

Modification of Chitosan onto PE by Irradiation in Salt Solutions and Possible Use as Cu²⁺ Complex Film for Pest Snail Control

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ABSTRACT: Chitosan-grafted-polyethylene (CS-g-PE) film was prepared using simultaneous radiation-inducing grafting technique. The copper ion (Cu²⁺) adsorptivity on the CS-g-PE film was determined. The CS-hydroxybenzyltriazole (CS-HOBt), CS-N-hydroxysuccinimide (CS-NHS), CS-acetic acid (CS-HOAc), and CS-glutathione (CS-GSH) were used as CS-salts in aqueous solutions. Among these grafting solution systems, the CS-g-PE film prepared from CS-HOBt solution showed the greatest grafting amount and highest Cu²⁺ adsorptivity, up to 30.2% (1.51 ppm, 7.56 μg cm⁻²). The effects of the CS-HOBt concentration and mixing solution on the grafting amount were also observed to clarify their efficacies to assist radiation-induced grafting reaction. SEM/EDS mapping, ICP and XRF were used to clarify the Cu²⁺ adsorption capacity of the CS-g-PE film and the stability of Cu²⁺ on the CS-g-PE complex film. The Cu²⁺ showed its stability on the CS-g-PE film at room temperature in neutral and basic solutions. The Cu²⁺---CS-g-PE complex film reduced pest snail breeding as high as 54%. The success of this observation is a new approach to modify PE surface with a functional biopolymer, CS containing Cu²⁺ complex for controlling pest snail breeding. © 2014 Wiley Periodicals, Inc. *J. Appl. Polym. Sci.* **2014**, *131*, 41204.

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INTRODUCTION

Copper (Cu) and cadmium (Cd), etc., have been used as pesticides for controlling populations of pest slugs and snails in agricultural field for many decades.^{1,2} In general, the uses of metal-based pesticides generally in the form of chloride salts (i.e., CuCl₂, CdCl₂).^{3,4} However, by directly spraying those metals to the field or soil initiates the environmental contamination. Cu-contaminated environment in soil, sediment and overlying water, as a result of Cu exposures to the pest snails, is a major concern.^{5,6} To avoid dramatically environmental problem by direct applying, a production of the Cu-based pesticide film would serve as a practical way for controlling pest snail and reducing the environmental impact. For example, Savenkova et al.⁷ reported that reduction of chemical pesticides could be accomplished by immobilizing the pesticides into a biodegradable polymeric matrix. This way could possess not only reducing pesticide contamination in the environment but also prolonging pesticide action due to controlled release mechanism.

Chitosan is a linear polysaccharide consisted of randomly distributed β-(1→4) linkage polysaccharide of D-glucosamine. Chitosan is derived from chitin, a major component of the

crustacean shell and the second most abundant amino copoly-saccharide next to cellulose.⁸ After discovering of chitosan, it has been paid attention according to its specific properties, such as bioactivity and non-toxicity due to pyranose ring, biodegradability because of glycosidic linking, functionality owing to amino acid, and hydroxyl group and metal ion chelating ability as a result of strong electrostatic interaction at the amino group (-NH₂). The amine group is one of the most efficient functional groups for heavy metal ion adsorption.⁹⁻¹¹ With such abundant and inexpensive natural resources containing many reactive sites especially the repetitive -NH₂, chitosan serves as an excellent metal adsorbent material.

From the past until now, there are many reports about the capacity of chitosan and its derivatives to adsorb metal ions, such as, Ag²⁺, Ni²⁺, Cu²⁺, Zn²⁺, and Hg²⁺.¹²⁻¹⁵ Chitosan had been developed as adsorbent materials for removing toxic heavy metal from waste water¹⁶⁻¹⁸ and recovering useful metal such as uranium in sea water.¹⁹

Chitosan derivatives have also extensively been researched by chemical modification to improve its chelating potential. For

example, Ding et al.,²⁰ proposed novel derivative of chitosan alpha-ketoglutaric acid and hydroxamated chitosan alpha-ketoglutaric acid exhibiting coordination with Zn^{2+} . Chitosan modified with ethylenediamine and with 3-amino-1,2,4-triazole-5-thiol have been produced as the adsorbent resins for Hg^{2+} and UO_2^{2+} .²¹ Chitosan as the adsorbent materials has been fabricated in various forms, such as, hydrogel,²² bead,²³ fiber,²⁴ and membrane.²⁵ Rodrigo et al.,¹² developed the chitosan bead and membrane for Hg^{2+} adsorption.

Although chitosan exhibits inherent good chelation of metal ions, it may not be practical to use for some applications due to mechanical property as well as usable shape. Therefore, another form of chitosan-modified substrate has also been reported, for example, chitosan were coated on the surface of polyethylene terephthalate granules.²⁶ The cross-linked chitosan was coated on nonporous silica gel for Cu^{2+} coordinating.²⁷ With covalent grafting, chitosan was grafted onto polyethylene,^{28,29} polylactic acid,³⁰ poly(acrylonitrile),³¹ and poly(hydroxyethyl methacrylate)³² films by radiation and plasma polymerization techniques. The chitosan coated films proposed as biocompatible and metal adsorbent materials. Various adsorbents with the amine functional groups have been developed from chitosan that contains the amine groups in its backbone. Besides, immobilization of the synthetic polymers with the amine groups is also possible improve metal ion adsorption property to the proper substrate. By considering a simple way to improve radiation-grafting system of chitosan solution, a series of different chitosan-salts in aqueous solutions is considered.

Radiation grafting is one of the most widely interesting methods for surface modification with desirable functions onto the appropriated substrate. It provides some advantages, such as simple, environmentally friendly and effective methods. It has been widely chosen as a source of energy for surface modification between non-compatible matrixes. Mao et al.,²⁸ conjugated chitosan with a photoinitiator, i.e., 4-azidobenzoic acid for modifying polyethylene surface via UV treatment. Based on this viewpoint, modifying chitosan onto PE surface using γ -rays induced grafting was possible when an appropriate chitosan solution was chosen.²⁹ With grafting by γ -irradiation, it is important to note that chitosan has not radiation reactive group, i.e., vinyl monomer. This, therefore, makes it more difficult to radiation grafting of chitosan onto other polymer substrates. Modifying radiation-reactive molecule onto chitosan would be a possible way before pursuing radiation-induced grafting of chitosan onto PE film via radical reaction. With modification of chitosan, the step of preparation would be increased. Although the photoinitiator-modified chitosan has been proposed for preparing chitosan-grafted-PE using UV treatment, the different types of aqueous chitosan-salts have not yet been reported for gamma-ray induced grafting of chitosan onto PE film.

This article showed an attempt to find different chemical molecules for preparing chitosan-salt solutions to assist surface modification via radiation-induced grafting reaction. Therefore, we investigate the performance of various aqueous chitosan-salt solutions on grafting amount and Cu^{2+} adsorption capacity. The stability of Cu^{2+} on the chitosan-grafted-polyethylene film was studied and determined using nondestructive X-ray fluorescence (XRF) technique. The performance of grafted film con-

taining Cu^{2+} complex to inhibit pest snails breeding is also demonstrated.

EXPERIMENTAL

Materials

Chitosan (CS) with percent degree of deacetylation (%DD) of 95% ($M_v = 7 \times 10^5$ dalton), was purchased from Seafresh Chitosan (Lab) (Thailand). 1-Hydroxy-benzyltriazole (HOBt), *N*-hydroxysuccinimide (NHS) were purchased from Wako (Japan). Glutathione (GSH) was obtained from Thermo Fisher Scientific Inc. Copper (II) sulphate ($CuSO_4$) was bought from Tokyo Chemical Industry (Japan). Acetic acid (HOAc), nitric acid (HNO_3), isopropanol and pH buffer solutions were purchased from Lab Scan (Thailand). A commercially available HDPE film with a thickness of 9 μm , MFR of 0.044 g/10 min (190°C, 2.16 kg, ASTM D1238) and impact strength of 139 kJ m⁻² (ASTM D1709), was used as a substrate film. All chemicals were used as received. The golden apple snails (*Pomacea canalicularata*) sample were collected from a river in central Thailand.

Instruments and Equipments

The γ -ray induced grafting was carried out using a ⁶⁰Co source via a Gamma Cell 220 at a dose rate of 10 kGy h⁻¹, which was supported by the Office of Atoms for Peace, Ministry of Science and Technology, Thailand. Fourier transform infrared spectrophotometer (FTIR) was carried out using a Thermo Nicolet Nexus 670 with an attenuated total reflection (ATR) accessory. The scanning was 32 times at a resolution of 2 cm⁻¹ in a frequency range of 4000–400 cm⁻¹. Morphology of film surface was observed with a Quanta 450, FEI scanning electron microscope (SEM) and elemental mapping of microstructures was investigated using scanning electron microscopy-energy dispersive x-ray spectrometry (SEM-EDS). Elemental analysis (EA) was carried out using a Perkin Elmer 2400 CHN. Contact angles were measured using a contact angle instrument model OCA-15EC, Germany. Deionize water (5.0 μl) was dropped onto film surface with a dosing rate of 1.0 μl -sec⁻¹ and the contact angle was measured at 25°C. Contact angle of five different positions were measured and calculated by sessile drop method by using SCA 20 software. The amount of Cu^{2+} adsorbed on the grafted film was desorbed in the aqueous nitric acid solution and determined by an inductively coupled plasma-optical emission spectrometry (ICP-OES) using a Perkin Elmer, Model Optima 5300 DV. The Cu^{2+} stability on the grafted films was determined via a non-destructive X-ray fluorescence (XRF) technique. The film was placed in a acrylic holder and measured using a GUL0055P XRF equipped with a CdTe γ /X-rays semiconductor detector (XR-100T), radioisotope ring source of plutonium-238 (²³⁸Pu), high voltage supplier and amplifier built in Model PX2T-CdTe and multi-channel analyzer (MCA) card. Data were recorded and processed using the Genie™ 2000 software (CANBERRA) and WinQxas program provided by International Atomic Energy Agency (IAEA). Three types of standardize metal, i.e., lead (Pb), iron (Fe), and copper (Cu), were used for energy/channel calibration.

Preparation of CS-Salt Solution

Chitosan (1 g, 6 mmole) was dissolved in HOAc (0.3678 g, 1 mole equiv. to CS), NHS (0.7055 g, 1 mole equiv. to CS), GSH (1.8838 g, 1 mole equiv. to CS), and HOBt (0.8282 g, 1 mole equiv. to CS) in distilled water (100 mL) to obtain CS aqueous solution (1% v/v) of

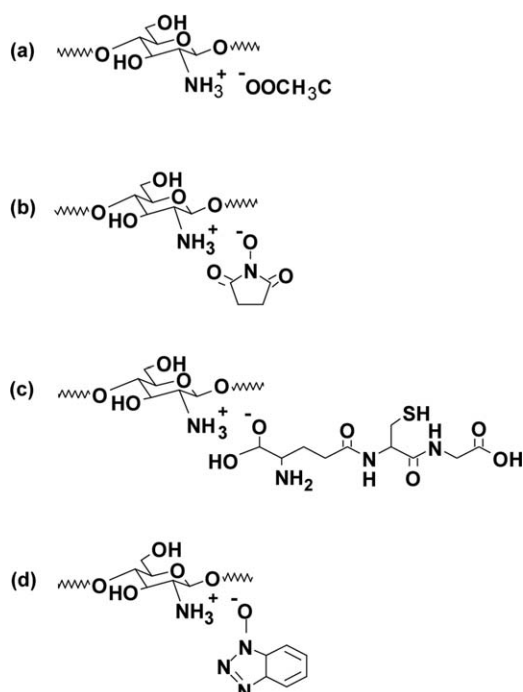


Figure 1. Chemical structures of CS-salts: (a) CS-HOAc, (b) CS-NHS, (c) CS-GSH, and (d) CS-HOBt.

CS-HOAc, CS-NHS, CS-GSH and CS-HOBt derivative salts, respectively (Figure 1). For CS-HOBt, CS with the concentrations of 0.1, 0.5, 1.0, and 2.0% (w/v) were prepared by dissolving CS (0.1, 0.5, 1.0, and 2.0 g) in HOBt (0.0828, 0.4144, 0.8282 and 1.6566 g, 1 mole equiv. to CS) in distilled water (100 mL). The mixture solution of CS-HOBt (0, 2, 5, 8, and 10 mL, 1% w/v) and CS-HOAc (1% w/v, 10, 8, 5, 2, and 0 mL) were prepared to obtain CS-HOBt in CS-HOAc solutions of 0, 20, 50, 80, and 100% (v/v). Similarly, the mixture solution of CS-NHS and CS-HOAc was carried out using CS-NHS (1% w/v, 0, 2, 5, 8, and 10 mL) and CS-HOAc (1% w/v, 10, 8, 5, 2, and 0 mL).

Preparation of Chitosan-Modified-PE Film via Radiation Induction

PE films were cut into 10×10 cm strips and surface washed thoroughly with isopropanol at 70°C for 12 h, sonicated for 6 h and dried at 50°C for 3 h. The cleaned PE films were immersed in an excess amount of the CS aqueous solutions, i.e., CS-HOAc, CS-NHS, CS-GSH, and CS-HOBt (10 mL), for 24 h in glass tubes. The samples were irradiated with a γ -ray the dose of 80 kGy in aerobic condition at room temperature according to the previous research.²⁹ After irradiation, the samples were left for 48 h before washing thoroughly with acetic acid (1%, v/v), NaOH (1%, v/v) and distilled water for five times, followed by drying at 50°C to obtain chitosan-grafted-polyethylene (CS-g-PE) films. The relative degree of grafting on PE film surface was calculated from the following equation:

$$\left[\frac{(W_G - W_0)}{W_0}\right] \times 100 \quad (1)$$

where W_G and W_0 are the weights of CS-g-PE and blank PE films, respectively.

ATR-FTIR for PE: 2915 cm^{-1} and 2845 cm^{-1} (C—H stretching), 1463 cm^{-1} (C—H bending); for CS: 1653 cm^{-1} (amide I),

1580 cm^{-1} (amide II), 3464 cm^{-1} (OH), and $1200\text{--}895 \text{ cm}^{-1}$ (pyranose ring); for CS-g-PE film: $1200\text{--}800 \text{ cm}^{-1}$ (pyranose ring), 1463 cm^{-1} (C—H bending), 1649 cm^{-1} (amide bond), 2915 cm^{-1} , and 2845 cm^{-1} (C—H stretching) and 3440 cm^{-1} (OH).

Cu²⁺ Adsorptivity on the CS-g-PE Film

The CS-g-PE films (10×10 cm) were immersed in CuSO_4 aqueous solution (5 ppm, 100 mL) for 48 h at room temperature. The membranes were taken from the copper ion (Cu^{2+}) solution. The amount of Cu^{2+} remaining in the solution was measured using ICP technique. The relative percent of Cu^{2+} adsorption capacity of CS-g-PE film was calculated from the following equation:

$$\left[\frac{(C_I - C_R)}{C_I}\right] \times 100 \quad (2)$$

where C_I and C_R are the initial and remaining concentrations of Cu^{2+} in the solution, respectively.

Stability of Cu²⁺---CS-g-PE Complex Film

The CS-g-PE films containing Cu^{2+} complex (Cu^{2+} ---CS-g-PE film) were cut into 7×7 cm strips. The initial amount of Cu^{2+} on the films was measured using nondestructive XRF technique to obtain C_I . The films were incubated in buffer solution with various pHs (pH 4, 7, and 10) and taken out from the solution every 2 weeks within a period of 24 weeks. The taken film complexes were dried before analysis. The amount of Cu^{2+} remained on the CS-g-PE film was then measured by the XRF to obtain C_R . The relative percent of Cu^{2+} remaining on CS-g-PE film was calculated from the following equation:

$$C_R/C_I \times 100 \quad (3)$$

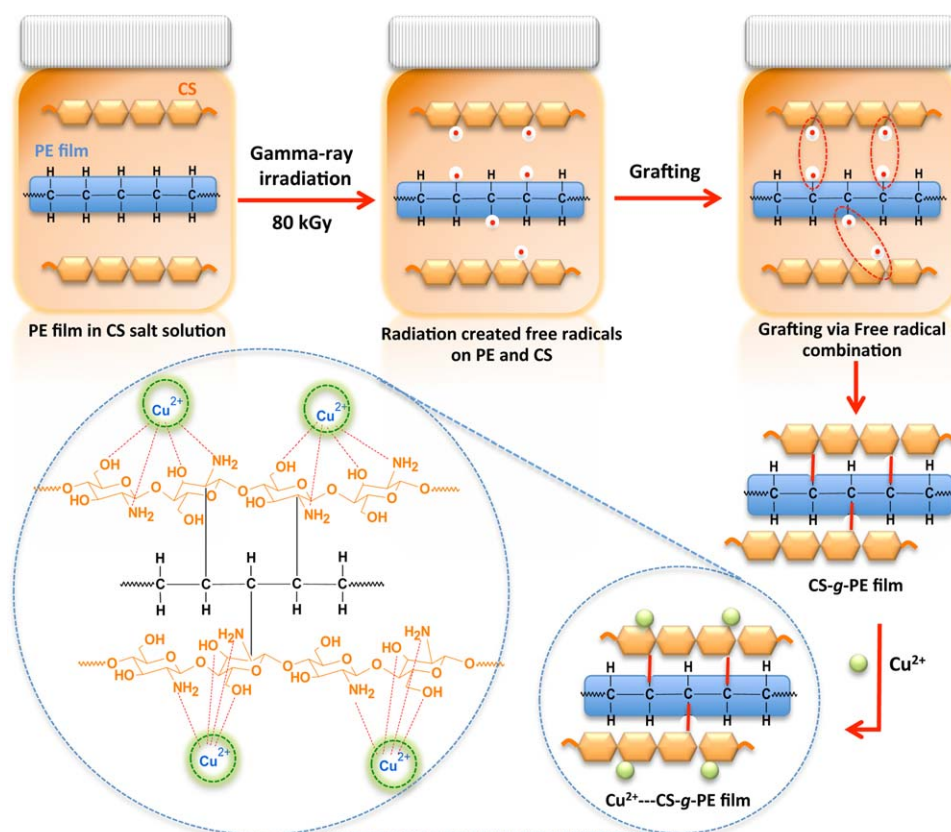
where C_I and C_R are the initial and remained count of Cu^{2+} on the CS-g-PE film, respectively. Similarly, the studies on stability of Cu^{2+} under different temperatures were carried out by incubating the films in distilled water under the temperatures of 25, 40, and 60°C .

Pest Snail Breeding Inhibition Behavior of Cu²⁺---CS-g-PE Complex Film

The golden apple snails (*Pomacea canaliculata*) were used as a model pest snail for studying apple snail breeding inhibition. The Cu^{2+} ---CS-g-PE film was prepared with 15×125 cm strip. It was covered inside the wall above the water level around the glass aquarium ($25 \times 35 \times 30$ cm) using adhesive tape. Water was filled and the five female and five male of the golden apple snails were put into that glass aquarium. Similarly, the glass aquariums without any film and with blank PE and CS-g-PE films were also carried out with the same procedure. The number of golden apple eggs laid above the water level was recorded in a period of 12 weeks.

RESULTS AND DISCUSSION

It is known that CS is well dissolved in HOAc aqueous solution, which is generally used to prepare CS solution for various reactions and applications. Therefore, the CS-HOAc solution was considered to be a simplest condition for radiation-induced grafting of CS onto PE film. Besides, NHS, an activating agent for carboxylic acid, was found to be able to prepare water-based CS solution.²⁹ Generally, NHS could assist esterification reaction



Scheme 1. Preparation of CS-g-PE film containing Cu²⁺ complex. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

between the hydroxyl group of CS and the carboxylic group of the conjugated molecule.³³ Since we also found out that CS exhibits good soluble in GSH aqueous solution, the CS-GSH salt solution was also prepared as the grafting solution in this study. In the past, the CS-HOBt has been proposed not only to assist the conjugation but also to serve as a good water-based CS system.³³ With such promising water soluble CS-salts, the present work is focused on preparation CS-g-PE film upon gamma-irradiation in the different CS-salts, i.e., CS-HOAc [Figure 1(a)], CS-NHS [Figure 1(b)], CS-GSH [Figure 1(c)], and CS-HOBt [Figure 1(d)]. Grafting reaction was carried out with the irradiation dose of 80 kGy in aerobic condition at room temperature because it is the most convenient procedure. After radiation-induced grafting process, the physisorbed CS-salt on PE film surface was removed by washing with good solvent (i.e., acetic acid) and neutralizing with NaOH and water before analyzing the grafted film.

When the aqueous solution of CS in the presence of PE film was subjected to ionizing radiation, hydrated electrons (e_{aq}^-), hydroxyl radicals ($HO\cdot$) and hydrogen radicals ($H\cdot$) were produced, along with other products, that is, hydrogen ions (H^+), hydrogen molecules (H_2), and hydrogen peroxide (H_2O_2), by a water radiolysis process. The $HO\cdot$ and $H\cdot$ species abstract hydrogen from the CS and PE backbone, rendering macroradicals of $CS\cdot$ and $PE\cdot$. Radical combination brought about grafting of CS onto the surface of PE film (Scheme 1).

The Cu²⁺ complex on CS-g-PE film is possibly formed via the effective amine groups. The nitrogen atom in the $-NH_2$ of CS acts as an electron donor and is presumably responsible for selective chelating with Cu²⁺. The metal ions (i.e., cupric ion, Cu²⁺) possibly coordinate with four $-NH_2$ ³⁴ as shown in Scheme 1. It was reported that the $-NH_2$ of CS was considered to be much more effective for binding metal ions than the acetyl group of chitin.³⁵

Figure 2 shows FTIR spectra of CS-g-PE films when the different CS-salt solutions were used in radiation-induced grafting system. The FTIR result for the grafted product was identified on the basis of spectrum analysis and comparison with relevant data reported in the literature.²⁹ It was clarified that CS-g-PE films obtained from CS-HOAc [Figure 2(c)] and CS-GSH [Figure 2(e)] show peaks similar to the blank PE film [Figure 2(a)]. The GSH molecule is an antioxidant compound; therefore, it acted as radiation protector by scavenging the free radicals produced upon irradiation rendering small grafting efficiency. For the CS-g-PE film prepared in CS-NHS [Figure 2(d)] and CS-HOBt [Figure 2(f)] solutions, the ATR-FTIR spectra show the peaks at 3440, 1649 cm^{-1} and 1200–800 cm^{-1} , indicating the $-OH$, amide bond, and pyranose ring of the CS characteristic function. This demonstrated that CS could graft onto PE film surface.

Surface Morphology and Cu²⁺ Mapping on CS-g-PE Film

The surface morphology of the CS-g-PE film was observed using SEM to identify how the PE surface changed when CS

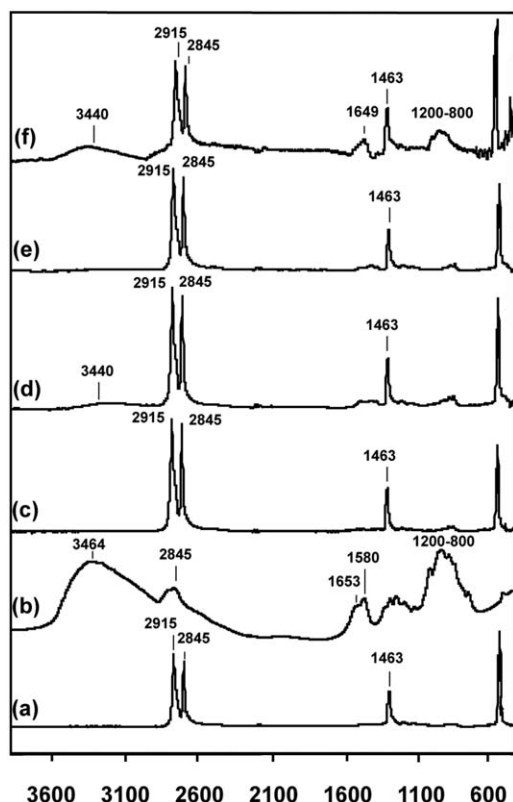


Figure 2. ATR-FTIR spectra of blank PE (a), CS (b), and CS-g-PE film obtained from CS-HOAc (c), CS-NSH (d), CS-GSH (e), and CS-HOBt (f) salt solutions.

was grafted onto PE. Both blank PE film [Figure 3(a)] and the irradiated PE film [Figure 3(b)] showed clear surface morphology. Compared with blank-PE surface, the CS-g-PE film exhibited significant rough surface due to the grafted CS [Figure 3(c)]. The different surface morphologies between PE film and the grafted film indicated that CS was successfully modified onto PE film via simultaneous radiation-induced grafting using gamma-rays.

To clarify the ability of the CS-g-PE film in chelating Cu^{2+} via CS function, elemental mapping of microstructures was

observed by SEM/EDS. The blank PE showed clear surface with no elemental spots indicating that Cu^{2+} could not be adsorbed on the non-functional PE surface [Figure 4A(a)]. By modifying CS onto the PE surface, Cu^{2+} complex was evidently seen on the CS-g-PE film surface [Figure 4A(b)]. The EDS mapping results demonstrated that Cu^{2+} homogeneously adsorbed on the surface of CS-g-PE via CS functional group. Figure 4B(b) showed the characteristic spectrum of Cu K α X-ray at 8.04 keV, which could not be observed in PE blank [Figure 4B(a)]. The results confirmed that Cu^{2+} could form complex on the surface of CS-g-PE film.

Surface Property by Water Contact Angle

To elucidate successful surface modification, the surface properties were investigated by measuring the water contact angles. For the blank PE film, the water droplet on the surface was mostly half-spherical shape with the contact angle of 95.72° [Figure 5(a)]. By modifying the PE film with CS, the water droplet as seen in Figure 5(b) became flatter and the contact angle of CS-g-PE film was observed to be 63.58° . In comparison with the blank PE film, a decrease in water contact angle of CS-g-PE film indicated the moiety of hydrophilic CS on PE film. The water contact angle of Cu^{2+} -CS-g-PE complex films was also determined and shown in Figure 5(c). Compared with the CS-g-PE film, the water contact angle of the Cu^{2+} -CS-g-PE complex films increased from 63.58° to 95.06° implying Cu^{2+} -coordinating surface. Generally, CS is hydrophilic polymer containing several -OH and -NH₂ groups. The hydrophilicity of CS decreased by covering with the metal ions complex. It is also suspected that addition of inorganic Cu^{2+} causes the surface roughness of the CS-g-PE film resulting in increase of the surface tension and the contact angle. The entropy of the solvent interface is controlled by surface tension. Therefore, the adsorption of Cu^{2+} on the CS-g-PE surface may increase the entropy due to the surface roughness.³⁶ The surface properties of the obtained film and water contact angle results verified the successful surface modification of PE with CS and Cu^{2+} .

Effect of CS-salt Form on Grafting Degree and Cu^{2+} Adsorptivity

To evaluate the relative amount of CS grafted onto PE film, a quantitative analysis based on the FTIR curve fitting method

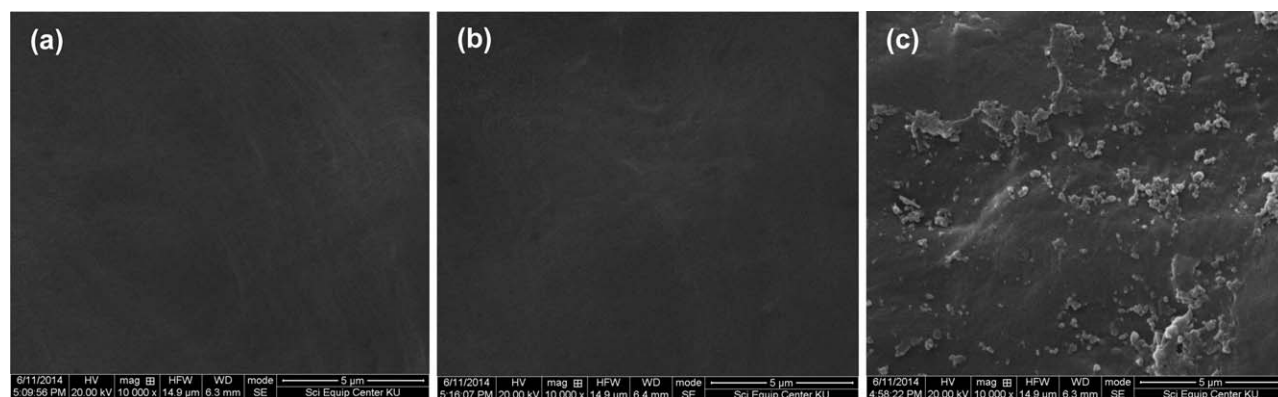


Figure 3. (A) SEM images of (a) blank PE, (b) 80 kGy irradiated PE, and (c) CS-g-PE films.

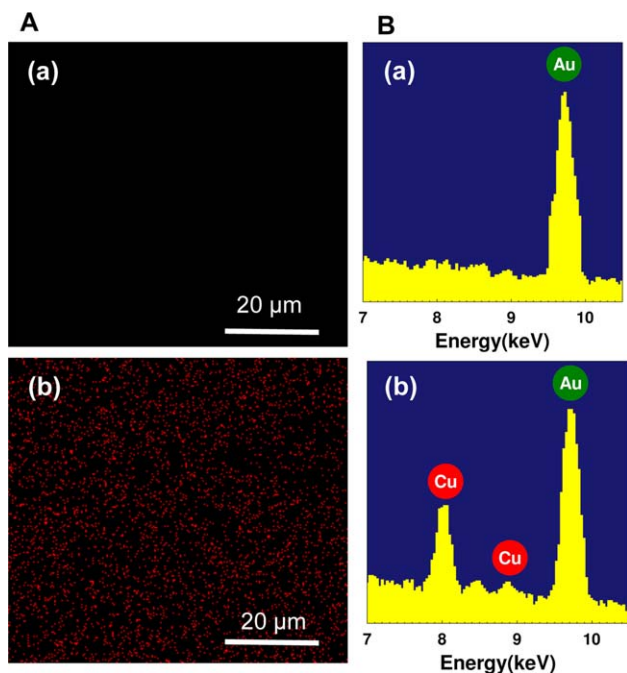


Figure 4. (A) EDS-SEM mapping micrographs and (B) EDS spectra of (a) blank PE (b) Cu^{2+} -CS-g-PE complex film. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

was carried out to determine an intensity ratio between I_{1649} (amide bond of CS) and I_{1463} (C—H bending of PE). The intensity ratios were 0.0375, 0.1504, 0.0619, and 0.5557 when the CS-HOAc, CS-NHS, CS-GSH, and CS-HOBT solutions were used as grafting solutions [Figure 6(A)]. The FTIR intensity

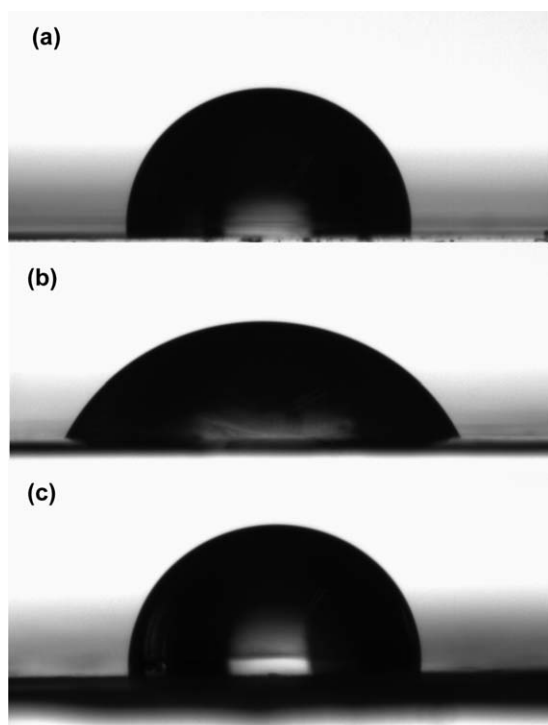


Figure 5. Digital images of water droplets of (a) blank PE, (b) CS-g-PE film and (c) Cu^{2+} -CS-g-PE complex film.

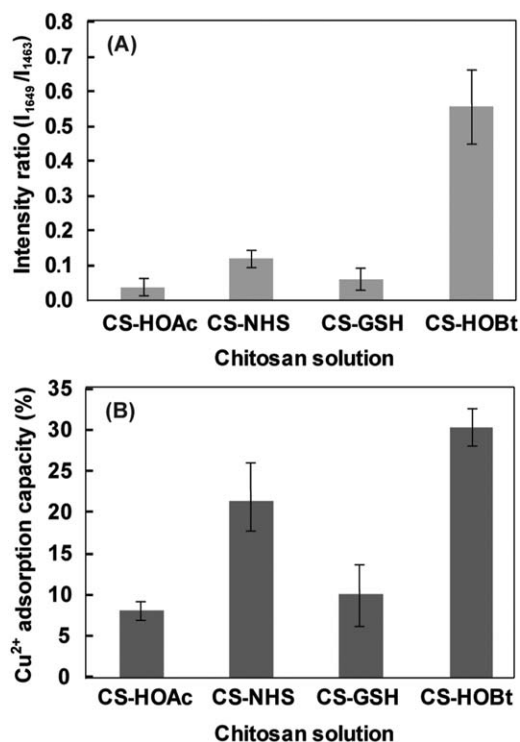


Figure 6. Type of CS-salt affects the amount of grafting and Cu^{2+} adsorptivity. (A) Relative intensity ratio (I_{1649}/I_{1463}) of the CS-g-PE films obtained from various CS-salts solutions (1% w/v) and (B) Cu^{2+} adsorptivities corresponding to (A).

ratio observed for the CS-g-PE film prepared from CS-NHS implied that CS-NHS might promote grafting; whereas, the CS-GSH and CS-HOAc solutions rarely assisted radiation-induced grafting reaction.

Grafting efficiencies of CS on PE film were also determined by weights and shown in Table I. It was found that the relative degrees of grafting were consistent with the FTIR intensity ratios. The CS-HOBT and CS-NHS solutions exhibited the grafting degree of $2.40 \pm 0.16\%$ and $1.64 \pm 0.33\%$, whereas the CS-HOAc and CS-GSH showed insignificant grafting degree of $0.26 \pm 0.09\%$ and $0.25 \pm 0.04\%$. The small amounts of the overall grafting degrees by weight were observed on the PE film because the grafting took place only on the surface of PE film.

The C, H, and N contents of the grafted films were also investigated to confirm the element of CS function on the PE film (Table I). Compared with the blank PE film, the CS-g-PE films displayed N content due to the $-\text{NH}_2$ of CS. It was found that CS-g-PE films prepared from CS-HOBT and CS-NHS show noticeable N content of 0.39 and 0.13% and C/N mass ratios of 0.0046 and 0.0015, respectively. The N/C mass ratios of CS-g-PE films are in agreement with the FTIR and degree of grafting by weight. The N/C mass ratio increased due to the amount of $-\text{NH}_2$ group of CS presented on the grafted film surface.

The degree of grafting determined from the FTIR intensity ratio and weight including the increase of the N/C mass ratio confirm that CS was modified on the PE film particularly when it was prepared in CS-HOBT. The results suggest that CS-HOBT

Table I. Grafting Degree and Elemental Analysis of the CS-g-PE Film from Different CS Salt Solutions

Samples	Grafting degree (%)	Elements				
		C	H	O	N	N/C
PE	0.00	85.78	14.22	0.00	0.00	0.0000
CS-g-PE (CS-HOAc)	0.26 ± 0.09	86.09	13.91	0.05	0.01	0.0001
CS-g-PE (CS-GSH)	0.25 ± 0.04	86.12	13.82	0.09	0.02	0.0002
CS-g-PE (CS-NHS)	1.64 ± 0.33	85.79	13.86	0.26	0.13	0.0015
CS-g-PE (CS-HOBt)	2.40 ± 0.16	84.61	13.63	1.38	0.39	0.0046

and CS-NHS solutions assist radiation-induced grafting and the chemical structures of CS-salts involved radiation-induced grafting system.

The GSH function is able to scavenge the free radicals presented in the reaction system as a result of the sulfhydryl (-SH) group³⁷; therefore, the free radicals generated during γ -ray irradiation were destroyed by hydrogen atom transfer mechanism. This brought about a low amount of grafting when the grafting reaction was carried out in the CS-GSH solution. By considering the color of grafting solution after irradiation (data not shown), the color of the irradiated CS-GSH solution remained original yellow implying the insignificant change of the CS chemical structure upon irradiation. The GSH can scavenge the

free radicals, therefore it could stabilize HO• and H• leading to low amount reactive species grafting remained in the reaction system. For the CS-HOAc, CS-NHS, CS-HOBt, the solution colors were changed from yellow to brown due to carbonyl group formation as a result of CS depolymerization.³⁸ From this experiment, it strongly validated the potential of benzotriazole group of HOBt and succinimide function of NHS, since it is known that HOBt and NHS are used to produce activated ester in the conjugation of carboxylic acid and amine group. Besides, the structure of HOBt is consistent with light absorbers assumed to be an important role in photografting via free radical reaction.³⁹ The function of HOBt and NHS presented in CS-HOBt and CS-NHS salts may also decrease the activation energy in the reaction system during gamma-ray irradiation. Therefore, the conjugating reaction through free radical system readily occurred.²⁹ It is important to note that imide and triazole compounds are known as photo acid generators,⁴⁰ which are found in chemically amplified photoresists because of their high photosensitivity.⁴¹ The γ -radiation or photon with the energy exceeding the ionizing potential (~ 5 eV)⁴² of the photoresist (i.e., NHS and HOBt) can eject electron. With the kinetic energy of the ejected electron, this is capable of additional exposure of the NHS and HOBt compounds. Besides, if the exposed energy is high enough to ionize the inner electron, the electron from the outer valence band consequently occupies the electron-hole leading to generate the additional x-ray photon. All absorbed energies can drive further reactions and ultimately dissipates as heat. It can be suggested that imide and triazole in NHS and HOBt efficiently assisted radiation-induced grafting of CS onto PE film.

To investigate Cu²⁺ adsorptivity, the blank PE and CS-g-PE films were immersed in the copper sulfate solution (5 ppm) for 48 h to obtain Cu²⁺---CS-g-PE complex films. The Cu²⁺ on the complex film was removed and measured using ICP. Figure 6(B) shows the Cu²⁺ adsorptivity of the CS-g-PE films prepared from different CS-salt solutions. For PE blank, the Cu²⁺ could not be observed on the PE film (data not shown). The CS-g-PE film prepared from CS-HOBt and CS-NHS solutions exhibited significant Cu²⁺ adsorptivity of 30.2% (1.51 ppm, 7.55 $\mu\text{g}\cdot\text{cm}^{-2}$) and 19.4% (0.97 ppm, 4.85 $\mu\text{g}\cdot\text{cm}^{-2}$), respectively. The Cu²⁺ adsorptivities of CS-g-PE films from CS-HOAc and CS-GSH were 8.1% (0.405 ppm, 2.02 $\mu\text{g}\cdot\text{cm}^{-2}$) and 10.1% (0.505 ppm, 2.52 $\mu\text{g}\cdot\text{cm}^{-2}$). The Cu²⁺ adsorptivity is consistent with the grafting amount of CS [Figure 6(A)]. The greater

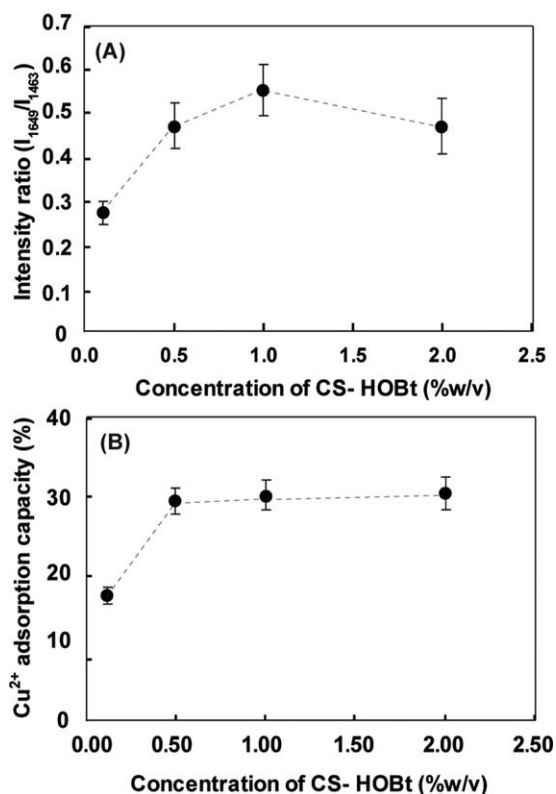


Figure 7. CS-HOBt concentration affects the amount of grafting and Cu²⁺ adsorptivity. (A) Relative intensity ratio (I_{1649}/I_{1469}) of the CS-g-PE films versus CS-HOBt concentration and (B) Cu²⁺ adsorptivities corresponding to (A).

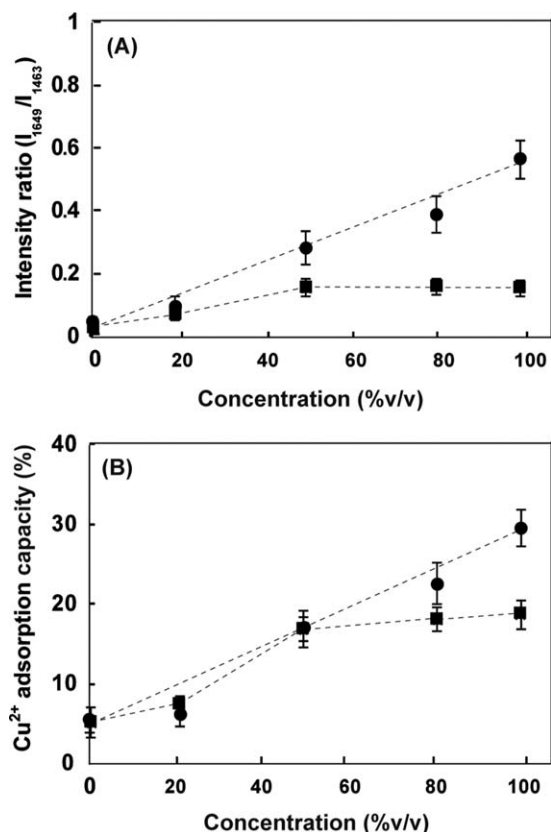


Figure 8. Concentration of the CS-HOBT (●) and CS-NHS (■) in the CS-HOAc solution affects the amount of grafting and Cu^{2+} adsorptivity. (A) Relative intensity ratio (I_{1649}/I_{1463}) of the CS-g-PE films versus concentration and (B) Cu^{2+} adsorptivities corresponding to (A).

amount of CS on PE film displayed the higher Cu^{2+} adsorptivity. This is due to nitrogen electron donor belonging to $-\text{NH}_2$ of CS to chelate ions.^{9,18}

Effect of CS Concentration on the Grafting Amount and Cu^{2+} Adsorptivity

As known from the previous results, the CS-HOBT was considered as the appropriated CS-salt solution for radiation-induced grafting. Here, the effect of CS-HOBT concentration on the grafting amount and Cu^{2+} adsorption capacity was elucidated. Figure 7(A) reveals that the relative amount of CS grafted on PE film increased with increasing the CS-HOBT concentration from 0.1 to 1.0% (w/v). When the given concentration is excessive over 1.0% (w/v), the grafting amount decreased. It is suspected that an excessive number of the free radicals on CS could not efficiently diffuse to combine with the free radical on the PE substrate.⁴³ This is attributed to the fact that at too high concentration the free radicals of CS hardly migrated to the reactive surface of PE because of viscous solution system. This obstructed diffusion of the CS free radicals approaching the surface of PE and creating the graft chain. Accordingly, this led to the decline of the intensity ratio when the concentration of CS-HOBT was as high as 2% (w/v). Likewise, the relative Cu^{2+} adsorption capacity [Figure 7(B)] is in accordance with the amount of CS grafted on the CS-g-PE films as seen in Figure 7(A). The Cu^{2+} adsorptivity was 29.2% and reaches the maxi-

imum value of 30.2 when the concentration of CS-HOBT approaches 0.5 and 1.0% (w/v), respectively. When the concentration was ranging from 0.5 to 2.0% (w/v), the Cu^{2+} adsorptivity is mostly identical [Figure 7(B)] because the amounts of CS grafted on the PE film were insignificantly different as seen in Figure 7(A).

Effect of Mixing Solution

It is important to confirm that the HOBT and NHS functions exhibited their ability to assist grafting reaction of the CS molecule onto PE film upon irradiation, as they show a noticeable amount of grafting and Cu^{2+} adsorptivity. Here, we observed the possibility to reduce the amount of HOBT or NHS in the grafting systems. The mixing solution system of CS-HOBT or CS-NHS with the CS-HOAc was prepared as grafting solutions. Different contents of CS-HOBT and CS-NHS solution (% v/v) were varied. Figure 7(A) shows that the intensity ratio of CS-HOBT/CS-HOAc increased, up to 0.5557 ± 0.0598 when the CS-HOBT content reached 100% (v/v). In the case of the CS-NHS/CS-HOAc mixed solution, the intensity ratio increased to a certain amount of 0.1513 ± 0.0279 when the CS-NHS content approached 50% (v/v). By increasing the CS-NHS contents up to 80 and 100% (v/v), the amount of CS grafted onto PE did not enhance. In comparison, the CS-HOBT shows mostly four times more effective grafting solution than that of CS-NHS. The results confirm that HOBT and NHS functions are able to assist radiation-induced grafting reaction. In addition, our observation also suggests that using the CS-NHS only 50% (v/v) in CS-HOAc solution bring about comparable grafting degree with absolute CS-NHS solution. The experiment regarded the use of mixing solution also confirms the capacity of HOBT and NHS in radiation-induced grafting system.

Similarly, the percent of Cu^{2+} adsorptivity as shown in Figure 8(B) corresponded to the amount of grafting [Figure 8(A)]. For the CS-g-PE film prepared from CS-HOBT/CS-HOAc mixed solution, the percent of Cu^{2+} adsorptivity increased from 5.8 to 30.2% (0.29–1.51 ppm) when the amount of grafting increased. In the case of CS-g-PE film obtained from CS-NHS/CS-HOAc,

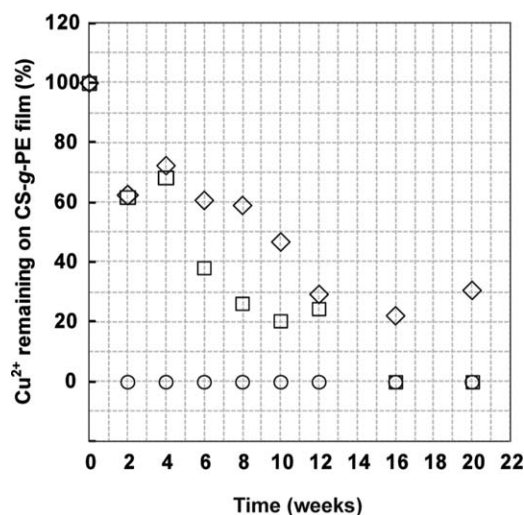


Figure 9. Stability of Cu^{2+} on the CS-g-PE film incubated in buffer solution with pH 4 (○), pH 7 (□), and pH 10 (◇).

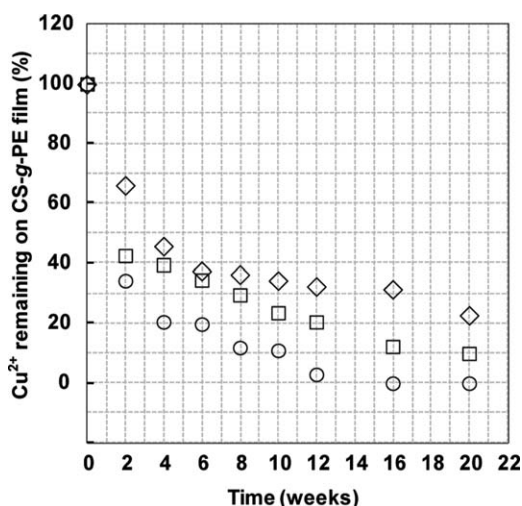


Figure 10. Stability of Cu^{2+} on the CS-g-PE film incubated in distilled water at the temperatures of 25°C (○), 40°C (□), and 60°C (◇).

the maximum percent of Cu^{2+} was observed around 19.4% (0.97 ppm) when the content of CS-NHS reached 100%. However, the Cu^{2+} adsorptivity was mostly similar in the range of 17.2–19.4% within the concentration range of 50–100%.

Stability of Cu^{2+} ...CS-g-PE Complex Film: Effects of pHs and Time

Although CS exhibited good metal ion adsorption capacity, it is important to clarify the stability of the Cu^{2+} adsorbed on the CS-g-PE film. In addition, good stability of the Cu^{2+} ...CS-g-PE complex film is an important property regarding potentially practical use including a cased model material for pest snail breeding inhibition. Therefore, the stability of Cu^{2+} on the CS-g-PE film surface was identified under incubation in different pHs at room temperature within a period of 20 weeks. The percent of Cu^{2+} remaining on the CS-g-PE film was determined every 2 weeks using non-destructive XRF technique. Figure 9 shows the percent of Cu^{2+} remaining on the films under basic (pH 10), neutral (pH 7) and acidic (pH 4) conditions. It was found that the Cu^{2+} continuously reduced with time. Under acidic environment (pH 4), the Cu^{2+} completely released after incubated over 2 weeks. In comparison with pH 4, the Cu^{2+} on the film remained higher than 50% after incubated at pH 7 and 10. By reaching 4 weeks, it was found that around 70% Cu^{2+} still maintained on the films when the Cu^{2+} ...CS-g-PE complex films were incubated in pH 7 and pH 10. Li and Bai,⁴⁴ explained that Cu^{2+} would be repulsive

Table II. Pest Apple Snail Breeding Inhibition of Cu^{2+} -CS-g-PE Complex Film

Samples	Laid eggs (%)
Without film (Control) ^a	0
Blank PE film	+58
CS-g-PE film ^b	+33
Cu^{2+} ...CS-g-PE film ^c	-54

^a Aquarium glass wall.

^b Prepared from the condition: 1% (w/v) CS-HOBT (1 : 1), 80 kGy.

^c Prepared from the condition b and containing 30% Cu^{2+} complex.

at $\text{pH} < 6.7$ but becomes attractive at $\text{pH} > 6.7$. At low pH values in acidic environment, a relatively high concentration of protons (H^+) would strongly compete with the Cu^{2+} for the amine group ($-\text{NH}_2$), and thus the stability of Cu^{2+} on the film was significantly decreased due to the Cu^{2+} desorption.⁴⁵ It can be described that the reactions taking place in the acidic desorption can be protonation of $-\text{NH}_2$ by H^+ to form $-\text{NH}_3^+$. Generally heavy ions including Cu^{2+} forms complex with CS at the NH_2 groups^{46,47}; therefore, at lower pH value, the Cu^{2+} desorbed from CS-g-PE surface due to protonation. At the higher solution pH, the electrostatic repulsion decreased. This possibly confirms that the higher amount of Cu^{2+} remained on the CS-g-PE films in the solution pH of 7 and 10. It can be summarized that Cu^{2+} adsorbed on CS-g-PE film is unstable under acidic pH and it is more stable under neutral and particular basic environment.

Stability of Cu^{2+} ...CS-g-PE Complex Film: Effects of Temperatures and Time

Similarly, thermal stability was investigated by incubating the Cu^{2+} ...CS-g-PE films at the temperatures of 25, 40, and 60°C in distilled water within a period of 20 weeks. Figure 10 demonstrated that storage temperature and time also influence the persistence of Cu^{2+} on the CS-g-PE film. The Cu^{2+} gradually reduced as the temperature increased, up to 60°C and the storage time extended, up to 20 weeks. The amount of Cu^{2+} remained almost 70% at room temperature, whereas, they were around 43% and 34% by increasing temperatures to 40°C and 60°C, respectively, within a period of 2 weeks. One can be seen that the Cu^{2+} still remained at a mostly constant amount of 30% in water over the period ranging from 1 to 4 months. A decrease in temperature results in greater stability of the Cu^{2+} on the CS-g-PE film. Likewise, Billir et al.⁴⁸ reported that with increased temperature, the adsorption of Cu^{2+} decreased. The results suggest that the Cu^{2+} ...CS-g-PE film is more stable at 25°C than 40 and 60°C.

Pest Snail Breeding Inhibition of Cu^{2+} ...CS-g-PE Complex Film

It has been reported that Cu-based substance is able to use as pesticide to inhibit pest snail in agricultural application.⁴⁹ To preliminarily investigate the efficiency of the Cu^{2+} ...CS-g-PE complex film to inhibit the golden apple snail breeding, the number of eggs laid on the glass aquarium wall (without film) and covered with blank PE, CS-g-PE, and Cu^{2+} ...CS-g-PE complex film was collected (Table II). All films were placed on the glass aquarium wall above the water level, since it is known from the literature that golden apple snails generally lay their eggs above the water level.⁵⁰ In comparison with the control condition, it was found that 54% reduction of breeding was observed. The results demonstrated that the Cu^{2+} ...CS-g-PE complex film exhibited effective apple snail breeding inhibition. This is due to the efficiency of the Cu^{2+} on the CS-g-PE complex film. It is suspected that the apple snails may be irritated with the Cu^{2+} adsorbed on CS-g-PE film surface; therefore, they avoided climbing up to the wall above water level. In the case of blank PE and CS-g-PE films, it was found an increase of egg breeding, up to 58% and 33%. The results confirm the efficiency of Cu^{2+} ...CS-g-PE film to inhibit apple snail breeding and it is possible to apply as a model pest snail control material.

CONCLUSIONS

In conclusion, we have originally demonstrated that CS-salt form in aqueous solution pay an important parameter for radiation-induced grafting of CS onto PE film. The CS-HOBt and CS-NHS are innovatively used as aqueous CS solution for modification of CS onto PE film surface. The photosensitive characteristic of triazole in HOBt and imide in NHS assists grafting via γ -irradiation. The CS modified PE film provides reactive surface for metal ion coordination complex. The CS-g-PE film showed effective and uniform Cu^{2+} adsorption on the surface of $7.55 \mu\text{g}\cdot\text{cm}^{-2}$. Furthermore, we envision from the investigation that our discovery will ultimately be applicable to use as a model material for pest snail control. Besides, this model material would be able to apply in other applications, such as biocompatible surface material.

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