q_t = \frac{m_t - m_0}{m_0} \quad (2)$$

where m_0 and m_t are the weights of the dry matrix and the water-swollen hydrogel, respectively.

2.7. Degradation of the hydrogels

Enzymatic degradation of the hydrogels was carried out according to the method reported by Amsden, Sukarto, Knight, and



(Az-CMCS);

Fig. 1. (A) Gelation route for photocrosslinkabe carboxymethyl chitosan (Az-CMCS); (B) a possible mechanism for the photocrtosslinking of Az-CMCS.

Shapka (2007). The dried hydrogels were incubated in a mixture of 4.0 mg/ml lysozyme and 0.1% (w/v) sodium azide in PBS (pH 7.4) at 37 °C. The lysozyme mixture solution was changed every week to maintain enzymatic activity. After a predetermined time, the hydrogel samples were taken out from the lysozyme solution, rinsed with double distilled water, and then freeze-dried and weighed to obtain the final weight after degradation. The extent of *in vitro* degradation was expressed as the percentage of the weight of a dried hydrogel film after lysozyme treatment.

2.8. Preparation of diuron-loaded hydrogel membranes

A 60.0 mg sample of diuron was added to 20.0 g of Az-CMCS aqueous solution (about 10%) and stirred until the diuron became dispersed. The rest of the procedures used were similar to those in the preparation of the hydrogel membrane without loading diuron. UV irradiation (20 W, 253.7 nm) was conducted for 10 seconds to induce the gelation of mixed AZ-CMCS/diuron solution. The formed hydrogel membrane was cut into circular disks (φ = 10 mm), and used for the release study of diuron. The diuron-loaded hydrogel membranes prepared using Az-CMCS-1 was named as G(d)-1.

By solar-simulated UV radiation, the diuron-loaded hydrogel samples were also prepared according to the above method. The used solar simulator is a 300 W xenon arc lamp (200–2500 nm spectral output, 50 W radiant output). The irradiation was also conducted for 10 seconds to induce the gelation of mixed AZ-CMCS-2 or AZ-CMCS-3/diuron solution. The obtained samples were named as G(d)-2 and G(d)-3, respectively.

2.9. Analysis of diuron in hydrogel membranes

Diuron-loaded hydrogel membranes were weighed (about 20 mg), and then treated with methanol in an ultrasound bath for 10 min. This led to the complete disintegration of the membrane. After 24 h at room temperature the mixture was sonicated for a further 10 min, and was then filtered quantitatively through a syringe filter (0.22 μm). The volume was made up to 50 ml with methanol, and the resulting solution was diluted 1:4 with methanol and analyzed for diuron by high-performance liquid chromatography (HPLC), using a LC-18 bonded-phase column, with a mobile phase of acetonitrile-water mix (80:20), at a flow rate of 1 ml/min. Detection was using a UV detector at 250 nm.

2.10. Controlled-release behavior of diuron

The release experiment of diuron in the pH6.0 buffer from diuron-loaded hydrogel samples was carried out in a thermostatic shaker bath at 25 °C. Each of diuron-loaded hydrogel membranes was soaked in 10 ml buffer solution of pH 6.0, and placed into stoppered conical flasks, which were put in the shaker bath for continuous shaking. At different time intervals, aliquots of 1.0 ml were removed for determination of diuron by HPLC as described above. Then for the samples all the buffer solution was replaced to minimize saturation effects.

In the final stage of the experiment, the hydrogel samples were removed from each flask to determine the amount of diuron that had remained incorporated in the samples. Release data were normalized at 100% by taking into account the amount of diuron

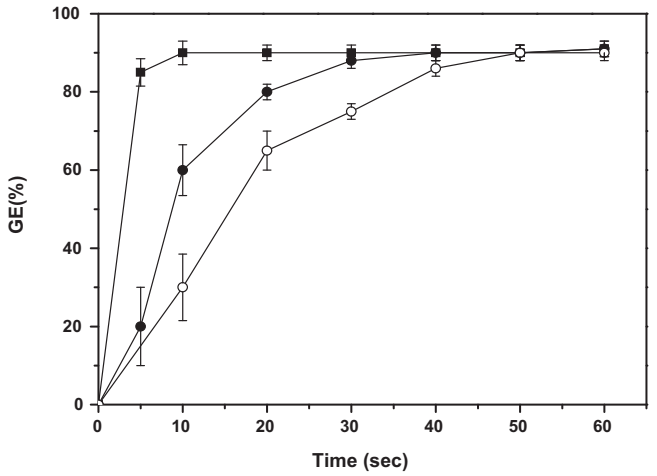


Fig. 2. Gelation efficiency as a function of irradiation time for 10% aqueous solutions of Az-CMCS samples upon exposure to 273.5 nm irradiation of UV lamp (4 W or 20 W). (■) Az-CMCS-1 solution radiated under a 20 W UV lamp; (○) Az-CMCS-1 solution radiated under a 4 W UV lamp; (●) Az-CMCS-2 solution radiated under a 4 W UV lamp. The data represent the mean ± SD of triplicate determinations.

released and the amount remaining incorporated in the hydrogel samples when the experiment finished.

3. Results and discussion

3.1. Gelation of photocrosslinkabe carboxymethyl chitosan (Az-CMCS)

The gelation route for photocrosslinkabe carboxymethyl chitosan (Az-CMCS) was illustrated in Fig. 1A. The compound Az-CMCS is a Schiff base, which can be synthesized by mixing azidobenzaldehyde (Az) and carboxymethyl chitosan (CMCS) in water solution at room temperature. For aqueous solutions of Az-CMCS samples with various substitution degrees of Az, their photocross-linking reactions occurred immediately upon exposure to 253.7 nm radiation in the absence of the photoinitiator. Their gelation efficiency (GE) as a function of UV irradiation time was shown in Fig. 2. Generally, the photocross-linking reaction kinetics were rapid, and the time for completely converting into the insoluble hydrogels was less than 60 s for 10% aqueous solutions of Az-CMCS samples upon exposure to 273.5 nm irradiation of UV lamp (4 W or 20 W). With the increase of irradiation time, the GE of aqueous Az-CMCS samples increase. Under the same irradiation conditions, aqueous solution of Az-CMCS-1 with a higher DS of AZ (see middle curve) has a higher GE value than that of Az-CMCS-2 with a lower DS of AZ (see bottom curve), indicating the contribution of the incorporated photocrosslinkng groups (AZ) to the UV-induced gelation.

To study the influence of the irradiation intensity on the GE, 10.0% aqueous solution of Az-CMCS-1 was irradiated with two kinds of different power of UV lamps (253.7 nm, 4 W and 20 W), respectively. Its gelation efficiency (GE) as a function of UV irradiation time was also shown in Fig. 2 (see top and middle curves). In contrast, with increasing intensity of UV lamp power, the time for completely converting into the insoluble hydrogels was further shortened (<10 s, see top curve).

According to the literature (Akio et al., 1992), an azidobenzoate group can produce a nitrene and N_2 when irradiated with light of an appropriate wavelength. The nitrene can be converted to primary, and secondary aminobenzoate and azobenzoate groups by hydrogen abstraction and coupling. Based on the structure of Az-CMCS, we propose that the secondary aminobenzoate and azobenzoate cross-linking structures are formed between Az-CMCS chains under UV irradiation, as shown in Fig. 1B.

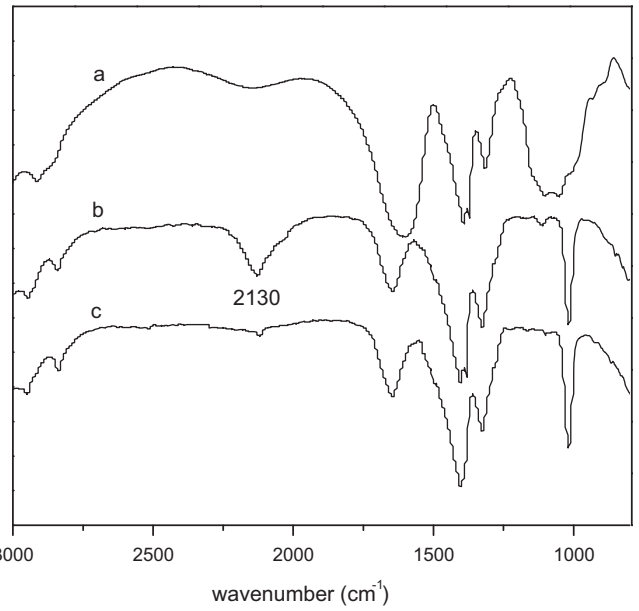


Fig. 3. FT-IR spectra of (A) CMCS, (B) Az-CMCS and (C) photocrosslinked carboxymethyl chitosan.

The FT-IR spectra of CMCS (see curve a), Az-CMCS (see curve b), and photocrosslinked carboxymethyl chitosan hydrogel (see curve c) are shown in Fig. 3. Upon comparing the IR spectrum of curve b with that of curve a, a characteristic absorption band at 2127 cm^{-1} is observed to be present in curve b but not in curve a, which indicates that azide substitution occurred. The az-substitution peak disappears in spectrum c, which indicates the formation of cross-linking between az groups after UV irradiation. After UV irradiation, it was observed that the color of the photocross-linked hydrogel turned yellow. The observed yellow color was a result of the formation of azobenzoate cross-linking structure.

3.2. Surface topographies of photocrosslinked hydrogels

To observe surface topography of swollen hydrogel, G-1, G-2 and G-3 swollen to equilibrium in a buffer of pH 6.0 at 25 °C were lyophilized. Scanning electron microscope observation shows that the swollen hydrogels exhibit a high level of porosity (see Fig. 4B–D). By contrast, the gel membrane obtained by heat-drying treatment for G-1 swollen in distilled water at 25 °C has a uniform and smooth surface morphology (see Fig. 4A). The formation of a porous structure on swelling is very important for enhancement of pesticide release.

3.3. pH sensitivities of the hydrogels

The changes in the degree of swelling at equilibrium for the three gels (G-1, G-2 and G-3) as a function of the pH value of the buffer solution are shown in Fig. 5. At pH = 3.0, each of the three gels exhibits the lowest degree of equilibrium; at pH > 3.0 or pH < 3.0, the degrees of equilibrium swelling of the gels increase. A sudden increase is observed in the pH range from 4.0 to 6.0, which shows good pH sensitivities of these hydrogels. Because the networks of the gels contain carboxyl groups and amino groups, when the positive charges of the amino groups are equal to the negative charges of the carboxyl groups, electrovalent bonds between $-NH^{3+}$ and

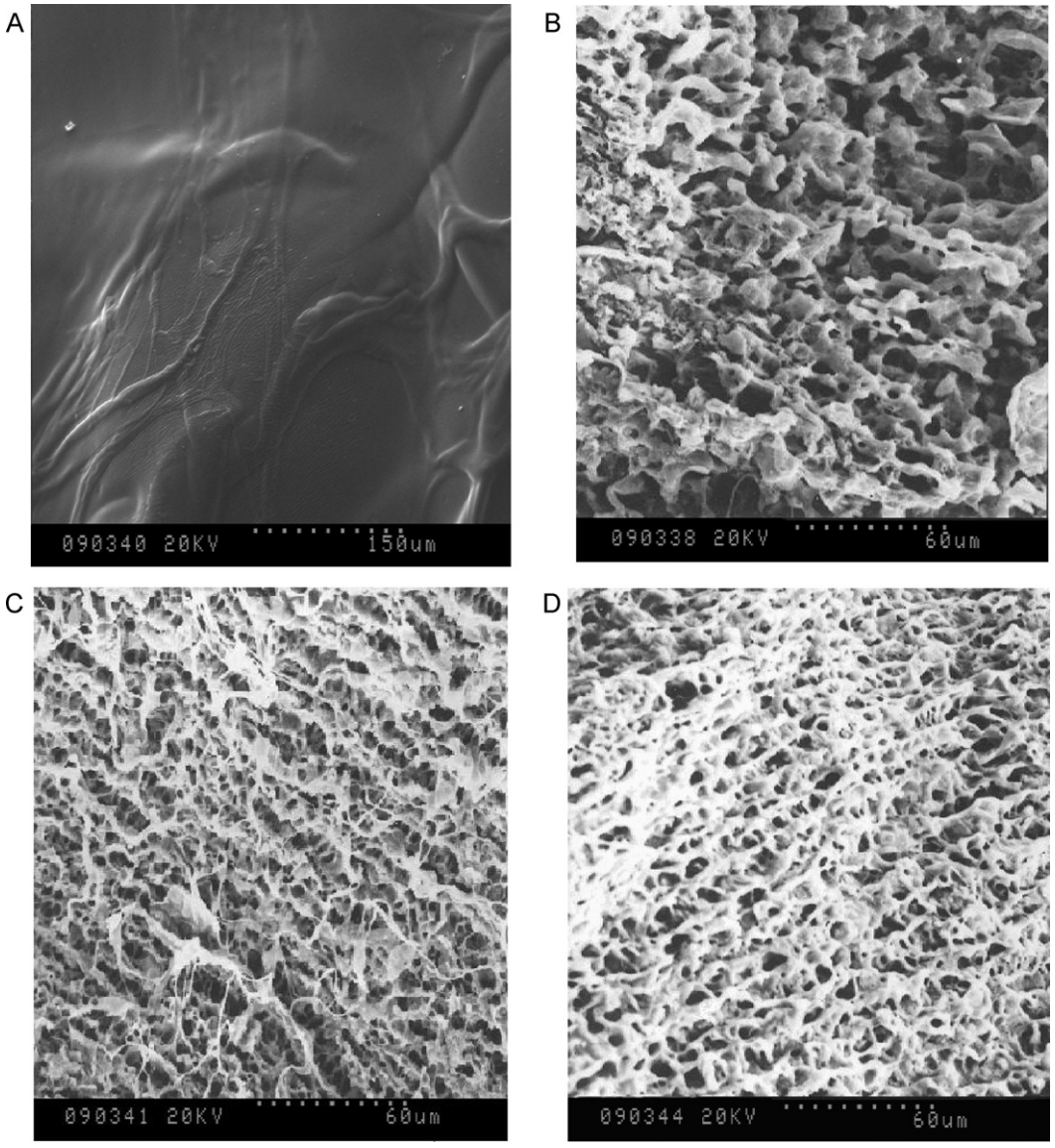


Fig. 4. SEM photographs of the hydrogel membranes obtained using different drying methods: (A) heat-drying treatment for G-1 swollen in distilled water at 25 °C; (B)–(D) freeze-drying treatment for G-1, G-2, and G-3 swollen to equilibrium in a buffer of pH 6.0 at 25 °C.

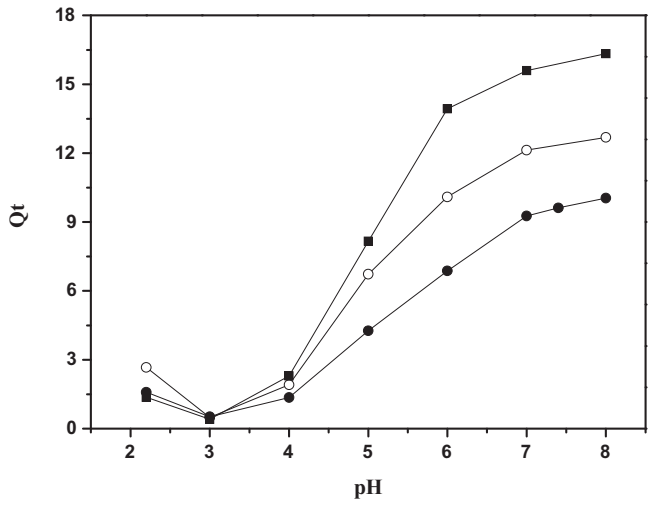


Fig. 5. Degree of equilibrium swelling curves for gels G-1–G-3 in the pH 2.2–8.0 buffer solutions at 37 °C. (■) G-1; (○) G-2; (●) G-3.

$-COO^-$ make the gels relatively compact, and so the degrees of their equilibrium swelling are lowest at pH 3.0. At pH > 3.0 or pH < 3.0, the networks are negatively or positively charged, and electrostatic repulsion and hydrophilicity cause the degrees of equilibrium swelling to increase. At pH 7.0–8.0, maximum ionization of the carboxyl groups occurs, giving rise to the highest degrees of equilibrium swelling of the gels.

It is also shown in Fig. 5 that the pH sensitive degree of three hydrogels decreases in the following order: G-1 > G-2 > G-3, which indicates that the pH sensitivities are related to their composition. Since the COOH/NH2 molar ratios of Az-CMCS increase in the following order: Az-CMCS-1 < Az-CMCS-2 < Az-CMCS-3; while the DS of Az decreases in the following order: Az-CMCS-1 > Az-CMCS-2 > Az-CMCS-3, we suppose that the decreased degree of equilibrium swelling of hydrogel results from the increase in the degree of cross-linking caused by the DS of Az.

The pH range of dew is between 6.0 and 7.7, in which the gels show very high degree of swelling, and the swollen hydrogels show a high level of porosity (see Fig. 4B–D). Therefore, using the gels as the pesticide carriers, pesticides could slowly be released by swelling matrices.

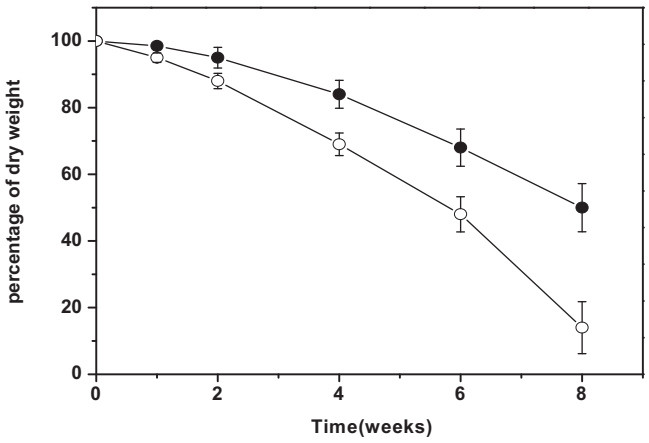


Fig. 6. Dry weight after *in vitro* degradation of hydrogel samples with different cross-linking degree in 4 mg/ml lysozyme solution in PBS (pH 7.4) incubated at 37 °C as a function of time. (◯) G-1 and (●) G-2.

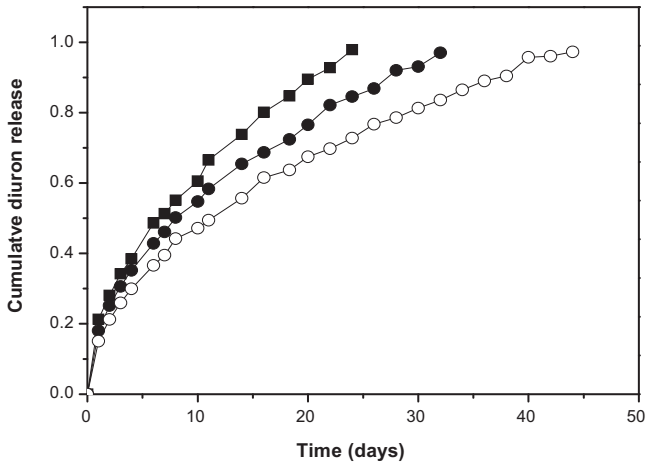


Fig. 7. Cumulative release of diuron in a buffer of pH 6.0 from diuron-loaded hydrogel samples with various AZ contents prepared by 253.7 nm UV or solar-simulated UV radiation. (■) G(d)-1; (●) G(d)-2; (◯) G(d)-3.

3.4. Degradaton of the hydrogels

The biodegradation of polymeric matrices in controlled release of pesticides is very important for avoiding pollution of the environment. Carboxymethyl chitosan can be degraded by lysozyme when dissolved in water (Ragnhild, Hjerde, & Varum, 1997). In this study, we found that carboxymethylchitosan-based photocrosslinked hydrogels were also biodegradable. For the resultant G-1 and G-2 samples, their *in vitro* degradation was shown in Fig. 6. By comparison, G-1 with a higher swelling degree had a faster degradation rate.

The degradation of the carboxymethylchitosan-based hydrogels results from the cleavage or hydrolysis of the glucosidic linkages in the hydrogels by the enzyme. Because of steric hindrance, an enzyme will hardly permeate into the polymer matrix prior to swelling, and diffusion is mainly dependent on the swelling of a hydrogel. A higher degree of swelling indicates that there are bigger or more pores in the network structure. More enzymes can approach the sites of glucosidic linkages consequently increasing the rate of degradation. G-1 with higher swelling degree showing bigger pores in its network (see Fig. 4B), so it has a faster degradation rate.

3.5. Controlled-release of diuron-loaded hydrogels

Fig. 7 shows the cumulative release curves of diuron from diuron-loaded hydrogels in a buffer of pH 6.0. The sample (G(d)-1) was prepared by UV irradiation at 253.7 nm, G(d)-2 and G(d)-3 were prepared by solar-simulated UV radiation. As can be seen, the three formulations had an excellent slow-release property, their cumulative release rates with time increasing in the following order: G(d)-3 < G(d)-2 < G(d)-1. Hydrogel with lower swelling degree exhibits smaller or fewer pores on its network structure (see Fig. 4C or D), which would make it more difficult for the diuron to dissolve and diffuse.

It has been reported in the literature (Cottefill, Wilkins, & da Silva, 1996) that the hydroxyl groups of the lignin (compared to other groups such as methoxyl, carbonyl groups, etc.) are the most important in forming hydrogen bonds with the imido and carbonyl groups of diuron. The extent of interaction between the lignin and diuron can affect the release of diuron from the matrix. A high energy of interaction would result in a slower release of diuron. On the basis of that there exists a much higher percentage of hydroxyl groups on the network structure for hydrogels studied here, compared to pendant carbonyl carboxyl groups and amine groups, we proposed the hydrogen bond interaction between the hydroxyl groups and the imido and carbonyl groups of diuron was a major cause of an excellent slow-release of diuron from the hydrogels. In addition, the hydrophobic interaction between phenyl groups in diuron and those in the hydrogels also contributed to this slow-release effect.

To understand the release mechanism of the entrapped diuron molecules from the diuron-loaded hydrogels, the release rates were analyzed by applying to the following empirical equation proposed by Ritger and Peppas (1987):

$$\frac{M_t}{M_0} = Kt^n \tag{3}$$

where M_t/M_0 is the percentage of diuron released at time t, and K is the kinetic constant. The exponent n is a parameter that is indicative of the transport mechanism. Only in two cases of $n = 0.5$ (pure diffusion controlled drug release) and $n = 1$ (swelling-controlled drug release or Case II transport), does this exponent give a clear indication of the mechanism. Other values for n indicate anomalous transport kinetics (Ritger & Peppas, 1987). Rate constants K, n and determination coefficients R^2 calculated according to Eq. (3) for the diuron-loaded hydrogels are gathered in Table 1. As it can be seen, the values of the determination coefficients are quite good, in all the cases $R^2 > 0.99$. The n values range from 0.4855 to 0.4954. Values close to 0.5 indicate that the release is diffusion controlled. From the constants in Table 1 the T_{50} value (the time taken for 50% of the diuron to be released) was calculated for the various formulations. The formulations can be ranked in order of increasing T_{50} values: G(d)-1 < G(d)-2 < G(d)-3. The formation of a porous structure in the hydrogels on swelling in water (see Fig. 4B–D) is expected to enhance the release. Water uptake and swelling of the matrices was shown to be fast compared to the rate of release of diuron from the hydrogels. This results in the release of diuron being controlled by diffusion through the matrix rather than by the swelling of matrix.

Table 1
Constants from fitting the empirical equation (3) to release date of diuron for diuron-loaded hydrogels in a buffer of pH 6.0.

Samples	K	n	R^2	T_{50} (days)
G(d)-1	0.2020	0.4909	0.9974	6.33
G(d)-2	0.1801.	0.4855	0.9996	8.19
G(d)-3	0.1513	0.4954	0.9994	11.16

4. Conclusions

In this work, the gelation of photocrosslinkable carboxymethyl chitosan (AZ-CMCS) was directly carried out by UV irradiation of its aqueous solution. It not only avoids using toxic cross-linkers, but also does not require any photoinitiators, toxic organic solvents. It was found that the photogelation reaction kinetics was rapid, and the photoirradiation intensity and the azidobenzaldehyde (Az) content incorporated in AZ-CMCS had obvious influences on the gelation efficiency. A relatively high irradiation intensity and Az content could obviously shorten the time required for complete gelation. The obtained hydrogels showed good pH sensitivity and the lysozyme degradability.

Diuron-loaded hydrogel membranes could be fast *in situ* formed by solar-simulated UV irradiation of a mixed aqueous solution of AZ-CMCS and diuron. The release of diuron from the various diuron-loaded hydrogel samples into a buffer of pH 6.0 has been shown to be diffusion controlled. The formation of a porous structure in the hydrogels on swelling in the buffer was assumed to be responsible for this diffusion behavior. AZ-CMCS may be useful in the controlled release of pesticide Amsden et al. (2007).

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