Synthesis and Property Evaluations of Photocrosslinkable Chitosan Derivative and its Photocopolymerization with Poly(ethylene glycol)

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ABSTRACT: Photocrosslinkable polymers are clean and convenient materials for the biomedical uses. Chitosan, which owns excellent biocompatible and antimicrobial properties, is one of the choices while it is introduced with photosensitive functional groups. In this study, chitosan was *N*-phthaloylated to react with acryloyl chloride in the organic solvent homogeneously and the result has been verified by the solubility test. Fourier transformed infrared spectrometry and nuclear magnetic resonance spectrometer analysis indicated that the modification with the attachment of the photosensitive functional group, the acryloyl group, onto chitosan and poly(ethylene glycol) (PEG) was feasible. The differential scanning calorimetry analysis further indicated that the melting points of the *N*-phthaloylated chitosan were decreased as compared with the untreated chitosan control. Then photocrosslinkable chitosan derivative (CH-PAA) was photocopolymerized with PEG diacrylate (PEGA) under UV irradiation. The adhesion strength characterization and swelling capacity evaluation for this photocopolymer have shown obvious raises of the adhesiveness and water-adsorption abilities as compared with the photopolymer of CH-PAA. © 2006 Wiley Periodicals, Inc. J Appl Polym Sci 100: 1794–1801, 2006

Key words: chitosan; poly(ethylene glycol); photocopolymerization; crosslinking; water affinity

INTRODUCTION

In decades, photocrosslinkable polymers were of the opinion that could provide a clean and convenient biomedical-related application. Photopolymerization is a process that uses light to convert a liquid macromer/prepolymer to a solid gel. With the control of photoinitiating conditions, this process can be carried out under mild and cytocompatible conditions.[1] Nevertheless, several synthetic polymers, due to unreacted toxic monomers remained during polymerization procedures, might contain carcinogenic or toxic substances. Hence, the use of natural biodegradable materials has received lots of attention recently.[2–5]

Chitosan, a linear nature polyaminosaccharide, is obtained by *N*-deacetylation of chitin, which is the main skeletal structure component in crustaceans. Chitosan exhibits good biodegradable, biocompatible, and antimicrobial abilities.[6–8] Many reports have discussed the safety of its photoreactive derivatives in medical uses such as wound dressing, biological adhesive, and drug delivery of photocrosslinkable chitosan.[9–11] Ono et al. introduced lactose moieties and photoreactive azide groups into chitosan. This photocrosslinkable chitosan may be used for biological adhesive[9] and dressing for wound-contraction acceleration.[10,12] Moreover, Jameela et al. evaluated the possibility of photocrosslinkable chitosan attached with aliphatic acid as a drug delivery matrix.[11] However, the utilization of chitosan is limited by its insolubility in general organic solvents except for some acids. Since solubility of chitosan derivatives in organic solvents is essential for many homogeneous reaction conditions, *N*-phthaloylation of chitosan with phthalic anhydride is an effective method to improve their organic solubility.[7,8,13]

Furthermore, chitosan and its derivatives have low water solubility attributed to their rigid structure and crystallinity. To enhance the solubility in water, chitosan is modified by chemical reaction or blended/copolymerized with hydrophilic polymers such as poly(vinyl alcohol) (PVA) and poly(ethylene glycol) (PEG).[14] Although several polymers are evaluated for chitosan copolymerization, PEG is of the most interest, owing to its hydrophilicity, biocompatibility, and biodegradability.[14–16] In addition, PEG could minimize the interaction between the components because it is an uncharged polymer.[17] Therefore, PEG diacrylate (PEGA), a photocrosslinkable PEG derivative (Scheme 1), was widely used in photochemical modification for the use of hydrogel scaffold, membrane material, and drug delivery.[15,18–21]

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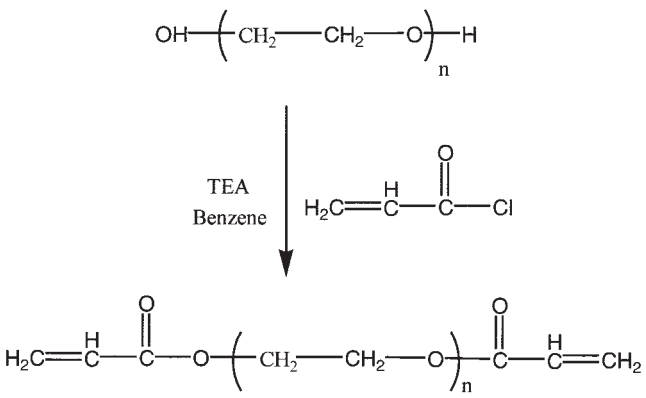


Scheme 1. Synthesis of PEGA.

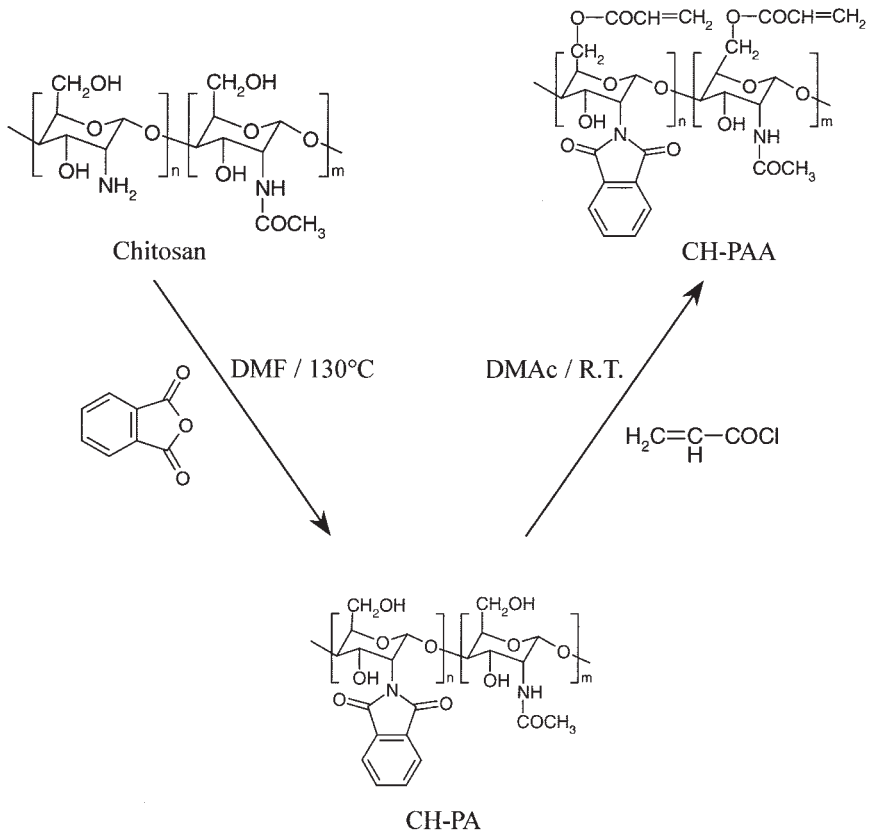
In this study, chitosan was *N*-phthaloylated to react with acryloyl chloride in the organic solvent homogeneously to obtain the photocrosslinkable chitosan (CH-PAA) (Scheme 2). Then CH-PAA was photocopolymerized with PEGA to improve the water affinity of chitosan. The chemical characteristics of products were analyzed with fourier transformed infrared spectrometer (FTIR) and nuclear magnetic resonance spectrometer (NMR). Additionally, the adhesion strength, thermal properties, and swelling ratios of samples were also measured.

EXPERIMENTAL

Preparation of CH-PAA

Chitosan (5 g) (viscosity-average molecular weight ~8500, deacetylation degree ~82%; Koyo Chemical, Tokyo, Japan) was mixed with 100 mL *N*,*N*-dimethyl formamide (DMF; TEDIA, Fairfield, OH) in a 250-mL reactor under nitrogen condition at 80°C for 1 h. Then 13.8 g phthalic anhydride (J. T. Baker, Phillipsburg, NJ) was added into the reactor for further reaction at 130°C. After 8-h reaction, the solution was poured into the iced water to precipitate out the product, *N*-phathaloylated chitosan (CH-PA). Unreacted reactants were removed by ether followed by alcohol for 24 h in Soxhlet extraction apparatus, respectively. Then CH-PA was dried in vacuum at 70°C for 24 h.

Appropriate amount of triethyl amine (TEA, HPLC grade; Ridel-de-Haën, Seelze, Germany) based on the stoichiometry of HCl generated was added into CH-PA/DMAc (*N*,*N*-dimethyl acetamide; TEDIA, Fairfield, OH) solution at 0°C under nitrogen environment. Then fourfolds of acryloyl chloride in accordance with the hydroxyl groups on the CH-PA molecule was added dropwise and the solution was kept at room temperature for 24 h. Finally, the solution was slowly poured into acetone, and the precipitate, CH-PAA, was purified with dialytic membrane



Scheme 2. Synthesis of chitosan derivatives.

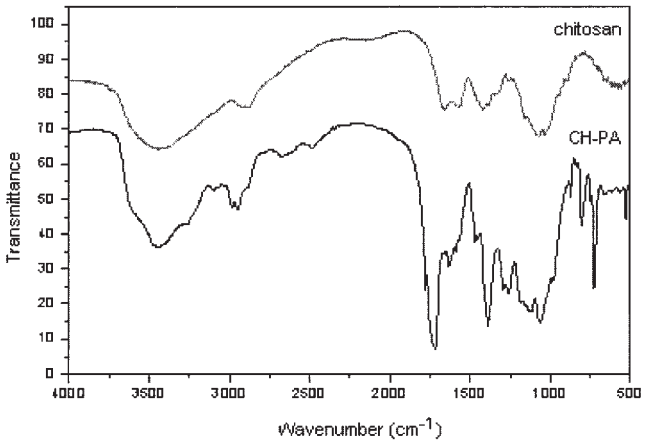


Figure 1 FTIR spectra of chitosan and CH-PA.

(MWCO = 3500; Spectrum®, Rancho Dominguez, CA) in deionized water and dried at room temperature.

Photopolymerization of CH-PAA

CH-PAA solution [50 wt % in *N*-methyl pyrrole (NMP, HPLC grade; Ridel-de-Haën, Seelze, Germany)] was mixed with 3 wt % of photoinitiator, 2,2-dimethoxy-2-phenyl acetophenone (Reagent grade; Aldrich, St. Louis, MO). This mixture was poured into a petri dish and exposed to the full-wavelength UV radiation for 1 h at a fixed distance of 15 cm.

Preparation of PEGA

The PEG (weight-average molecular weight ~1500; Ridel-de-Haën, Seelze, Germany) was first dried by azeotropic distillation with benzene under nitrogen atmosphere. According to the amount of HCl generated, appropriate amount of TEA was then added slowly to the PEG reactant. Then fourfolds of acryloyl chloride (based on the stoichiometric amount of hydroxyl groups of PEG) were added dropwise in nitrogen condition with the reactor cooled by the iced bath. After 24 h, 500 mL of cold ether was added and the precipitate was separated by filtration. The filtrate was cooled to 0°C and the precipitate was collected. This precipitate was dissolved in benzene followed by precipitating in cold ether. Finally, the precipitate, PEGA, was filtered out, dried at room temperature, and stored in dark and dry environment until used.

Photocopolymerization of CH-PEG

In a similar photocuring procedure as described earlier, CH-PAA blended with PEGA at different weight ratios was exposed to the full-wavelength UV radiation for 1 h at a fixed distance of 15 cm. The product was labeled as CH-PEG in the following discussion.

TABLE I
Chemical Characterization of ^{13}C CP-MAS Solid-State NMR Spectrum of Chitosan

Carbon number[a]	Chemical shift (ppm)
1	105 for glucosamine unit; 99 for acetylglucosamine unit
2	58
4	83
3, 5, 6	60–78
7	174
8	24

[a] Carbon number is shown in Figure 2.

Sample analysis

The sample chemical characteristics were analyzed by FTIR and NMR. The FTIR spectra were collected with BIO-RAD FTS-40 (Bio-Rad, Cambridge, MA). The ^1H NMR analysis was carried out by using the BRUKER AMX400 solution-NMR spectrometer (Bruker, Rheinstetten, Germany), with the samples dissolved in D_2O solution containing 2% DCl at 80°C. The solid-state NMR analysis was examined by the Bruker Avance 400 solid state NMR spectrometer (Bruker, Rheinstetten, Germany) at an operation frequency of 100.63 MHz at 300 K. The solid-state NMR technique was used to overcome the sample solubility problems. In addition, it can also provide information about the original molecule behavior as well as chemical struc-

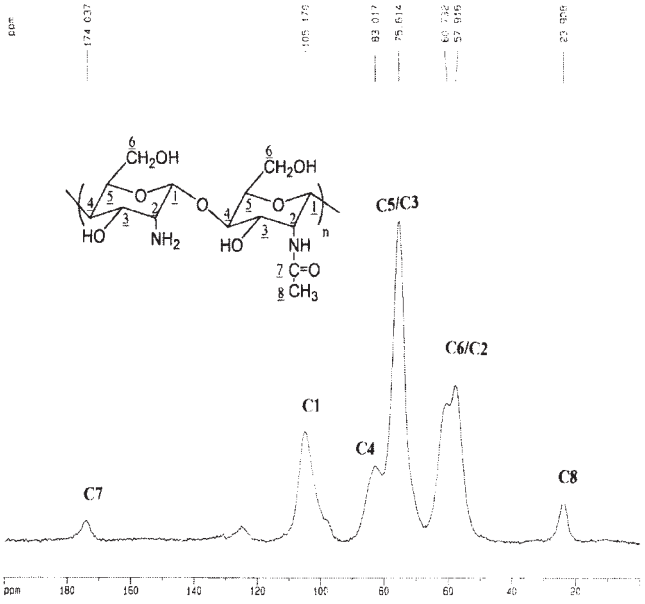


Figure 2 ^{13}C CP-MAS solid-state NMR spectrum of chitosan.

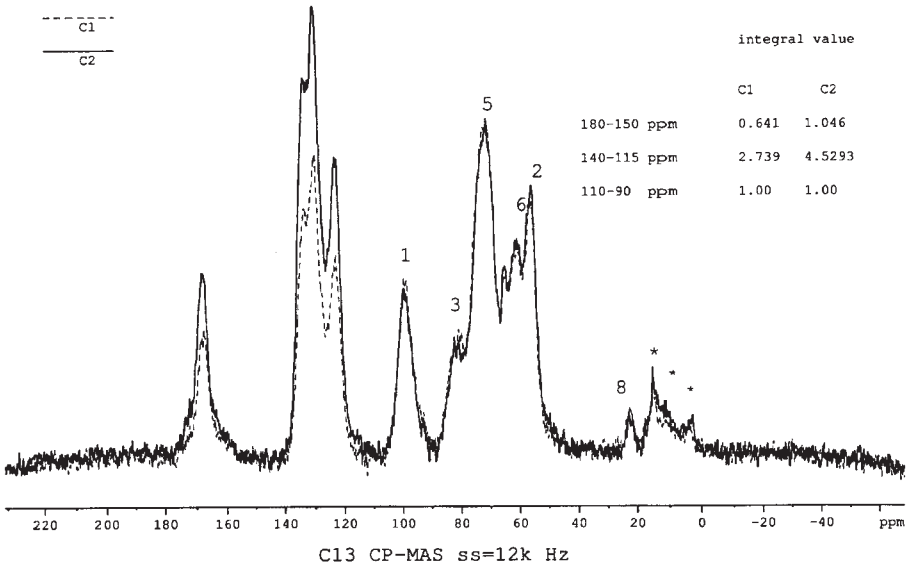


Figure 3 ^{13}C CP-MAS solid-state NMR spectra of (C1)CH-PA and (C2)CH-PAA.

ture without disturbing the interactions caused by the deuterium solvents as in ^{1}H NMR analysis.

The thermal property of tested specimens was examined by differential scanning calorimetry (DSC; Perkin–Elmer DSC 7; Perkin–Elmer, Norwalk, CT) in the temperature range from 50 to 400°C with the heating rate of 20°C/min.

To measure the adhesion strength of samples, 50 wt % CH-PAA, 50 wt % PEGA, or 25 wt % PAA mixed with 25 wt % PEGA solution was evenly spread between two glass cover slides. Then, one glass cover slide was fixed while the other one was pulled with different weight. With the weight increasing, the weight (i.e., the adhesion strength) was recorded when the cover slide was begun sliding after the UV radiation for various duration. As the adhesion strength is no longer increased with the curing time, the sample was regarded as fully crosslinked.

The swelling properties of samples were studied by immersing the samples in deionized water at room temperature at different periods of time. The swelling ratio of these samples was calculated by using the equation as follows:

$$\text{Swelling ratio}(\%) = (W_s - W_d)/W_d \times 100$$

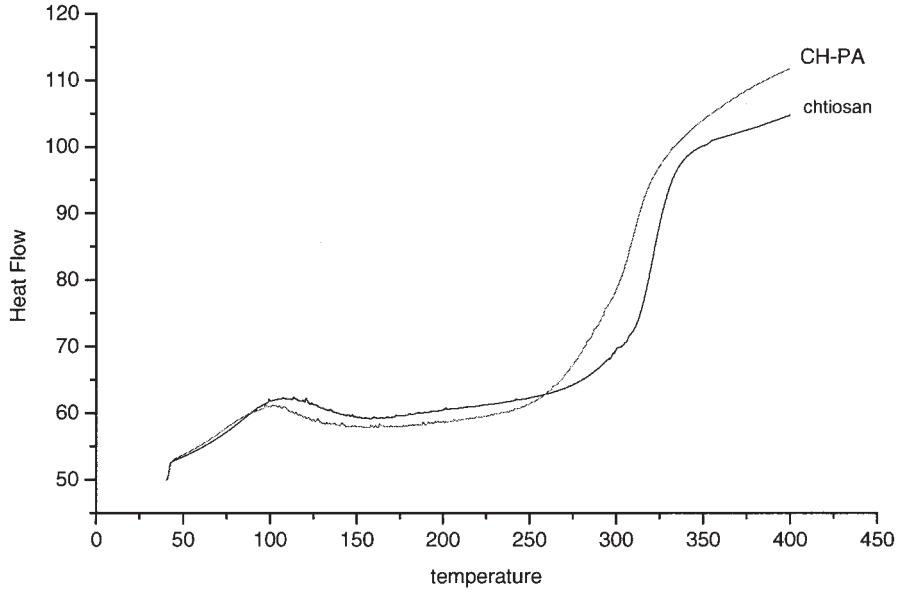


Figure 4 DSC analysis of chitosan and CH-PA.

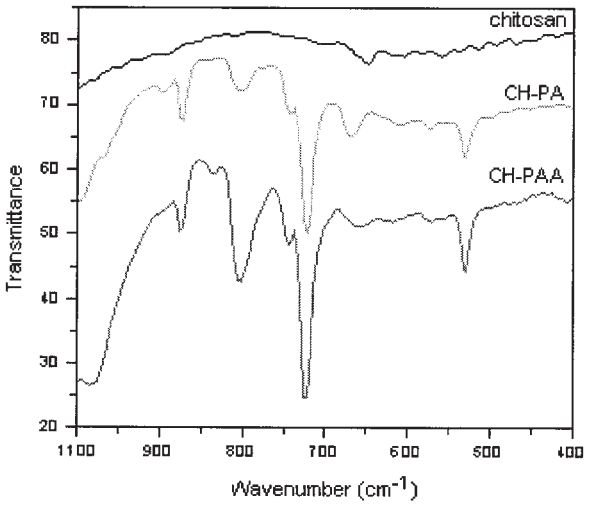


Figure 5 FTIR spectra of chitosan and its derivatives.

where W_s and W_d is the weight of the swollen and dry samples, respectively.

RESULTS AND DISCUSSION

Analyses of CH-PA and CH-PAA

FTIR spectra of CH-PA (Fig. 1) showed strong peaks (1770 and 1710 cm^{-1}) revealing phthalimido functional groups that were not observed in chitosan spectrum.[8] This finding indicated that the phthalimido group was attached to the chitosan chain. Solid-state NMR can provide more detailed information about molecule structure than FTIR. However, overlapping of the spinning side bands and the complex chitosan proton signals made it difficult to assign chitosan structure. To solve these problems, each NMR ^{13}C signal of chitosan could be recognized with the application of cross polarization-simultaneous phase inversion (CP-SPI)[13,22] and Table I listed the ^{13}C chemical shifts of chitosan. As a result, it was noted that the C^{13} chemical shift of CH-PA (Fig. 2) at 115–180 ppm, which represented the aromatic rings and carbon–carbon double bonds, appeared after the attachment of phthalimido group [as compared with chitosan (Fig. 3)].[23]

The crystallization characteristic of chitosan and its derivatives were examined by DSC. It indicated that original chitosan bears a transition state, which may be the melting point, at 309°C while this state shifts downwards for CH-PA (Fig. 4) to 289°C. This implicated that the incorporated phthalimido group could change the crystal arrangement and density of molecules within the chitosan structure.

Next, from FTIR spectra of CH-PAA (Fig. 5), characteristic peaks of alkene C—H (723 and 802 cm^{-1}) were noted and this indicated the successful attachment of the photosensitive acryloyl functionality into the CH-PA structure.[24] Furthermore, as compared with the solid-state NMR spectrum of CH-PA (Fig. 2), the increasing peak area of CH-PAA in chemical shift at 115–180 ppm was considered as resulted from the contribution of acryloyl groups. Therefore, the degree of substitution (DS) of acryloyl group could be calculated as follows:

$$\mathrm{DS}(\%) = \frac{(1.046 - 0.641) + (4.5293 - 2.739)}{(0.641 + 2.739)} = 64.9\%$$

The solubility of these two chitosan derivatives, CH-PA and CH-PAA, was tested with various organic solvents as shown in Table II. This indicated that the phthalimido group could improve the solubility in organic solvents, such as DMF, DMAc, *N*,*N*-dimethyl sulfone (DMSO), and pyridine, possibly through the modification of the chitosan crystal structure. In contrast, the introduction of photosensitive groups (acryloyl in CH-PAA) reduced the solubility of chitosan derivatives in organic solvents (compared with CH-PA). It might be attributed to the formation of new interchain or intrachain hydrogen bondings within the chitosan structure.

Analysis of PEGA

Characteristic peaks (1730 and 1633 cm^{-1}) of —O—C═O group were found in FTIR spectrum of PEGA (Fig. 6) in comparison to PEG, and this finding supported the attachment of acryloyl groups.[15,20] Additionally, the chemical shifts at 3.5–3.8 ppm related to the PEG backbone and those at 5.8–6.4 ppm represented that the acryloyl groups were noted in ^1H NMR spectrum of PEGA (Fig. 7). The degree of acrylation

TABLE II
Solubility Test of Chitosan and its Derivatives

Material	DMSO	DMAc	DMF	Pyridine	NMP	Methanol
Chitosan	−	−	−	−	−	−
CH-PA	+	+	+	+	+	−
CH-PAA	−	−	−	−	+	±

+, soluble; ±, partially soluble; −, insoluble.

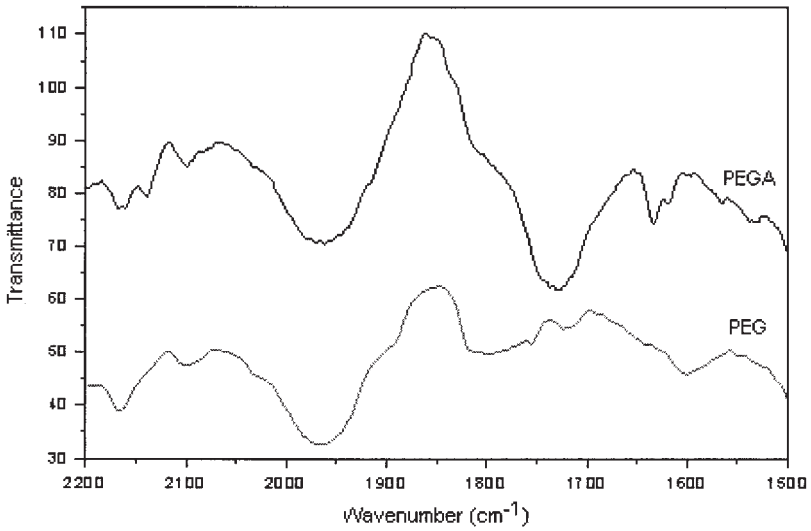


Figure 6 FTIR spectra of PEG and PEGA.

was calculated to be 83.5% according to the equation suggested by previous studies.[19,25]

Adhesion strength for photopolymerized samples

As shown in Figure 8, the testing indicated that the adhesion strength for photopolymerized CH-PAA reached a plateau value after 40 min of UV exposure, while it took 80 min for the PEGA. Less-extended UV exposure time was needed for the UV-crosslinked CH-PEG. In addition, the adhesion strength increased for the UV-crosslinked CH-PEG as compared with that for the photopolymerized single component counterpart. This implicated that the addition of PEGA into CH-PAA (or vice versa) could lead to the formation of interpenetrating crosslinking between these two constituents and resulted in adhesiveness reinforcement of UV-cured photopolymers.

Swelling property of photopolymerized CH-PAA and CH-PEG

The swelling characterization of photopolymerized CH-PAA (Fig. 9) showed that only 3.5% of the original weight of water was absorbed into the crosslinked CH-PAA chains after 24 h. This could be attributed to the introduction of hydrophobic aromatic phthalimido

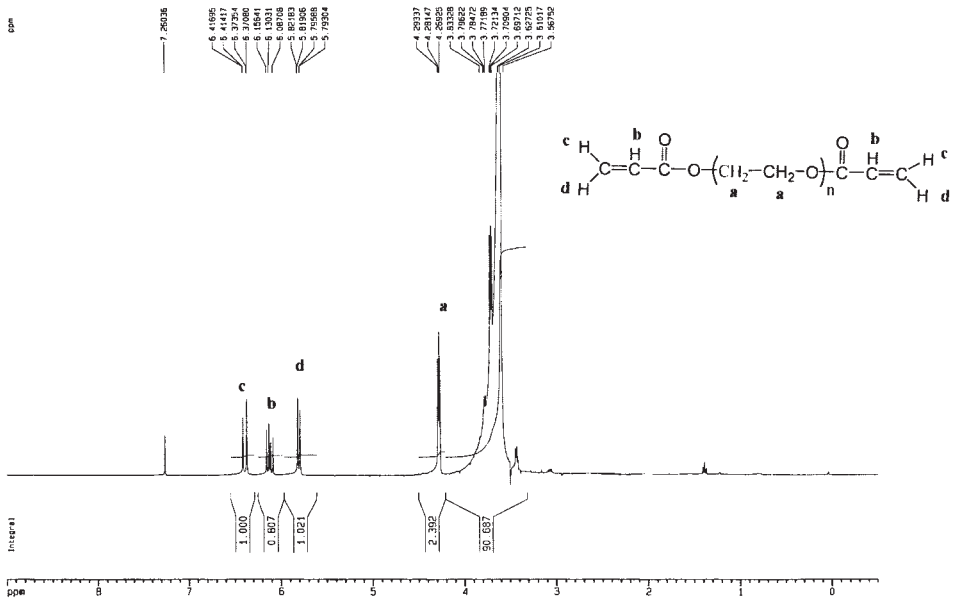


Figure 7 ^{1}H NMR spectrum of PEGA.

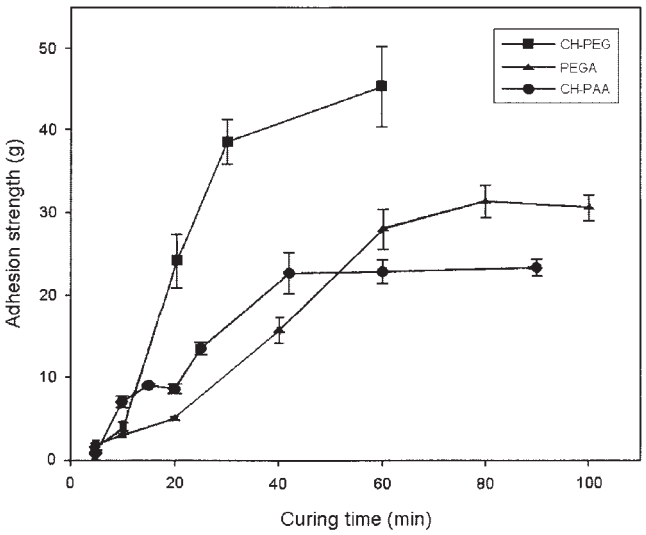


Figure 8 Adhesion strength for UV-crosslinked samples.

functionality to the chitosan main chain as well as the dense packing formed after UV crosslinking.

In contrast, for CH-PEG photocopolymerized samples, the swelling ratio of UV-crosslinked sample at 24 h was greatly increased with more PEGA incorporated into the CH-PAA/PEGA mixture (Fig. 10). This could be ascribed to the great water-absorption capability associated with the PEG.[20]

CONCLUSIONS

The photocrosslinkable chitosan may be of great use in biomedical applications because of its potential biodegradability, biocompatibility, and antimicrobial capability. In this study, *N*-phathaloylated chitosan (CH-PA) was reacted with acryloyl chloride to obtain photocrosslinkable chitosan (CH-PAA). Then CH-PAA was photocopolymerized with PEGA to enhance the water-adsorption ability of chitosan. The characterization results of FTIR and solid-state NMR for CH-PA and CH-PAA indicated that the phthalimido and acryloyl groups were successfully introduced into the chitosan and CH-PA structure, respectively. In addition, the attachment of the phthalimido group onto chitosan was further implicated by the shift of the melting point as revealed with DSC analysis. Moreover, the solubility test has shown that the CH-PA was more soluble in organic solvents than chitosan via the introduction of bulky phthalimido group, resulting in the variation of molecular chain packing in the chitosan structure. In contrast, the subsequently attached acryloyl group lowered the solubility of CH-PA in the organic solvents due to the formation of hydrogen bondings of acryloyl group. The adhesion strength of photocopolymerized CH-PEG was stronger than the UV-cured CH-PAA and PEGA at the same weight percentage. Furthermore, photocopolymerized CH-PEG had higher swelling ratio than UV-cured CH-PAA because of the water affinity of PEG segment This finding indicated that the copolymerized PEGA greatly improves the water-adsorption capability of photocrosslinkable chitosan. The research of the physical properties and potential *in vitro* and *in vivo* biomedical applications of CH-PEG, such as UV curing for wound dressing and tissue adhesive application during the surgery, is currently proceeding.

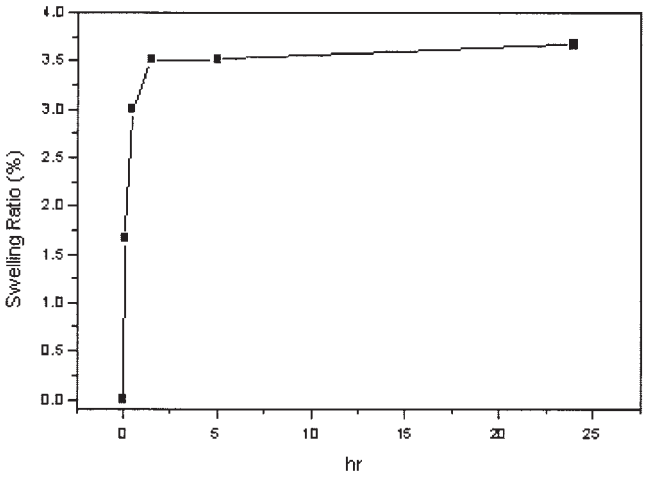


Figure 9 The swelling ratio of photopolymerized CH-PAA.

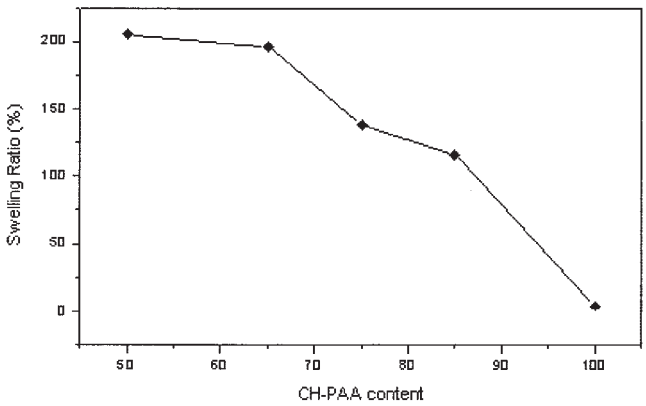


Figure 10 The swelling ratio of photocopolymerized CH-PEG with different CH-PAA content.

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