การเตรียมและการปลดปล่อย 8-ไฮดรอกซีควิโนลีนแบบควบคุมจากฟิล์มไคโทซานแตกสลายทางชีวภาพได้

นางสาวศรัณย์รัตน์ ภิรมย์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2554

ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย
บทคัดย่อและแฟ้มข้อมูลฉบับเต็มของวิทยานิพนธ์ตั้งแต่ปีการศึกษา 2554 ที่ให้บริการในคลังปัญญาจุฬาฯ (CUIR)
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PREPARATION AND CONTROLLED RELEASE OF 8-HYDROXYQUINOLINE FROM BIODEGRADABLE CHITOSAN FILMS

Miss Sarunrat Phirom

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Petrochemistry and Polymer Science
Faculty of Science
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Thesis Title	PREPARATION AND CONTROLLED RELEASE OF 8-HYDROXYQUINOLINE FROM BIODEGRADABLE CHITOSAN FILMS
By	Miss Sarunrat Phirom
Field of Study	Petrochemistry and Polymer Science
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ศรัณย์รัตน์ ภิรมย์ : การเตรียมและการปลดปล่อย 8-ไฮดรอกซีควิโนลีนแบบควบคุมจากฟิล์มไคโทซานแตกสลายทางชีวภาพได้. (PREPARATION AND CONTROLLED RELEASE OF 8-HYDROXYQUINOLINE FROM BIODEGRADABLE CHITOSAN FILMS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : ผศ.ดร.วรินทร ชวศิริ, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ดร.กฤษณา ศิรเลิศมุกุล, 87 หน้า.

งานวิจัยนี้ได้ศึกษาการเตรียมฟิล์มไคโทซานที่กักเก็บ 8-ไฮดรอกซีควิโนลีนสำหรับควบคุมการปลดปล่อยเพื่อใช้ยืดอายุการปักแจกันของดอกกุหลาบ ฟิล์มไคโทซาน/8-ไฮดรอกซีควิโนลีนถูกเตรียมโดยวิธีการแคสท์ด้วยตัวทำละลาย ผลกระทบของความเข้มข้นของไคโทซานและ 8-ไฮดรอกซีควิโนลีนได้ถูกตรวจ ผลกระทบของความเข้มข้นของไทรโพลีฟอสเฟตซึ่งเป็นสารเชื่อมขวางสำหรับไคโทซานฟิล์มรวมถึงระยะเวลาในการเชื่อมขวางได้ถูกศึกษา การปลดปล่อยที่ยาวนานขึ้นกับการเตรียมฟิล์มไคโทซาน/8-ไฮดรอกซีควิโนลีน โดยใช้ความเข้มข้นของไคโทซานและปริมาณสารเชื่อมขวางสูง และระยะเวลาในการเชื่อมขวางที่นานขึ้น ได้ศึกษาลักษณะทางกายภาพของฟิล์มไคโทซาน/8-ไฮดรอกซีควิโนลีน โดยเทคนิคเอกซเรย์ดิฟแฟรกชัน ฟูเรียร์ทรานสฟอร์มอินฟราเรดสเปคโทรสโกปีและสแกนนิงอิเล็คตรอนไมโครสโกปี สภาวะที่เหมาะสมสำหรับการยืดอายุการปักแจกันของดอกกุหลาบ10วัน คือ200มิลลิกรัมต่อลิตรของฟิล์มไคโทซาน/8-ไฮดรอกซีควิโนลีนร่วมกับสารละลายน้ำตาล 20% โดยน้ำหนักต่อปริมาตร นอกจากนี้สามารถนำสารละลายชุดเดิมมาใช้กับดอกกุหลาบชุดใหม่ซึ่งสามารถยืดอายุการปักแจกันของดอกกุหลาบได้7วัน ข้อได้เปรียบที่ได้รับคือ สามารถลดการใช้ 8-ไฮดรอกซีควิโนลีนและฟิล์มไคโทซาน/8-ไฮดรอกซีควิโนลีนใช้งานง่ายและนำกลับมาใช้ใหม่ได้

สาขาวิชา ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ ลายมือชื่อนิสิต.....

ปีการศึกษา 2554 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม.....

5273410223 : PETROCHEMISTRY AND POLYMER SCIENCE

KEYWORDS : 8-HYDROXYQUINOLINE/CONTROLLED RELEASE/ BIODEGRADABLE POLYMER/CHITOSAN

SARUNRAT PHIROM : PREPARATION AND CONTROLLED RELEASE OF 8-HYDROXYQUINOLINE FROM BIODEGRADABLE CHITOSAN FILMS. ADVISOR: ASST. PROF. WARINTHORN CHAVASIRI, Ph.D., CO-ADVISOR: KRISANA SIRALERTMUKUL, Ph.D., 87 pp.

This research explores the preparation of 8-hydroxyquinoline (8-HQ) loaded in chitosan films for controlled release to extend vase life of rose. 8-HQ/chitosan films were prepared by using solvent casting method. The effects of the concentration of chitosan films and 8-HQ were investigated. The effect of the concentration of tripolyphosphate (TPP) used as the crosslinking agent for chitosan films was studied including the crosslinking time. Slow release was achieved from 8-HQ/chitosan films prepared using a higher concentrations of chitosan, TPP and longer crosslink time. The characteristics of film was determined by X-ray diffraction, Fourier transform infrared spectroscopy and scanning electron microscopy. Optimum conditions of prolong vase life of rose for 10 days is 200 ppm of 8-HQ loaded chitosan films combined with 20% w/v of sugar solution. Furthermore, the film solution could be reused for another set of rose which could extend the vase life of another set of rose up to 7 days. This advantage can reduce the use of 8-HQ and chitosan films were easy to use and reusable.

Field of Study : Petrochemistry and Polymer Science Student's Signature……...

Academic Year : 2011 Advisor's Signature …….....

Co-advisor's Signature ……

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LIST OF ABBREVATIONS

^{o}C	:	degree Celsius
mg	:	milligram (s)
g	:	gram (s)
ppm	:	parts per million
mL	:	milliliter (s)
mm	:	millimeter
cm	:	centimeter
cm^{-1}	:	unit of wavenumber (IR)
% w/w	:	percent weight by weight
%w/v	:	percent weight by volume
MW	:	molecular Weight
FT-IR	:	fourier transform infrared spectrophotometry
HPLC	:	high performance liquid chromatography
XRD	:	x-ray diffraction
SEM	:	scanning electron microscopy

CHAPTER I
INTRODUCTION

1.1 Background

Nowadays, roses have many benefits and are widely used in terms of decoration and trading. The popularity of roses comes from their variety of color and sizes, good scent, *etc*. However, the petals and leaves rapidly wither after cutting. Moreover, when putting cut roses in the vase, the stem can be contaminated by the microbe which shortens the vase life [1]. An antibacterial agent, 8-hydroxyquinoline (8-HQ) [2], is added in sucrose solution to extend vase life of the rose. Normally, sucrose is mainly used as a food source, water balancing agents and antimicrobial agents to prevent the blockage of xylem vessels. Currently, 8-HQ is used in form of solution, which is toxic and required high consumption per usage. Therefore, the search for a new releasing system was explored to control the release of 8-HQ from biodegradable polymer such as, chitosan film.

1.2 Objective of this research

In this research, the effects of the concentration of chitosan film and 8-HQ were also investigated. The effect of the concentration of tripolyphosphate (TPP) used as the crosslinking agent for chitosan film was examined including crosslinking time.

Moreover, physical and chemical properties of chitosan films containing 8-HQ were also studied compared with free 8-HQ or normal chitosan film.

1.3 Scope of this research

This research focused on the effects of various parameters, namely

Concentrations of chitosan (2 and 3% w/v), concentrations of 8-HQ (10, 20 and 30% w/w), concentrations of crosslinking agent, TPP (0, 3 and 5% w/v) and crosslinking time (30, 60 and 180 min).

The methodology of this study is as follows:

1. Gather concerning data, information and literature reviews.
2. Prepare normal chitosan film and chitosan film containing 8-HQ, crosslink and

non-crosslink film.

3. Examine the properties (both physical and chemical) of chitosan film and chitosan film containing 8-HQ.
4. Interpret the data and summarize the results.

CHAPTER II

THEORY AND LITERATURE REVIEWS

2.1 8-hydroxyquinoline (8-HQ) [2]

8-HQ is a derivative of quinoline with OH group at C-8. It is white to pale yellow crystal phenolic odor with the formula C_9H_7NO. This 8-HQ is widely used commercially, although under a variety of names such as oxyquinoline and 8-quinolinol. 8-HQ is a monoprotic bidentate chelating agent to separate metal and is effective to control microorganisms. It was used as a bacteriostatic and fungistatic agent. It is used antiseptics and deodorants. The sulfate salt of 8-HQ is used as a complexing agent for pharmaceuticals.

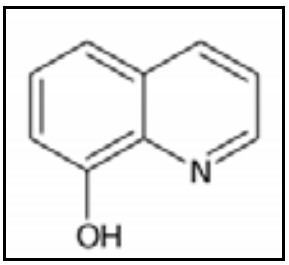


Figure 2.1 Chemical structure of 8-HQ [2].

2.2 Biodegradable polymer [3, 4]

Biodegradable polymers could be derived from synthetic or natural polymer. Biodegradation of polymeric biomaterials involves cleavage of hydrolytically or enzymatically sensitive bonds in polymer leading to polymer erosion. Polymeric biomaterials can be classified as hydrolytically degradable polymers and enzymatically degradable polymers. The most naturally occurring polymers undergo enzymatic degradation. The mechanism for degradation by hydrolysis or enzymatic cleavage resulted in a scission of polymer backbone. The hydrolysis of biodegradable polymers is extensively investigated for biomedical, pharmaceutical, agricultural and packing applications.

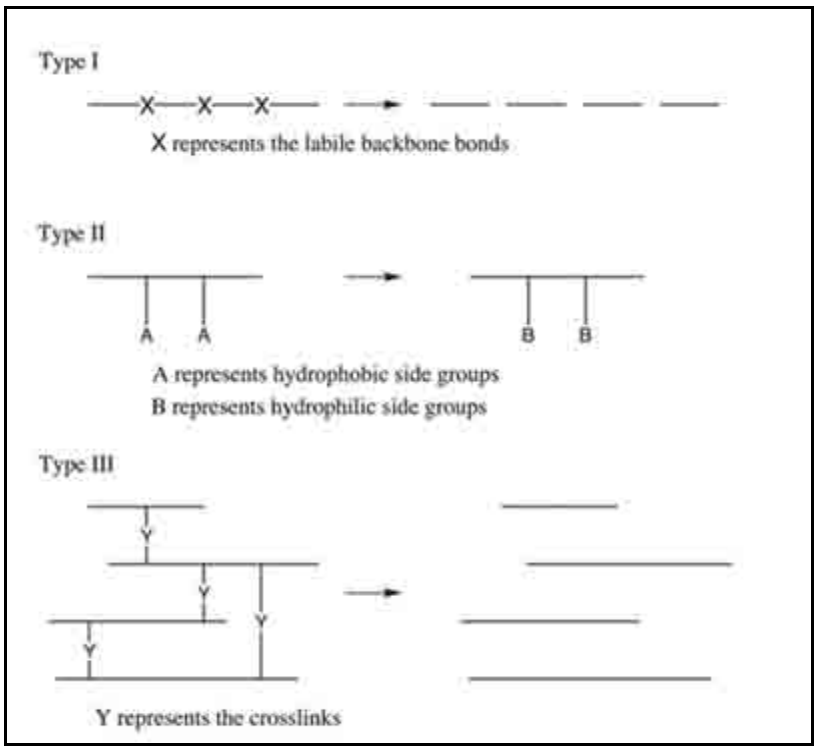


Figure 2.2 Schematic representation of the types of polymer degradation [4].

2.2.1 Chitosan [5]

Chitosan is a linear polysaccharide composed of randomly distributed ß-(1-4)-linked *D*-glucosamine and *N*-acetyl-*D*-glucosamine. It is a natural, non-toxic, biocompatible, biodegradable polysaccharide available as solution, flake, fine powder, bead and fiber. Chitosan reveals an ability to form film, chelate metal ions, *etc*. Chitosan possesses antimicrobial activity and filmogenic properties, besides being biocompatible and biodegradable. Chitosan films have been successfully probed at an experimental level on food such as eggs, fruits, vegetables, dairy products and meat, where it has been observed that chitosan treatment offers protection against contamination and microbial spoilage, increasing food quality.

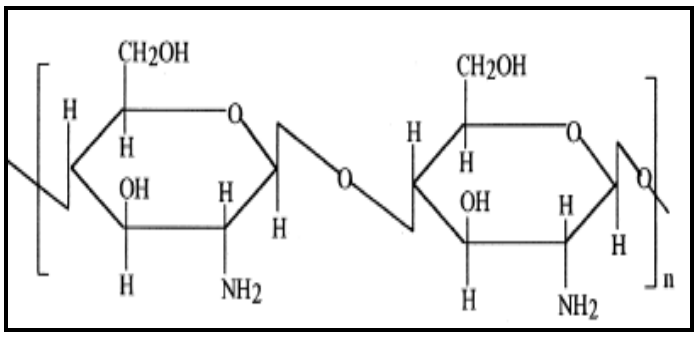


Figure 2.3 Chemical structure of chitosan [5].

2.2.2 Alginate [6]

Alginates are a linear copolymer containing β-(1,4)-linked D-mannuronic acid and α-(1,4)-linked L-guluronic acid residues isolated from brown seaweeds. Alginate and its derivatives have been utilized as a hydrocolloid in a variety of applications such as food additives, pharmaceuticals, cosmetics and textile manufacturing. For food: alginate has an excellent functionality as a thickening agent, gelling agent, emulsifier and stabilizer. Excellent properties of alginate could be applied to numerous kinds of food, such as ice cream, jelly, lactic drinks, beer, *etc*. For textile printing: alginate is used as a substrate of color paste when applying patterns to fabrics, scarf, *etc*. Alginate is easier to decompose compared with other substrates for textile printing, and gives easier waste water disposal. For pharmaceutical: alginate forms gel in the high-acidic stomach and protect stomach mucosa. For cosmetics: with its functionality of thickener and moisture retainer, alginate helps retaining the color of lipstick on lip surface by forming gel-network.

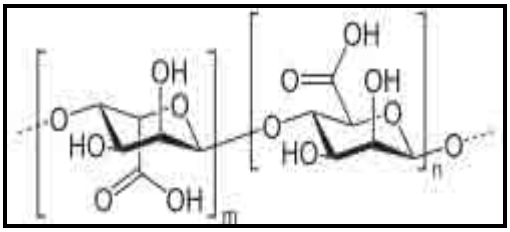


Figure 2.4 Chemical structure of alginate [6].

2.3 Crosslinkers [4]

Crosslinking are bonds that link one polymer chain to another, the chemical links between molecular chains form a three-dimensional network of connected molecules. The vulcanization of rubber using elemental sulfur is an example of crosslinking, converting raw rubber from a weak plastic to a highly resilient elastomer. The strategy of covalent crosslinking is used in several other technologies of commercial and scientific interest to control and enhance the properties of the resulting polymer system or interface, such as thermoset and coatings.

Crosslinking has been employed in the synthesis of ion-exchange resins and stimuli-responsive hydrogels made from polymers containing polar groups. As polyelectrolytes, hydrogels are inherently water soluble. To make them insoluble, they are chemically crosslinked during manufacture or by a second reaction following polymerization of starting monomers. The degree of crosslinking, quantified in terms of the crosslink density, together with the details of the molecular structure, has a profound impact on the swelling characteristics of the crosslinked system.

2.4 Controlled release mechanisms [7, 8]

A controlled release or drug delivery system occurs when the drug molecule is incorporated into a matrix polymer (natural or synthetic) and the dissolving drug can be released through the matrix with constant over a long period. In this case, there are three primary ways which active molecules can be released from each system: diffusion, degradation, and swelling followed by diffusion.

Diffusion can occur on a macroscopic scale through pores in the matrix or on a molecular level by passing between polymer chains (Figure 2.5). The reservoir systems as shown in Figure 2.6 indicate the controlled release system of implantable or oral drug Figure 2.6 (a) and transdermal drug 2.6 (b) through diffusion mechanism.

For the others system, controlled release based on swelling (Figure 2.7) and eroding matrix (Figure 2.8) allow diffusion of drug from degrading system.

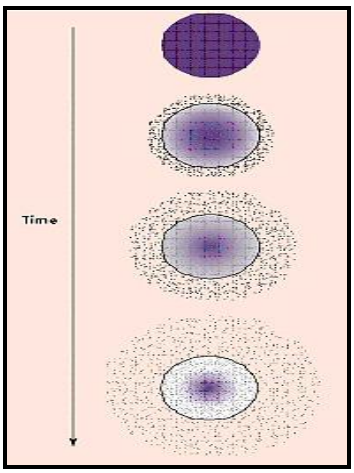


Figure 2.5 Drug delivery from a typical matrix drug delivery system [7].

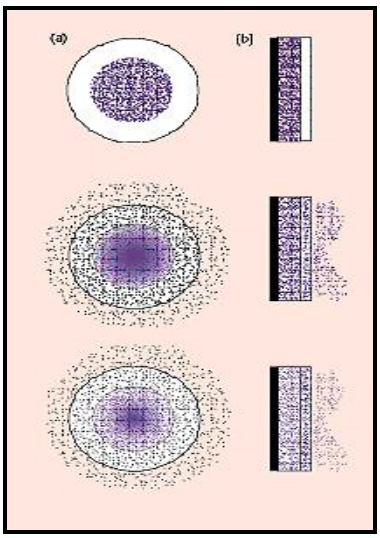


Figure 2.6 Drug delivery from typical reservoir devices: (a) implantable or oral systems, and (b) transdermal systems [7].

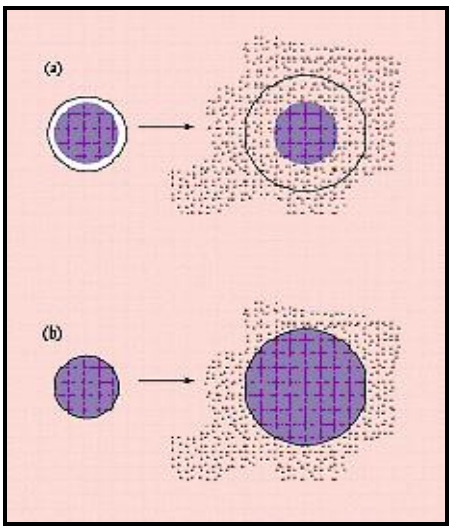


Figure 2.7 Drug delivery from (a) reservoir and (b) matrix swelling-controlled release systems [8].

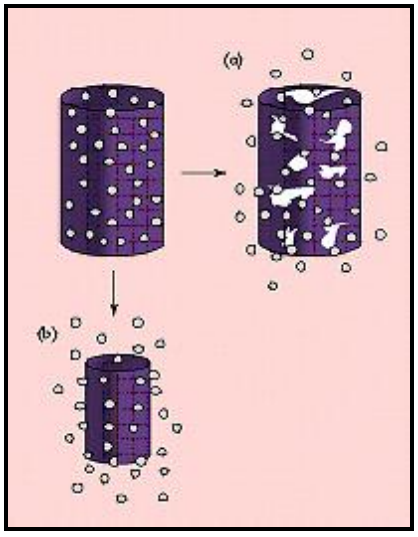


Figure 2.8 Drug delivery from (a) bulk-eroding and (b) surface-eroding biodegradable systems [8].

2.5 Literature reviews

Kazuo *et al.* [9] have studied the effects of temperature, 8-HQ sulfate (8-HQS) and sucrose on the vase life of cut rose cv. Sonia flowers. They suggested that the use of 200 ppm 8-HQS and sucrose combine with 200 ppm 8-HQS could extend the vase life at all temperatures. Percent fresh weights of rose in all solutions were found to

increase during the first 3-9 days of the experiment and then declined. Water uptake in the control and 8-HQS treatment was increased over the first 1-2 days, and then declined.

Daungporn *et al.* [10] studied on the effects of sucrose, $AgNO_3$ and 8-HQS on postharvest behavior of *Dendrobium pompadour* flowers. It was found that the solution of 10-30 mg/L $AgNO_3$ or 50-200 ppm of 8-HQS could extend vase life of orchid flowers while the use of 10% sucrose resulted in decreasing vase life of orchid flowers and the obstruction of xylem vessels due to microbial growth. The optimum holding solution was 200 mg/L HQS + 10 mg/L $AgNO_3$ + 2% sucrose showing the highest rate of water uptake.

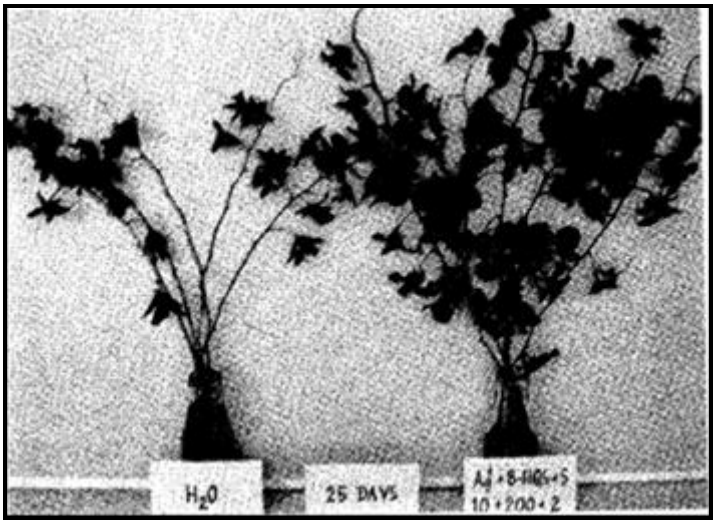


Figure 2.9 Orchid flower held in distilled water (left) and the solution containing 2% sucrose+10 mg/L $AgNO_3$+200 mg/L HQS (right) for 25 days at ambient temperature [10].

Mousa *et al*. [11] studied the effects of silver nanoparticles (SNP) and 8-hydroxyquinoline citrate(8-HQC) as antimicrobial agent on the extend vase life of gerbera. The result showed that the holding solution of 1 mg/L SNP in 6%sucrose solution could extend vase life gerbera due to induced blockage of vessel of gerbera.

Venkatesh *et al*. [12] studied the action of cobalt extending the vase life of cut roses. Cobalt (Co) prevented the blockage in stems of Rosa hybrid cultivar

‘Samantha’, leading to increase water uptake, maintained water in the cut rose. The results of water uptake and water loss, showed various Co^{2+}concentrations (mM) such as 0.0, 0.5, 1.0, 1.5 and 2.0 mM. Fresh weight showed the amount different Co concentrations, the lowest fresh weights occurred in 0.5 mM followed by 2.00 mM, while the highest fresh weight was maintained by 1.5 mM concentration. Co exerts a dual effect in delaying senescence of cut rose; first, by increasing water uptake, and second, by reducing water loss, thereby improving water balance and preventing water stress in cut flower, leading to increased fresh weight and vase life of flower.

Li-Jen *et al.* [13] reported the prolong vase life of postharvest cut rose flowers using sucrose and silver thiosulfate(STS) as extended agents. The use of sucrose solution combined with STS and 8-HQS will help to extend the vase life of cut roses to more than 13 days when comparison to the use of 0.2 mM STS alone.

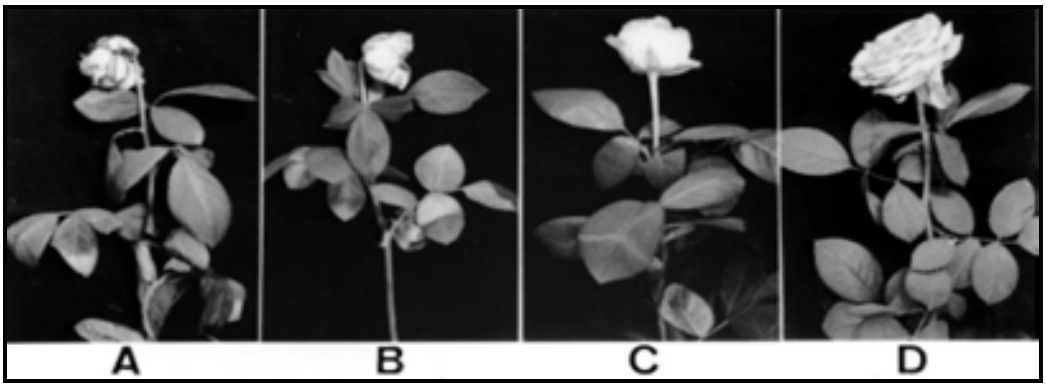


Figure 2.10 Flower longevity of cut roses as affect by a pulse treatment of sucrose,STS and STS followed by sucrose, water (A); sugar (B); STS (C); STS+sucrose (D). Photographs were taken 8 days after treatment [13].

Mohamadisona *et al*. [1] examined $AgNO_3$, 8-HQS, nano silver and distilled water to extend vase life of rose compared with tap water. The relative fresh weight, fresh weight to day ratio, flower diameter, flower opening and vase life were investigated. The duration of vase life could be observed with some symptoms such as necrosis, wilting and abscission of petals, chlorosis and abscission of leaves and bent

neck which caused to reduce flower attraction. The flowers treated with 8-HQS (250 mg/L) combined with nano silver (2 mg/L) had the highest water uptake of flower.

Mohy *et al*. [14] studied on the effects of different concentrations of $AgNO_3$ and sucrose on the vase life of cut rose (*Rosa hybrida*) in terms of wilting, chlorophyll retention and carbohydrate degradation. In this experiment various concentrations of $AgNO_3$ at 20, 30 or 50 ppm and sucrose at 1, 2 or 3% w/v were studied (Figure 2.11). The results showed that the percentage of wilting was minimized when using the combined treatments. However the percentage of wilting was found to increase with increasing in concentration of $AgNO_3$ and complete wilting occurred at 10, 8 and 7 days after treatment with 20, 30 and 50 ppm $AgNO_3$, respectively. Whereas sucrose showed the shortest time of wilting. The result showed the best condition for extending vase life of rose was 30 ppm of $AgNO_3$ in 3% sucrose.

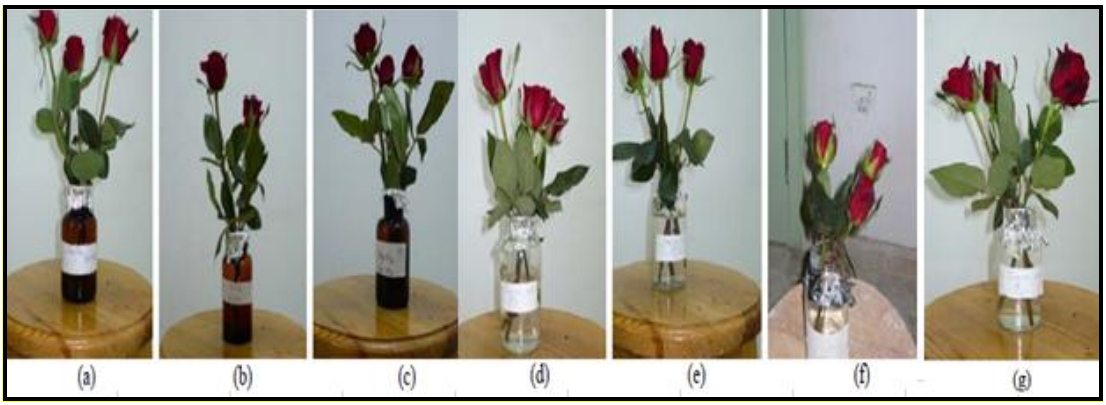


Figure 2.11 Effect of $AgNO_3$ and sucrose on vase life of rose cut flowers (a): $AgNO_3$ 20 ppm, (b): $AgNO_3$ 30 ppm, (c): $AgNO_3$ 50 ppm, (d): 1%sucrose, (e): 2% sucrose, (f): 3% sucrose, (g):control [14].

Fahmy [15] reported the effect of 8-HQS on the vase life of several flowers. In this experiment, various concentrations of 8-HQS at 100, 200 and 400 ppm with or without 50 g/L sucrose were investigated. The effect of 8-HQS on vase life of carnation cut flowers showed the best condition for treatment was 400 ppm 8-HQS combined with 50 g/L of sucrose could prolong vase life as 13.67 days compared with control solution (Figure 2.12). The effect of 8-HQS on vase life of chrysanthemum

cut flowers showed the best condition for treatment was 400 ppm 8-HQS without sucrose could prolong vase life as 22.67 days compared with control solution (11.33 days) (Figure 2.13). The effect of 8-HQS on vase life of roses, revealed that the vase life of rose was increasing when 8-HQS was used in the presence of sucrose. The best condition of 400 ppm 8-HQS combined with 50 g/L sucrose could prolong vase life of rose as 11 days compared with controlled solution as 5.3 days (Figure 2.14).

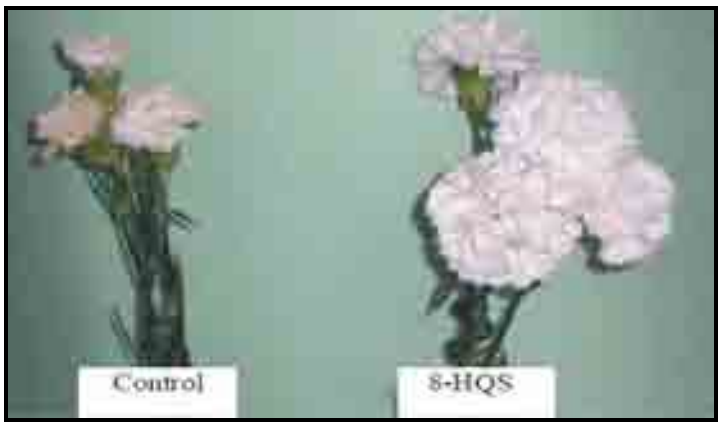


Figure 2.12 Effect of 8-HQS at 400 ppm plus 50 g/L sucrose on the vase life of cut carnations in comparison with untreated control. Photo was taken on day 7 of vase life [15].

Figure 2.13 Effect of 8-HQS at 400 ppm on the vase life of cut chrysanthemum in comparison with untreated control. Photo was taken on day 11 of vase life [15].

Figure 2.14 Effect of 8-HQS at 400 ppm+ 50 g/L sucrose on the vase life of cut roses in comparison with untreated control. Photo was taken on day 5 of vase life [15].

K.C. *et al*. [16] studied the efficiency release of centhroman (Figure 2.16) from microspheres with chitosan of different molecular weights and degrees of deacetylation (DDAs) used glutaraldehyde as crosslinker for encapsulation the beads. The microsphers prepared with variousdegrees of deacetylations with low DDAs (48%), medium DDAs (62%), high DDAs (75%) and molecular weight with low molecular weight (LMW) (260 kg mol^{-1}), medium molecular weight (MMW) (1134 kg mol^{-1}) and high molecular weight (HMW) (2224 kg mol^{-1}). The swelling and release of centchroman with 6% crosslinker showed the bead that prepared from HMW had a low degree of swelling (150%wt), MMW had degree of swelling (250%wt) and LMW had a high degree of swelling (287%wt). Microspheres was determined with shape factor (S) with using average parameter (L) and area (A) of about 20 microspheres and using equation 1.

$$S = \frac{L^2}{4\P A} \qquad (1)$$

Where L and A are the average perameter and area of the selected surface on microspheres. The result showed the microspheres with low-molecular weight of chitosan were rough, larger in size 62.20 µm due to poor molecular packing and cross-linking in comparison to microspheres obtained from medium and high molecular weight of chitosan was 39.78 and 22.60 µm, respectively. The degree of

deacetylation was low (48 wt%) showed degree of swelling of 282 wt% period of 40 hrs. The microspheres with a high degree of deacetylation (75 wt%) showed the least degree of swelling as 213 wt% due to more compact and then medium degree of deacetylation showed degree 250 wt%. This study had clearly indicated that microspheres with 62 wt% DDAs were suitable for the loading of centchroman due to the optimum degree of swelling and pore size.

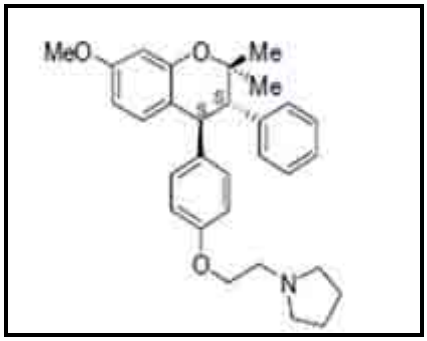


Figure 2.15 Chemical structure of centchroman [16].

Qun *et al.* [17] studied controlled release of ciprofloxacin hydrochloride from chitosan/polyethylene glycol blend film. The tests showed that the release amount of ciprofloxacin hydrochloride increased when increased ratio of PEG and decreased an amount of drug loaded in the film. Then, the release amount of ciprofloxacin hydrochloride increased when declined as the thickness of the time. The effect of pH showed that a higher pH led to a better solubility of ciprofloxacin hydrochloride, which resulted in higher drug release rate. Furthermore, the films that had longer cross-linking time in TPP solution, the drug release rate had lower within 24 h. The morphology of CP and CP-4 films were determined by Scanning Electron Microscope (SEM) and showed the cross section of CP (Blank matrix film, without drug, with PEG contents of 3.5 wt%) and CP-4 (PEG contents were 8.0 wt%) films are smooth and homogeneous, the result could indicate good compatibility between the matrix and drug (Figure 2.16).

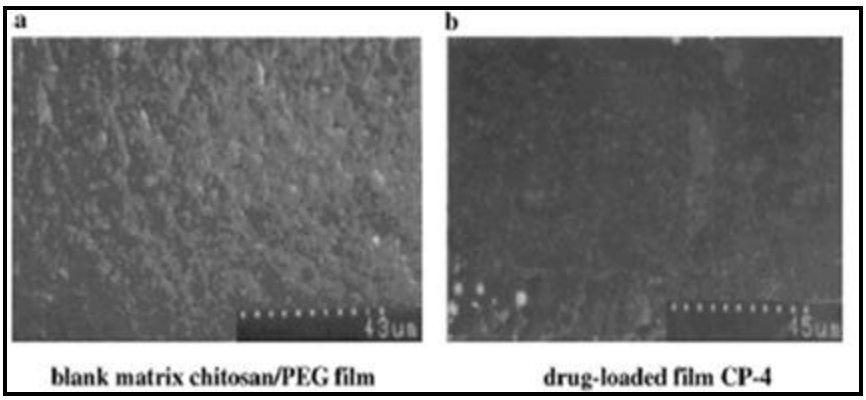


Figure 2.16 SEM photographs of blank matrix chitosan/PEG film CP and drug loaded film CP-4 (a) Blank matrix chitosan/PEG film and (b) drug-loaded film CP-4 [17].

X.Z. *et al.* [18] addressed the influence of multivalent phosphate structure on the properties of cross-linked chitosan films for controlled drug release. The electrostatic interactions between anions and chitosan have influence on the properties of cross-link chitosan. Phosphate, pyrophosphate and tripolyphosphate (Figure 2.17) as crosslinker were used for experiment, which controlled by pH solution due to pH being dependent change the number of anion and the degree of ionization of chitosan (Figure 2.18). The lower charge number of phosphate had low interaction with chitosan. While pyrophosphate and tripolyphosphate with more negative charges showed high ability of crosslink to chitosan. The result showed that the cross-linking time and anion concentration had an effect on the film swelling, and the prolongation of cross-linking time and the increase of anion concentration resulted in the decrease of the film swelling ratio. Chitosan film morphology also played an important role on the film swelling. The same conditions of the swelling ratio of drug loaded films were less than that of blank film because the former had a porous structure that facilitated the diffusion of anions into the inside of film to form more crosslinking sites.

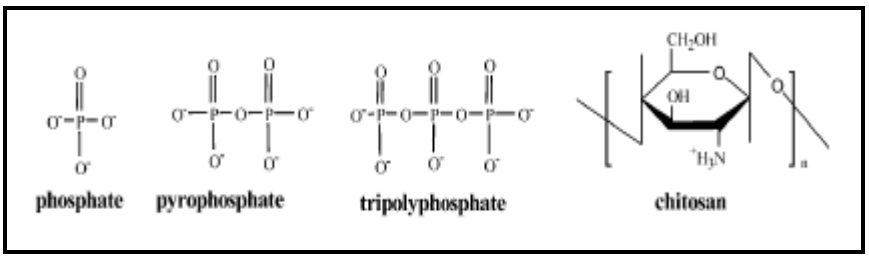


Figure 2.17 The structure of Phos, Pyro, TPP and chitosan [18].

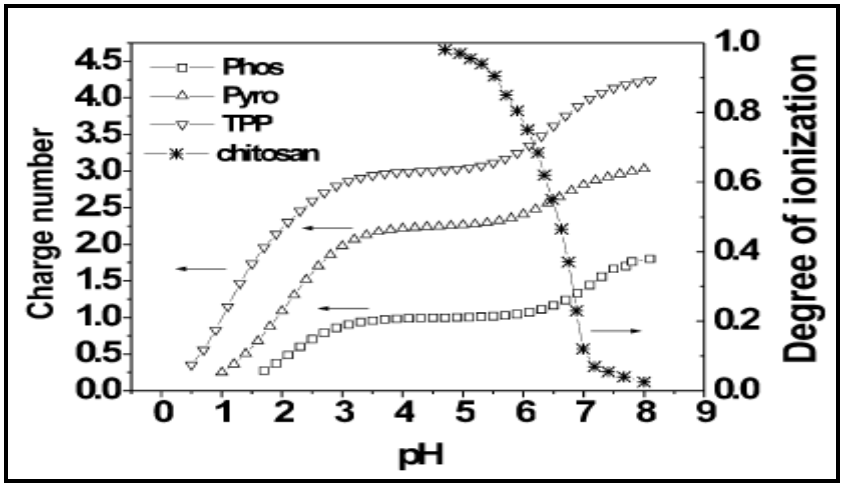


Figure 2.18 The pH-dependent charge number of Phos, Pyro and TPP, and the degree of ionization of chitosan [18].

Kashappa *et al.* [19] studied the preparation and characterization of drug-loaded chitosan-tripolyphosphate microspheres by spray drying method using acetaminophen as a model drug. The results showed that chitosan-TPP microparticles loaded with acetaminophen were obtained in the size range of 3.1-10.1 μm. The encapsulation efficiency of these microspheres was 48.9-99.5%. The swelling of chitosan-TPP microspheres increased with increasing in the molecular weight of chitosan due to the higher chain-relaxation ability in high-molecular weight chitosan as a result of increasing the entanglement of the polymeric chain. The swelling of chitosan-TPP microspheres was decreased with increasing volume of 1% w/v TPP solution as cross-linker because high cross-linked density. Concentration of chitosan, drug loading, amount of TPP solution and molecular weight of chitosan had

influenced with surface morphology and drug release. The result of drug release was relative to the viscosity of chitosan solution. The increased viscosity of chitosan solution relatively strong matrix upon interaction with TPP. High cross-linking density of chitosan-TPP had resulted in less swelling ability, the release of drug was decreased. When high concentration of chitosan was used for preparing microspheres, the drug was effectively entrapped into the chitosan-TPP and resulting in slower release rates. In that study, the release process of acetaminophen from spray-dried chitosan-TPP microspheres was somewhat biphasic with an initial burst effect, followed by slower release. The result of release of acetaminophen from chitosan-TPP microspheres through two steps was shown in Figure 2.19. Step 1: the release of surface adhered acetaminophen within 30 min (Figure 2.19, step 1). Step 2: diffusion of acetaminophen from swollen chitosan-TPP microsphere (Figure 2.19, step 2)

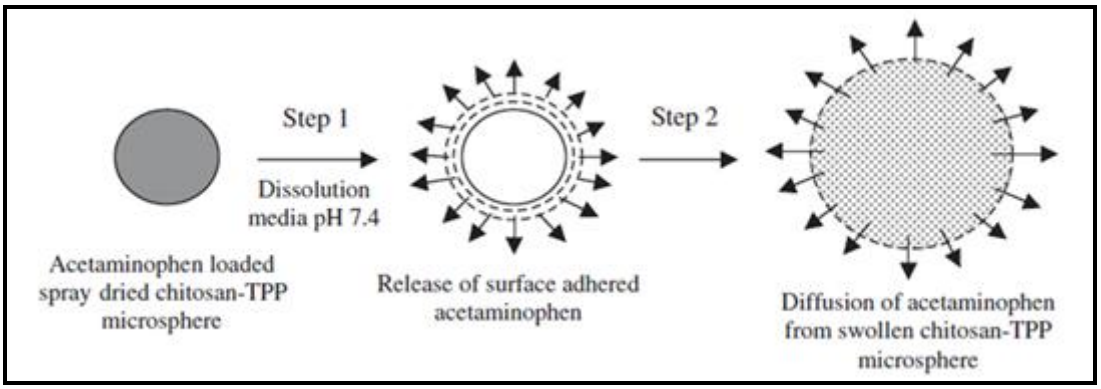


Figure 2.19 Predicted drug release process from spray-dried chitosan-TPP microsphere [19].

Thawien *et al*. [20] studied the preparation and characterization of biodegradable blend films from rice starch-chitosan. Rice starch-chitosan was developed by casting film-solution on leveled trays. The results showed that the film solubility of biodegradable blend films decreased with the addition of chitosan as biodegradable blend films with a rice starch and chitosan ratio decreasing from 2:1 to 0.5:1. These results could arise from the fact that higher chitosan content induced a rice–starch- chitosan interaction and resulted in decreasing in the film solubility. Water vapor permeability of rice starch-chitosan biodegradable blend film was lower than chitosan films but higher than polyolefin.

Carmen *et al.* [21] studied the films of chitosan glutamate and sodium alginate obtained by a casting/solvent evaporation method, and crosslinked, tripolyphosphate (TPP) and calcium chloride ($CaCl_2$), respectively. The water vapor transmission rate of chitosan films linearly decreased with increasing concentration of crosslinking agent. The swelling of the alginate films was independent of $CaCl_2$ concentration. Chitosan films showed swelling and permeability characteristics, which were dependent on pH and on the concentration of crosslinking agent.

Angela *et al*. [22] examined the development and characterization of chitosan/gelatin films as innovative mucoadhesive system for buccal delivery of propranolol hydrochloride. In vitro release studies showed that propranolol hydrochloride stopped the release in the first 30 min for all films analyzed. Films with amount of chitosan is an excess (r=0.2 and 0.4) showed a higher release of drug with a greater amount of gelatin allowing 83 and 66% of drug released in 30 min, respectively. On the contrary, films containing amount of gelatin is an excess (r=0.6 and 0.8) showed drug release of 54 and 48%, respectively in 30 min. Propanolol hydrochloride was not completely released from all the formulations containing chitosan/gelatin complexes; this behavior could be related to the presence of possible interactions between drug and gelatin, which proportionally to gelatin increase in the films, limit drug diffusion through the chitosan/gelatin polymeric network.

Devika *et al*., [23] examined the effect of pH on cross-linking of chitosan with TPP. The cross-linking of chitosan was dependent on cationic and negative charged species. Two conditions of pH 3 and 9 were studied. When pH of TPP was 3, phosphoric ion was present and pH of TPP was 9, OH^- and phosphoric ion were present. The OH^- ions were linked to amino group by deprotonation. These results showed the mechanism of cross-linking of chitosan with TPP by deprotonation or ionic interaction as shown in Figure 2.20. This could be explained that the cross-linking was affected by deprotonation at higher pH of TPP, but the chitosan crosslink with TPP at lower pH was affected by ionic interaction between positive charged chitosan and negatively charged phosphoric ions. Then the swelling of cross-linked chitosan was depended on pH of TPP. At pH 3 and 9, this swelling as 668 and 157% was observed because at lower pH a greater number phosphoric ion led to high cross-linking density and result of morphology (Figure 2.21). At pH 9, it showed that

chitosan crosslinked was more porous, the structure was loose could be explained its lower crosslink density.

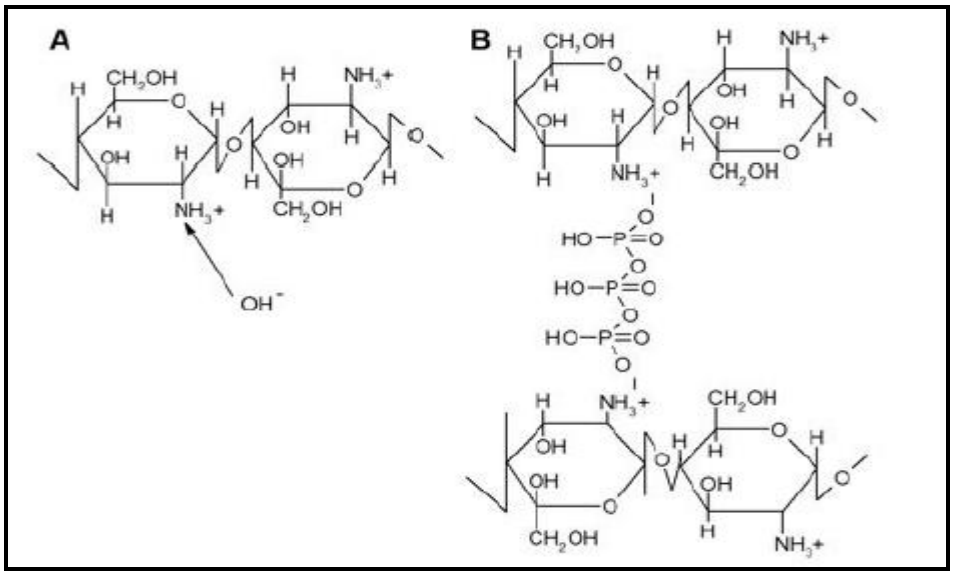


Figure 2.20 Interaction of chitosan with TPP (a) deprotonation, (b) ionic cross-linking [23].

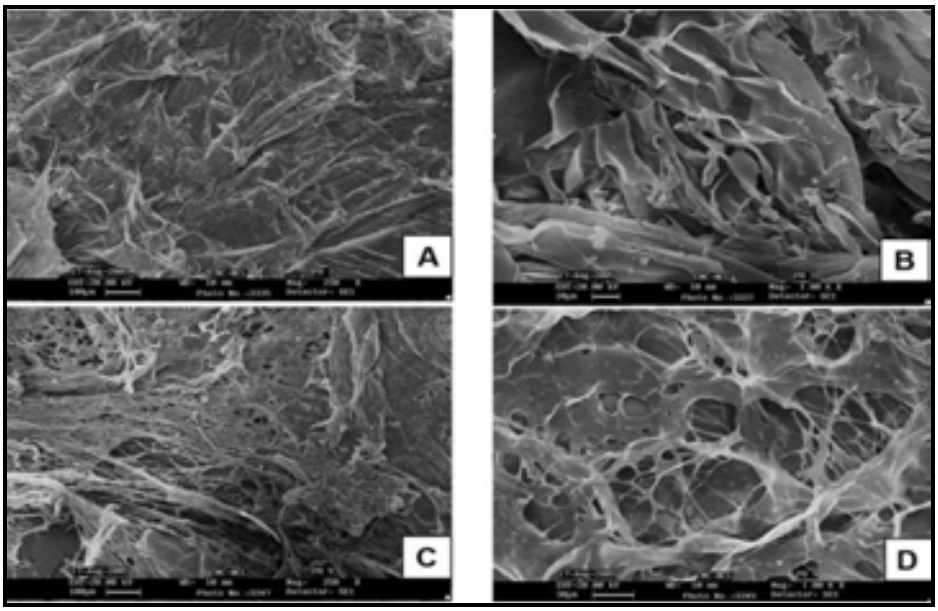


Figure 2.21 Scanning electron microphotographs of chitosan cross-linked with (a) TPP at pH 3, x250, (b) TPP at pH 3, 1KK, (c) TPP at pH 9, x250, (d) TPP at pH 9, 1 KK [23].

Ahmed [24] studied the influence of alginate concentration, amount of $CaCl_2$, crosslink time and molecular weight of alginate for release of theophyline loaded sodium alginate microspheres. Beads prepared with high concentration of alginate and $CaCl_2$ could prolong the release rate of theophylline. At low molecular weight, the results showed high amount of released theophyline. The curing time as 15 min had optimum curing time for maximum drug loading efficiency.

Pimwipha *et al.* [25] studied controlled release of sodium diclofenac (DFNa) from chitosan (CS) and carrageenan (CR) beads. Various factors influencing drug release such as proportion CS/CR, amount of DFNa, type and amount of cross-linking agent were investigated. Optimum formulation: the ratio of CS/CR of 2:1 and 5% w/v DFNa. The resulting crosslinked beads with glutaric acid and glutaraldehyde were more efficient prolonged drug release than non-crosslinking. The beads cross-linked with glutaradehyde were able to control the release of drug over 24 h.

Georage *et al*. [26] studied about swelling and vitro release of verapamil from calcium alginate and calcium alginate-chitosan beads. The result revealed that the swelling ability of beads was dependent on the presence of polyelectrolyte complex between alginate and chitosan, pH of aqueous media. The encapsulation efficiency of verapamil in both calcium-alginate and chitosan-alginate-chitosan mixed beads exceeded 80%.

CHAPTER III

EXPERIMENTAL

3.1 Material and chemicals

Chitosan (MW 500,000-1,000,000 Daltons, 95% min deacetylation) from shrimp shell was purchased from Bonafiles Marketing Co., Ltd (Bangkok, Thailand). Methanol (HPLC grade) and water (HPLC grade) were purchased from RCI Labscan Limited (Bangkok, Thailand). Acetic acid was purchased from Merck Co., Ltd (Germany). Ethylenediaminetetraacetic acid disodium salt (EDTA-di-sodium salt) was purchased from Ajax Finechem Pty Ltd. (Australia). 8-hydroxyquinoline (8-HQ) was purchased from Fluka Chemical Company. Tripolyphosphate (TPP) was purchased from Grand Chemical Co., Ltd (Bangkok, Thailand).

3.2 Instruments and equipments

The infrared spectra of all samples were recorded with Fourier transform infrared spectroscopy (FTIR) (Nicolet Instruments Technologies, Inc. WI, USA). High Performance Liquid Chromatography (HPLC) (Aglilent Co., Ltd) was used to determine 8-HQ loading efficiency and solubility release of 8-HQ from chitosan film. From the results of chitosan and 8-HQ loaded chitosan films, the effects of 8-HQ on degree of crystallization of blend films were determined with X-ray diffractometer at 40 kV, 30 mA and analyzed between 4-40º (2θ). The surface and cross-sectional morphologies of chitosan films were determined with Scanning Electron Microscope (SEM) was acquired through the JSM-5410LV (JEOL, Tokyo, Japan).

3.3 General procedure

3.3.1 Preparation of chitosan beads

The preparation of 8-HQ loaded chitosan beads was performed by dissolving chitosan in 1% v/v acetic acid solution with constant stirring. 8-HQ was dissolved in 20 mL EtOH, added into chitosan solution and dropped this solution through syringe with diameter 0.2 mm into 0.1 N NaOH solution contain 0.5% glutaraldehyde. The

distance between the edge of the syringe and the surface of 0.1 N NaOH solution was 21 cm. The beads were left in 0.1 N NaOH for 0.5 h., washed with distilled water, and dried in air for 48 h.

3.3.2 Preparation of alginate beads

Alginate beads were prepared by dissolving sodium alginate in distilled water. 8-HQ was dissolved in 20 mL EtOH then 8-HQ was completely added to sodium alginate solution, then dropped this solution through syringe with diameter 0.2 mm into 0.5 M $CaCl_2$ solution. This distance between the edge of the syringe and surface of 0.5 M $CaCl_2$ solution was 21 cm. The beads were left in 0.5 M. $CaCl_2$ solution for 0.5 h, washed with distilled water, dried in air for 48 h.

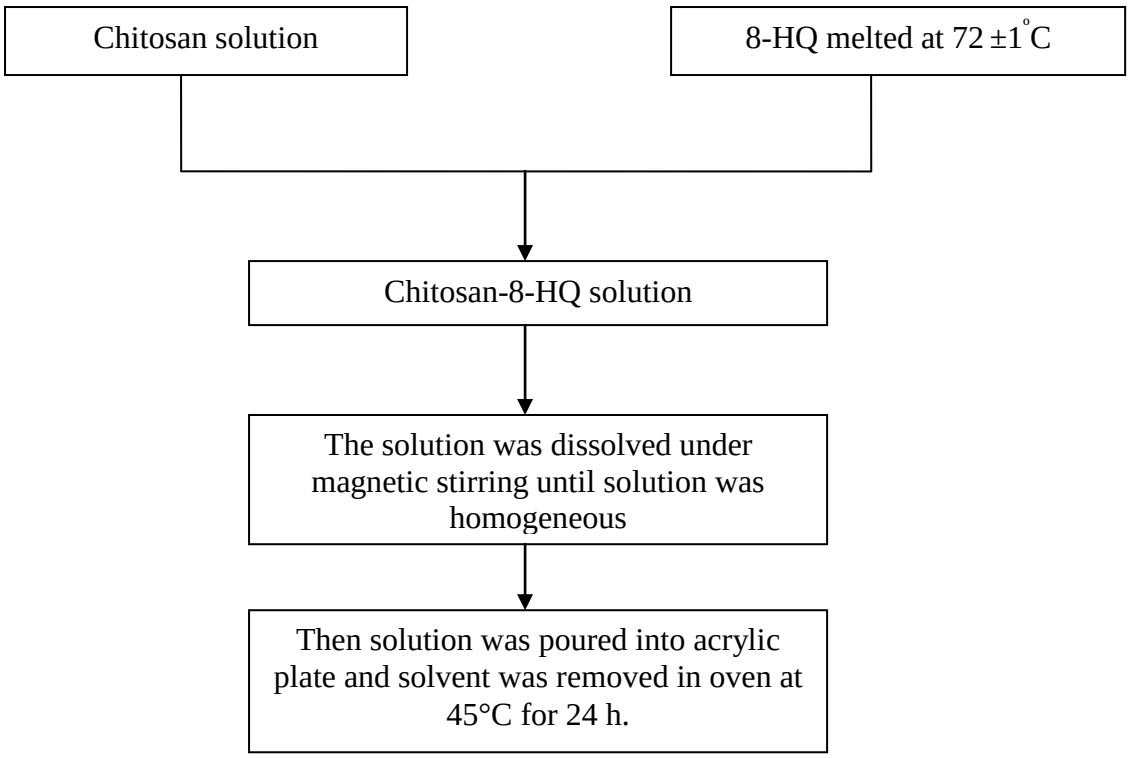
3.3.3 Preparation of chitosan film, 8-HQ loaded chitosan film and crosslink chitosan film.

All chitosan films were prepared by using solvent-casting method. The chitosan films with various concentrations including 1, 2 and 3% w/v in 1% acetic acid were prepared and 8-HQ loaded in film was done by incorporated 8-HQ (10, 20 and 30% w/w of 8-HQ) into the solution. (Step I)

The solutions were then poured in acrylic plate (size 10.5x12.0x1.0 cm) and solvent was removed in oven at 45ºC for 24 h. Cross-linking chitosan film was obtained by immersing chitosan film into TPP solutions (3 and 5% w/v) at 30, 60 and 180 min. (step II)

All of the samples were kept in dessicator before further analyzed.

Step I: Preparation of 8-HQ loaded chitosan films



Step II: Process of crosslinking

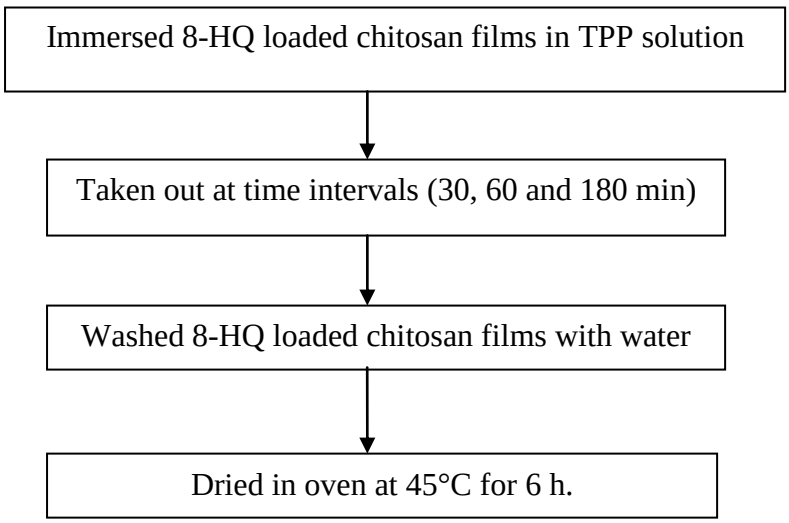


Figure 3.1 Preparation process of 8-HQ loaded crosslink and non-crosslink chitosan films.

3.4. Testing

3.4.1 8-HQ loading efficiency studies. (For testing chitosan beads and alginate beads)

Weigh beads (*ca.* 30 mg) into volumetric flask and dilute to 25 mL with MeOH. The sample was sonicated for 48 h and determined the amount of 8-HQ loading efficiency by high performance liquid chromatography (HPLC).

Condition of HPLC

Column	: Eclipse XDB-C18 5µm 4.6X250 mm.
Guard Column	: Eclipse XDB-C18 Analytical Guard Column
Detector	: VWD
Mobile phase	: 60% 2mM ethylenediaminetetraacetic acid disodium salt in water-acetic acid (99:1) ; 40 % MeOH-acetic acid (99:1)
Flow rate	: 1.00 mL/min

3.4.2 FT-IR analysis

The FT-IR spectra of chitosan, 8-HQ, 8-HQ loaded chitosan films were recorded on Nicolet Fourier Transform Infrared Spectrophotometer: Impact 410 (Nicolet Instruments Technologies, Inc. WI, USA). Infrared spectra were recorded between 400 cm^{-1} to 4000 cm^{-1} in transmittance mode.

Figure 3.2 Nicolet Impact 410 FT-IR

3.4.3 X-ray diffraction studies

The X-ray diffraction patterns of chitosan, 8-HQ and 8-HQ loaded chitosan films were determined on PW3710 BASED diffractometer using CuKα radiation. The voltage and current used were 40 kV and 30 mA, respectively and analyzed between 4- 40º (2θ). From the results of chitosan and 8-HQ loaded chitosan films, the effect of 8-HQ on degree of crystallinity of blend films was determined.

Figure 3.3 X-ray diffractometer

3.4.4 Scanning electron microscopy studies

The surface and cross-sectional morphologies of chitosan film was studied by SEM analysis. Chitosan films were fixed on supports and coated with gold-palladium under an argon atmosphere using a gold sputter module in a high-vacuum evaporator. Samples were then observed with JSM-5410LV scanning microscope using secondary electron imaging at 15 kV in order to examine the structure of the films.

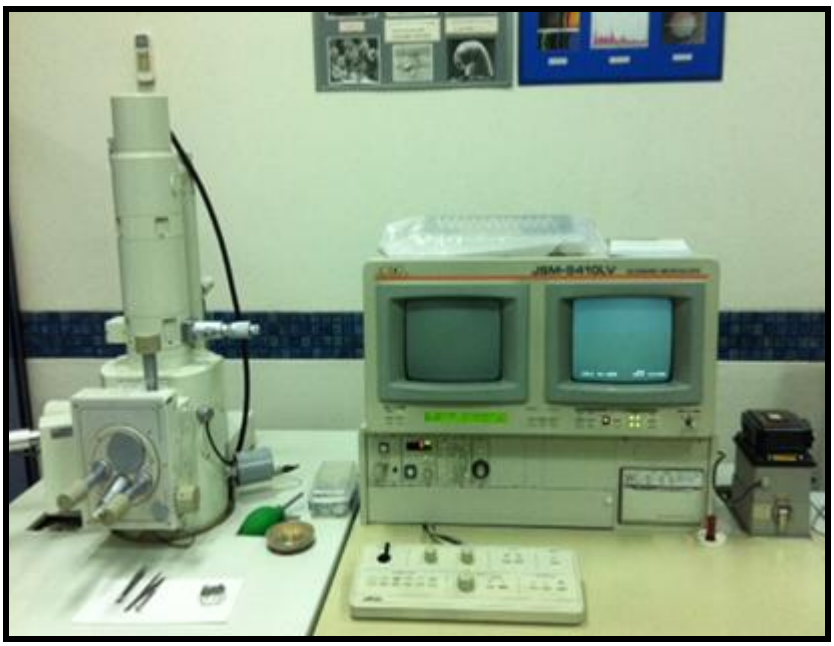


Figure 3.4 Scanning electron microscope

3.4.5 8-HQ loading efficiency studies.

Weigh 8-HQ loaded chitosan films (*ca*.30 mg) into volumetric flask and dilute to 25 mL with MeOH. The sample was sonicated for 48 h. and determined drug loading efficiency by HPLC.

Condition of HPLC

Column	: Eclipse XDB-C18 5µm 4.6X250 mm.
Guard Column	: Eclipse XDB-C18 Analytical Guard Column
Detector	: VWD
Mobile phase	: 60% 2mM ethylenediaminetetra acetic acid disodium salt in water-acetic acid (99:1); 40 % MeOH-acetic acid (99:1)
Flow rate	: 1.00 mL/min

Figure 3.5 High performance liquid chromatography (HPLC)

3.4.6 Release profile of 8-HQ from chitosan films

Weigh chitosan films (*ca*.33mg) in glass bottles containing 50 mL of 20% sugar at 25±2°C. Pipetted solution 1 mL, fixed time period, into volumetric flask 25 mL dilute to volume with MeOH. Determination the amount of 8-HQ released from the films by HPLC. Then an equal volume of the same dissolution medium was added back to maintain a constant volume.

In this study, the effect of various factors on 8-HQ release profile consist of

1. Crosslink and non-crosslink chitosan films
2. Chitosan concentration
   - Release studies of 8-HQ that the film fixed concentration of 8-HQ (30%) and various concentration of chitosan 2 and 3% w/v.
3. 8-HQ concentration
   - Release studies of 8-HQ that the film fixed concentration of chitosan (3%) and various concentrations of 8-HQ 20 and 30% w/w.
4. TPP concentration
   - Release studies of 8-HQ that film had 30% 8-HQ, 3% chitosan and various amounts of TPP as crosslinker 3 and 5% w/v.
5. Crosslink time
   - Release studies of 8-HQ that the film had 30% 8-HQ, 3% chitosan and

5% TPP and various crosslink times 30, 60 and 180 min.

3.4.7 Swell studies [20]

Weigh the film pieces, 2 cm x 2 cm (*ca*. 0.3 g) and immersed in a definite volume of water at definite pH=5.5±0.5 and temperature at 25±1.0 ºC, then took out at time intervals (1, 5, 10, 20, 30, 60, 120, 180 and 300 min). The swollen film was gently pressed with filter papers to remove excess water and finally weighed the film. The swelling ratio was calculated by the following equation.

$$\text{Swelling} = \frac{\text{Wt. of swollen films } (W_f)}{\text{Wt. of dry films } (W_d)}$$

where W_f and W_d were the weights of swollen and dry films at interval time, respectively.

3.4.8 Efficacy studies of prolongation of vase- life [10]

Cut roses (*Rosa hybrid* L.) cv. Grand Gala were selected in this study. Roses were chosen with the in same size and fixed date of cut off rose 3 days. Rose stems were trimmed to 38 cm, and all leaves except for the upper two were removed. Cut roses were placed in each of 600 mL bottles with 400 mL of tap water (controlled) and other concentrations of 8-HQ in sugar solution as following.

3.4.8.1 1st experiment of prolongation of vase-life

3.4.8.1.1 The effect of tap water, 10 and 20% w/v of sugar on rose vase life

Added 400 ml of each solution (tap water, 10 and 20 % w/v of sugar) into the vase, then placed a rose into the vase.

3.4.8.1.2 The effect of 8-HQ in tap water and 20% sugar solution on rose vase life

Added 400 mL of each solution such as 400 ppm of 8-HQ in tap water and 400 ppm 8-HQ in 20% sugar, then placed a rose into the vase.

3.4.8.1.3 The effect of non-crosslink and TPP crosslink film on rose vase life

Added 400 mL of 20% w/v sugar plus 200 ppm of 8-HQ loaded crosslink chitosan films and non-crosslink chitosan films in 20% sugar, then placed a rose into the vase.

3.4.8.1.4 The effect of crosslink time on rose vase life

Added 400 mL of 20% sugar plus 200 ppm of 8-HQ loaded crosslink chitosan films with difference of crosslink time as 30, 60 and 180 min, then placed a rose into the vase.

All this experiment had to keep condition at 25±2ºC and humidity of 45± 5%. The end of vase-life was investigated the decline of rose showed symptoms of petal wilting, stem bending (≥90ºC) or breaking and relative fresh weight [1] over the first five days of the experiments.

3.4.8.2 2nd experiment of prolongation of vase-life

Placed a rose into the vase contained various solution from 1st experiment. Record the end of vase-life and relative fresh weight of roses in several parameters solutions as below;

3.4.8.2.1 The effect of tap water, 10 and 20% w/v of sugar solution on rose vase life

3.4.8.2.2 The effect of 8-HQ in tap water and 20% sugar solution on rose vase life

3.4.8.2.3 The effect of non-crosslink and TPP crosslink film on rose vase life

3.4.8.2.4 The effect of crosslink time on rose vase life

3.4.9 Relative fresh weight [1]

Weighted the rose in each vase and recorded. The relative fresh weight was calculated by following equation.

% Relative fresh weight = $W_t / W_{t=0}$ X100

W_t = fresh weight of rose in 2, 3, 4 and 5 days

$W_{t=0}$ = fresh weight of rose in first

CHAPTER IV
RESULTS AND DISCUSSION

This research explores the preparation, loading efficiency and controlled release of 8-HQ from chitosan and alginate beads. The prepared beads however contained a little amount of 8-HQ. Thus, a new approach was examined by preparing of 8-HQ/chitosan as film. Nonetheless, the study on control release has never been researched. Several parameters such as the amount of chitosan, concentration of TPP and crosslink time were investigated. Physical properties of prepared chitosan films were characterized by XRD, FTIR and SEM. The prepared chitosan films were eventually investigated for 8-HQ loading efficiency, their swelling, releasing of 8-HQ, and rose vase life.

4.1 8-HQ loading efficiency

The evaluation of 8-HQ loading efficiency into chitosan and alginate was analyzed using HPLC. The content of 8-HQ was determined from its supernatant. Table 4.1 shows 8-HQ loading efficiency of two different polymers: the beads containing 3% chitosan and 30% 8-HQ (%8-HQ loading efficiency of 2.1%), and those with 6% alginate and 30% 8-HQ (%8-HQ loading efficiency of 2.6%).

Table 4.1 8-HQ loading efficiency of chitosan and alginate beads.

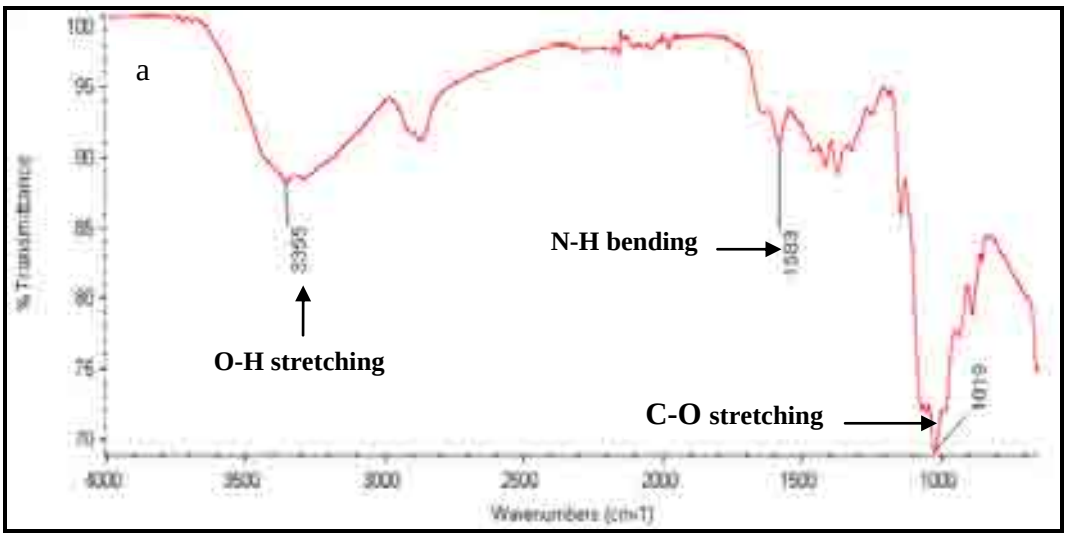
Formulation	%8-HQ loading efficiency	
	in bead	in crosslink solution
30% 8-HQ loaded in chitosan beads	2.06 ± 0.11	28.27 ± 0.27
30% 8-HQ loaded in alginate beads	2.62 ± 0.36	26.80 ± 0.47

The amounts of 8-HQ in crosslink solution: 28.3 and 26.8% were analyzed by HPLC from chitosan and alginate beads, respectively (Table 4.1). The results revealed low % 8-HQ loading in both chitosan and alginate beads. This was mainly because 8-HQ was slightly soluble in H_2O, thus the process for preparing the beads had to alter

by dissolving 8-HQ in EtOH and added into polymer solution (3% chitosan and 6% alginate solutions); however, this solution was still non homogeneous. When the former solution was dropped into the crosslink solution, 8-HQ was precipitated out resulting that little 8-HQ was encapsulated in the beads. Therefore, the encapsulation using this mentioned procedure was not successful. The preparation and controlled release of 8-HQ as film was then selected for further investigation.

4.2 FT-IR analysis

FT-IR was used to determine the interaction between chitosan and 8-HQ. Figure 4.1 shows the FT-IR spectra of chitosan, 8-HQ, and 8-HQ loaded in crosslink chitosan film. The FT-IR spectra of chitosan showed in Figure 4.1(a) by the broad brand at 3355 cm^{-1} was O-H stretching, while the band at 1583 cm^{-1} was N-H bending and a peak at 1019 cm^{-1} was C-O stretching. According to the FT-IR spectrum of 8-HQ (Figure 4.1 (b)), the characteristic absorption band at 3038 cm^{-1} was O-H stretching, while the band at 1573 cm^{-1} was assigned to C=C stretching of aromatics. In Figure 4.1(c), the presence of both chitosan and 8-HQ was detected, *i.e.*, C=C stretching at 1540 cm^{-1} which was absent in the spectrum of pure chitosan. All major peaks of chitosan were found in the mixed system. This could be an evidence that 8-HQ was loaded in chitosan film.



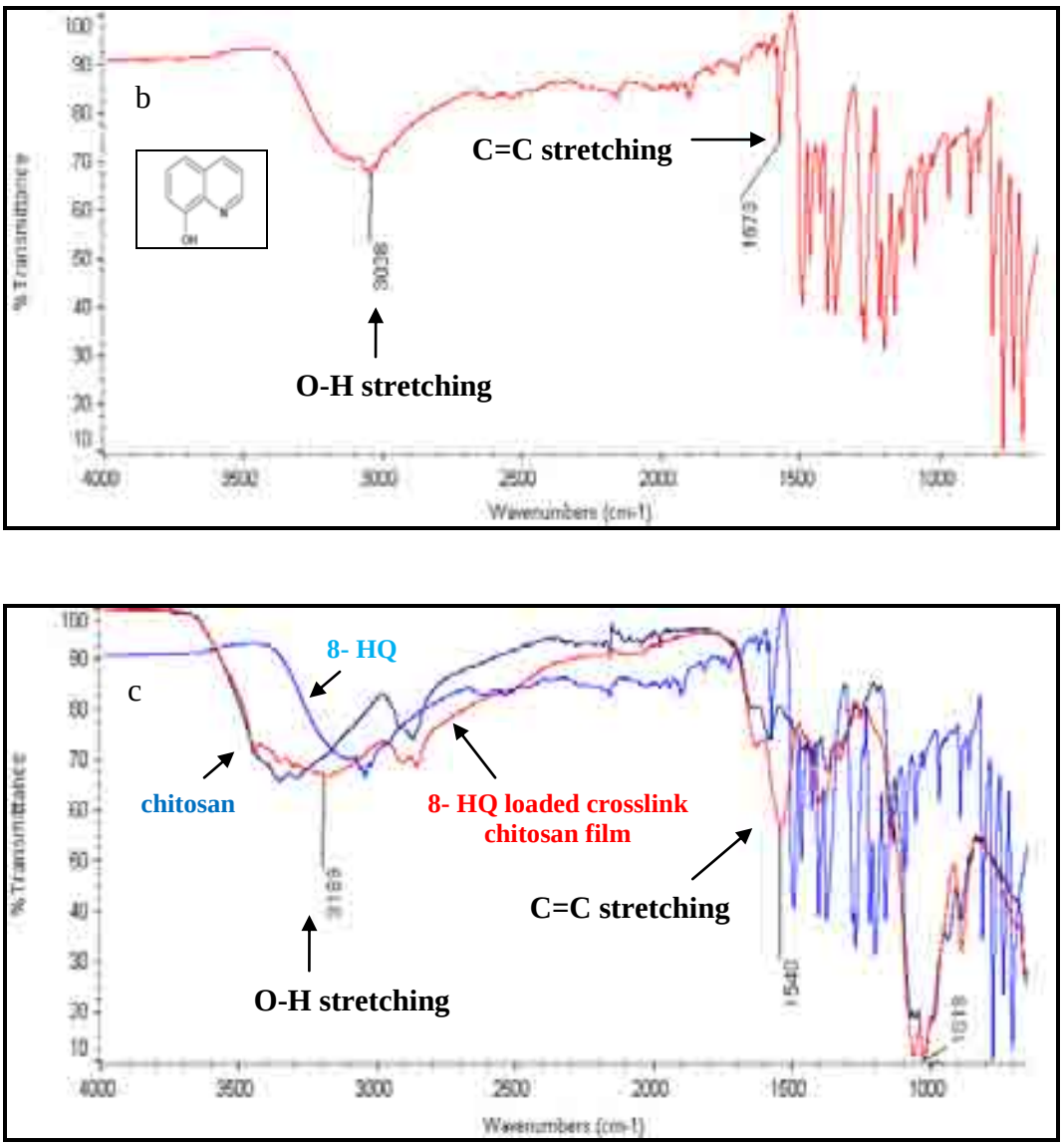
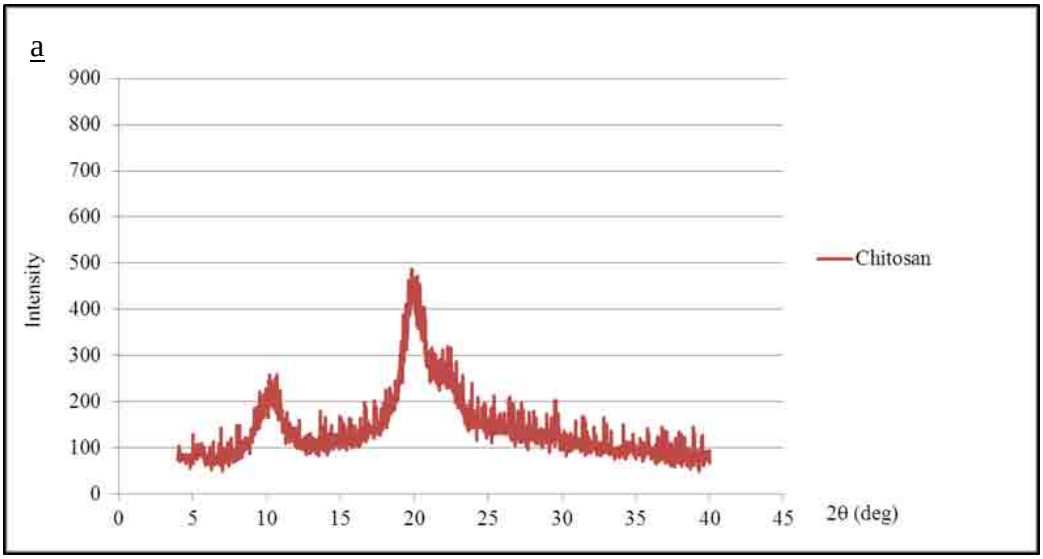


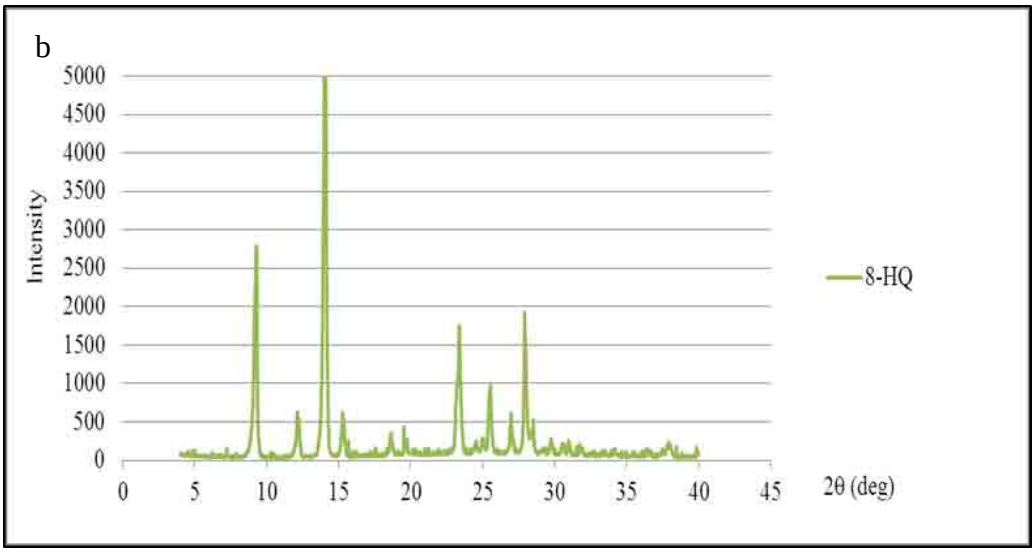
Figure 4.1 The FT-IR spectrums of chitosan (a), 8-HQ (b) and FT-IR spectrums including of chitosan, 8-HQ and 8-HQ loaded crosslink chitosan films (c).

4.3 X-ray diffraction studies

The comparison of X-ray diffractograms of chitosan, 8-HQ and 8-HQ loaded crosslink chitosan films are shown in Figure 4.2. As observed, the diffractograms of chitosan consisted of two typical crystalline peaks at 2θ = 10.15º and 19.63º (Figure 4.2 (a)), while 8-HQ had typical crystalline peaks at 2θ = 9.25º, 12.11º, 14.07º, 15.27º, 23.35º, 26.95º and 27.91º (Figure 4.2 (b)). From the X-ray diffraction patterns of 8-

HQ loaded crosslink chitosan film (Figure 4.2 (c)), 8-HQ had specific sharp peaks and chitosan had a broad peak, indicating the presence of crystalline and amorphous structure, respectively. This indicated that when 8-HQ was mixed into chitosan, sharp crystalline peaks disappeared. This could be explained that 8-HQ dispersed in chitosan chains and caused crystallinity decrease.





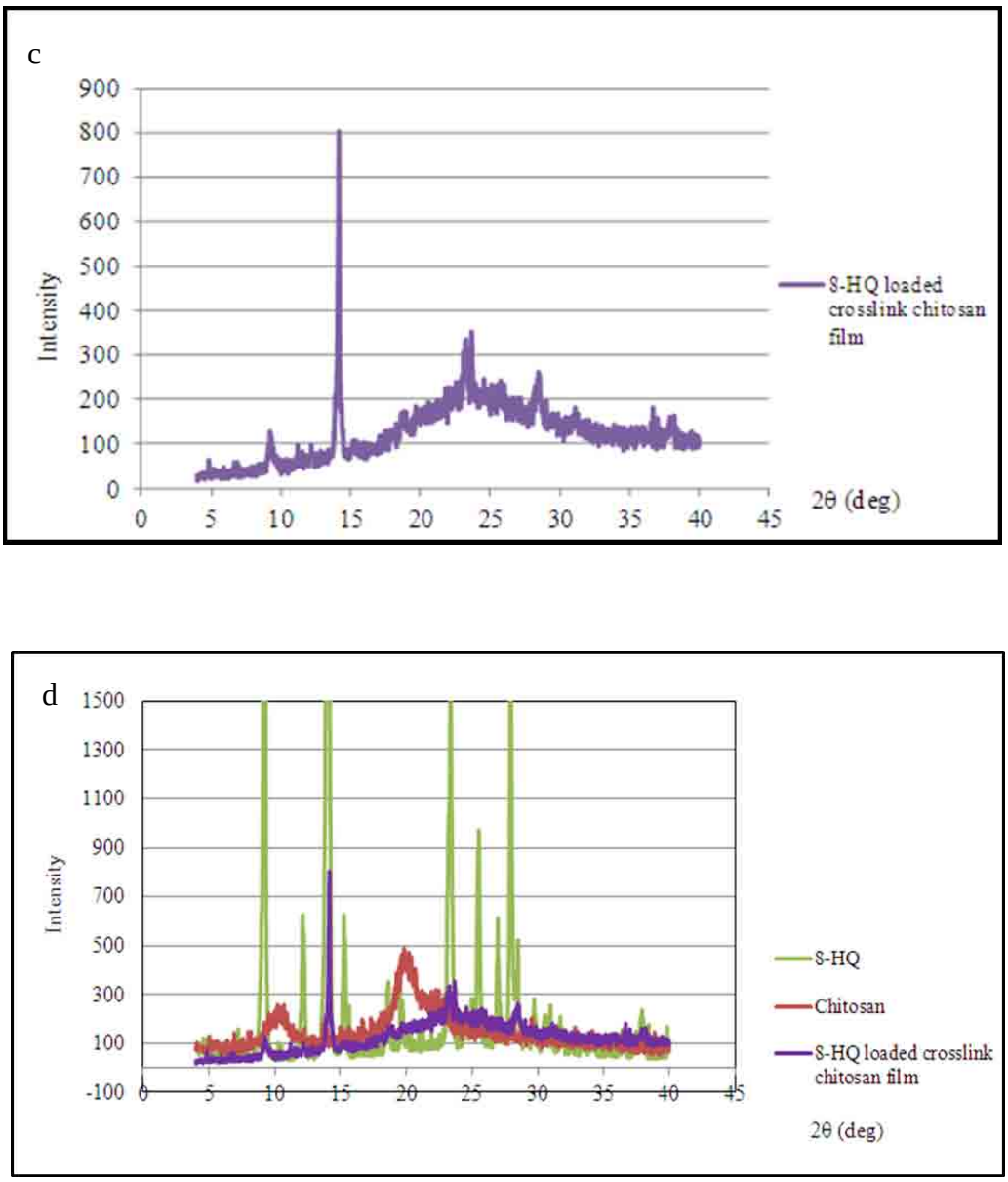


Figure 4.2 The X-ray diffractograms of chitosan (a), 8-HQ (b), 8-HQ loaded crosslink chitosan film (c) and X-ray diffractograms including of chitosan, 8-HQ and 8-HQ loaded crosslink chitosan films.

4.4 Scanning electron microscopy studies

The incorporation of 8-HQ in chitosan films resulted in a significant change of the surface and cross-section morphologies. The micrographs of 8-HQ loaded chitosan films showed large pore of 8-HQ in loaded chitosan films (Figure 4.3B and 4.4 B), while that of the chitosan films was smooth and homogeneous, with the

absence of pores on chitosan films (Figure 4.3A and 4.4A). The micrographs of 8-HQ loaded crosslink chitosan films with crosslink time as 30 min (Figure 4.3C and 4.4C) showed porous structure of 8-HQ loaded chitosan films, whereas Figure 4.3D and 4.4D showed the surface of used 8-HQ loaded crosslink chitosan films which were coarse and porous.

The cross-section morphologies of chitosan film in Figure 4.5A and 4.6A were smooth and quite dense. However, the incorporation of 8-HQ loaded chitosan film (Figure 4.5B,C and 4.6B,C) resulted in significant change of cross-section morphologies. The cross-section morphologies of 8-HQ loaded chitosan film showed more porous than in original chitosan (Figure 4.5D and 4.6D).

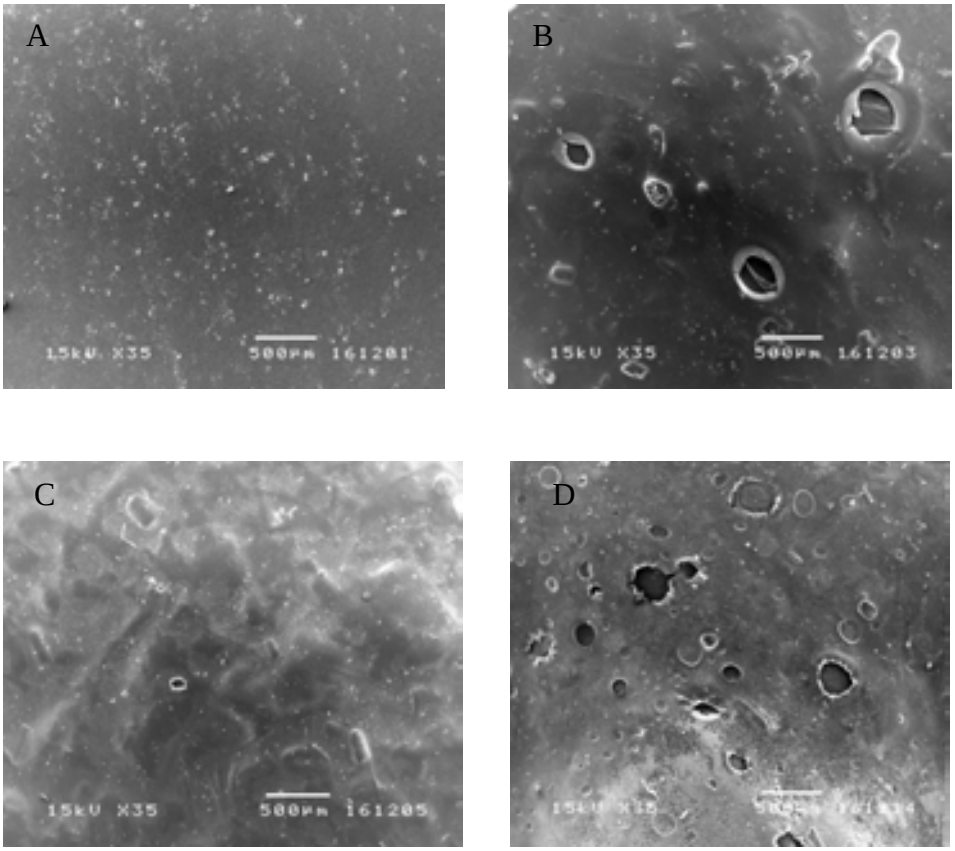


Figure 4.3 SEM micrographs of films (35X): chitosan films (A), 8-HQ loaded chitosan films (B), 8-HQ loaded crosslink chitosan films (C), and used 8-HQ loaded crosslink chitosan films (D).

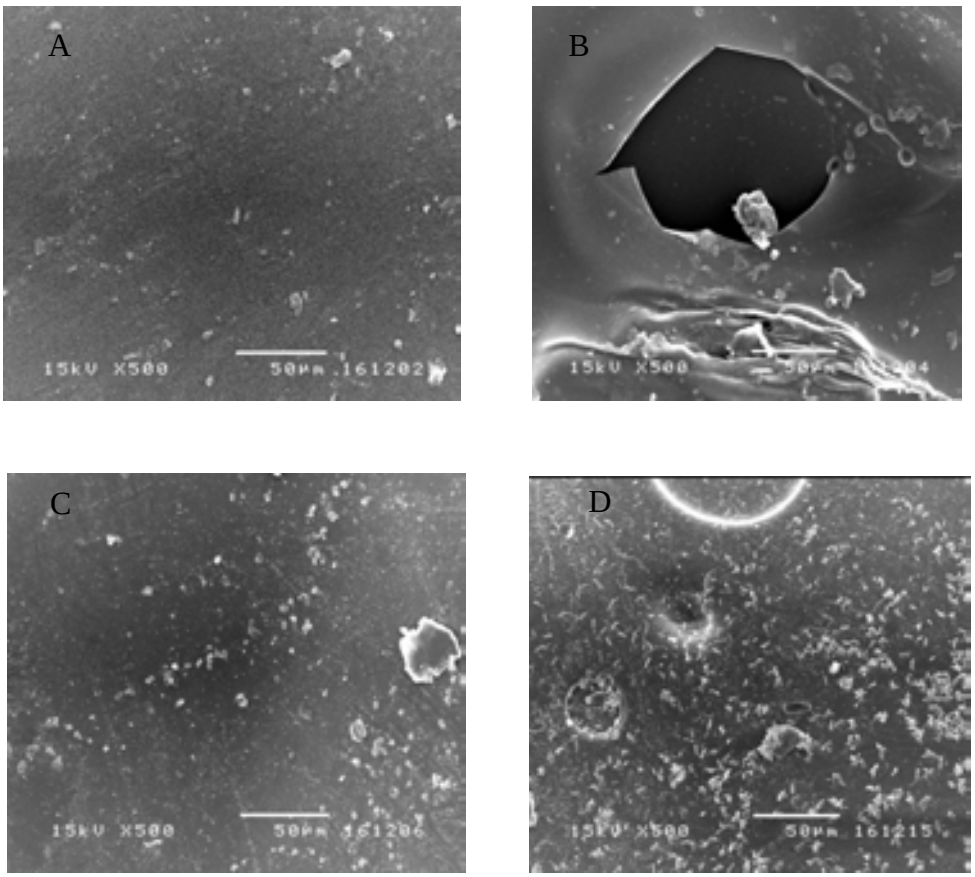


Figure 4.4 SEM micrographs of films (500X): chitosan films (A), 8-HQ loaded chitosan films (B), 8-HQ loaded crosslink chitosan films (C), and used 8-HQ loaded crosslink chitosan films (D).

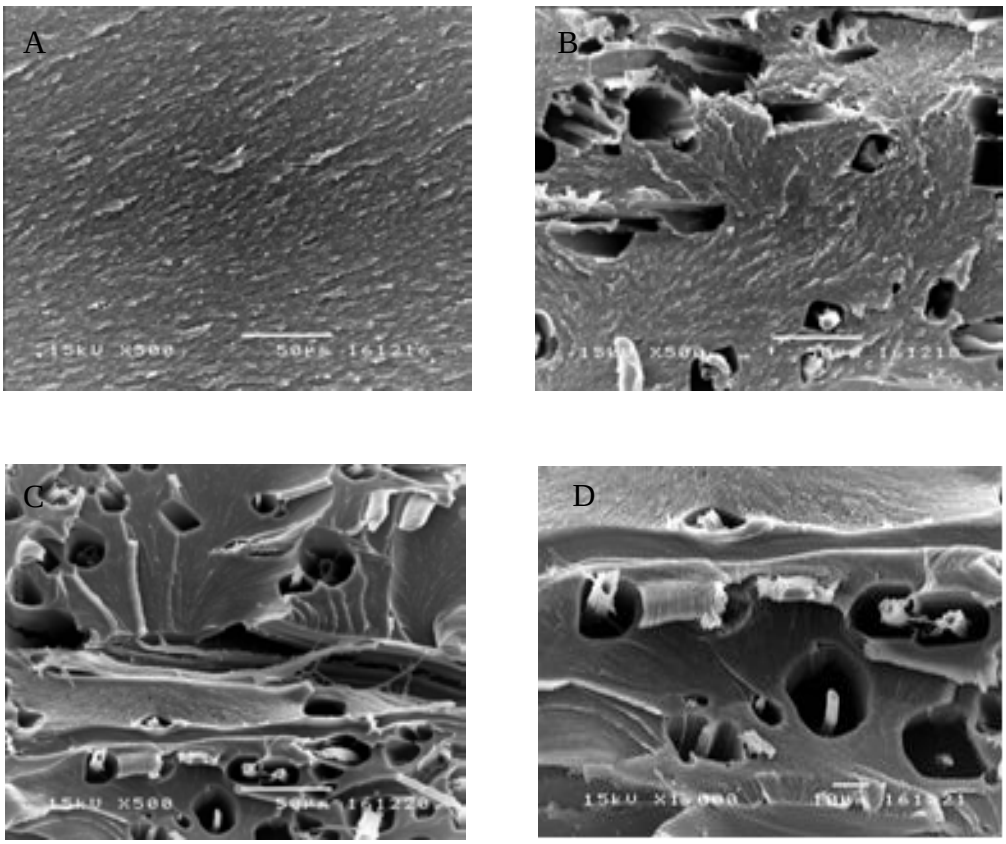


Figure 4.5 The cross-section morphology of films (500X): chitosan films (A), 8-HQ loaded chitosan films (B), 8-HQ loaded crosslink chitosan films (C), and used 8-HQ loaded crosslink chitosan films (D).

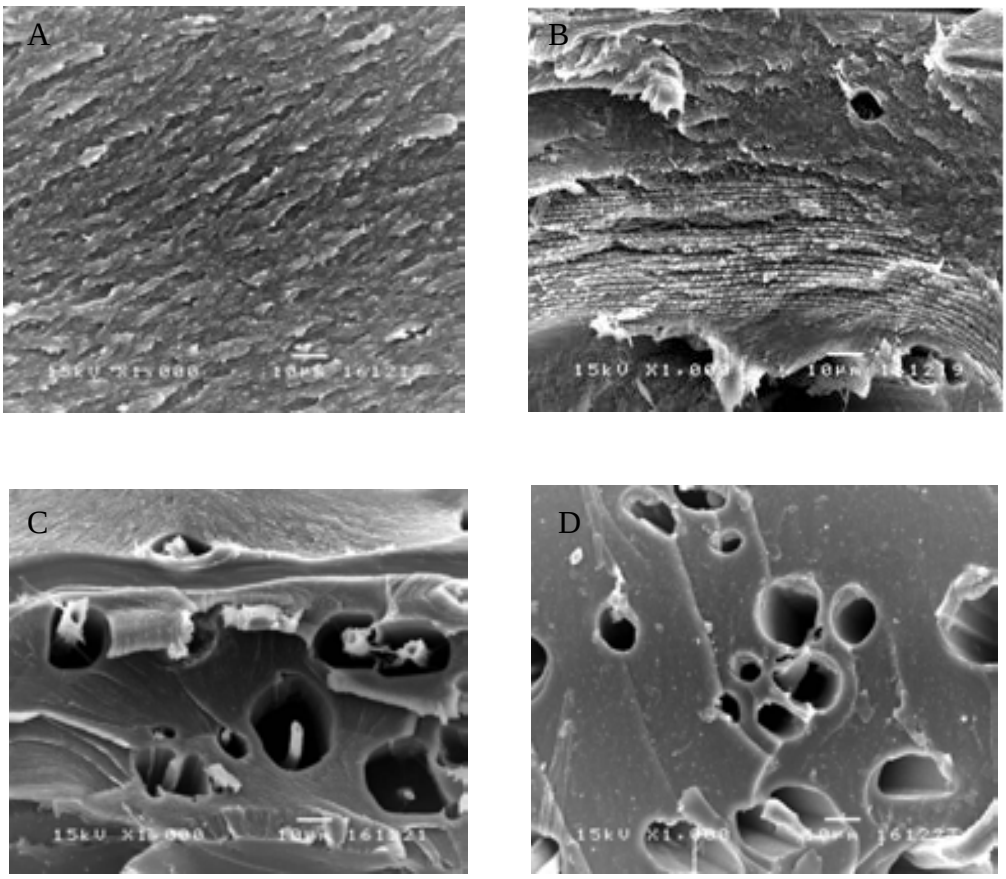


Figure 4.6 The cross-section morphology of films (1000X): chitosan films (A), 8-HQ loaded chitosan films (B), 8-HQ loaded crosslink chitosan films (C) and used 8-HQ loaded crosslink chitosan films (D).

4.5 8-HQ loading efficiency studies

8-HQ loading efficiency study, the concentration of chitosan: 1, 2 and 3%w/v was varied, while the amount of 8-HQ was fixed at 30%. The evaluation of 8-HQ loading efficiency into chitosan was analyzed using HPLC. The 8-HQ content was determined from its supernatant. The results are presented in Table 4.2.

Table 4.2 8-HQ loading efficiency in chitosan films.

Entry	Concentration		% 8-HQ loading efficiency	
	Chitosan (%w/v)	8-HQ (%w/w)	Theory	Experimental
1	1	30	33.03	15.01±0.34
2	2	30	29.79	22.19±0.38
3	3	30	30.23	28.41±0.48
4	3	10	10.69	7.35± 0.33
5	3	20	20.03	15.72±0.41
6	3	30	30.23	28.41±0.47

Table 4.2 reveals that % 8-HQ loading in chitosan film increased from 15.0, 22.2 and 28.4%, respectively when the amount of chitosan increased. When the chitosan concentration was fixed at 3% and varied the amount of 8-HQ from 10, 20 and 30% w/w, the content of 8-HQ in matrix film was increased with % 8-HQ loading in chitosan film from 7.4, 15.7 and 28.4% w/w, respectively. Thus, the optimum conditions for the preparation of 8-HQ loaded chitosan film were 30% 8-HQ and 3%w/v chitosan. This can be explained by the fact that the high concentration of chitosan caused the polymer network rigid resulting in decreasing the release amount of 8-HQ outside the film.

4.6 Release studies

The release profile of 8-HQ from matrix film chitosan was analyzed using HPLC. The 8-HQ release from film was plotted as function of time.

4.6.1 Effect of chitosan concentration

The concentration of chitosan was one of major factors to influence the release of 8-HQ. The effect of chitosan concentration on the release of 8-HQ was explored as presented in Figure 4.7.

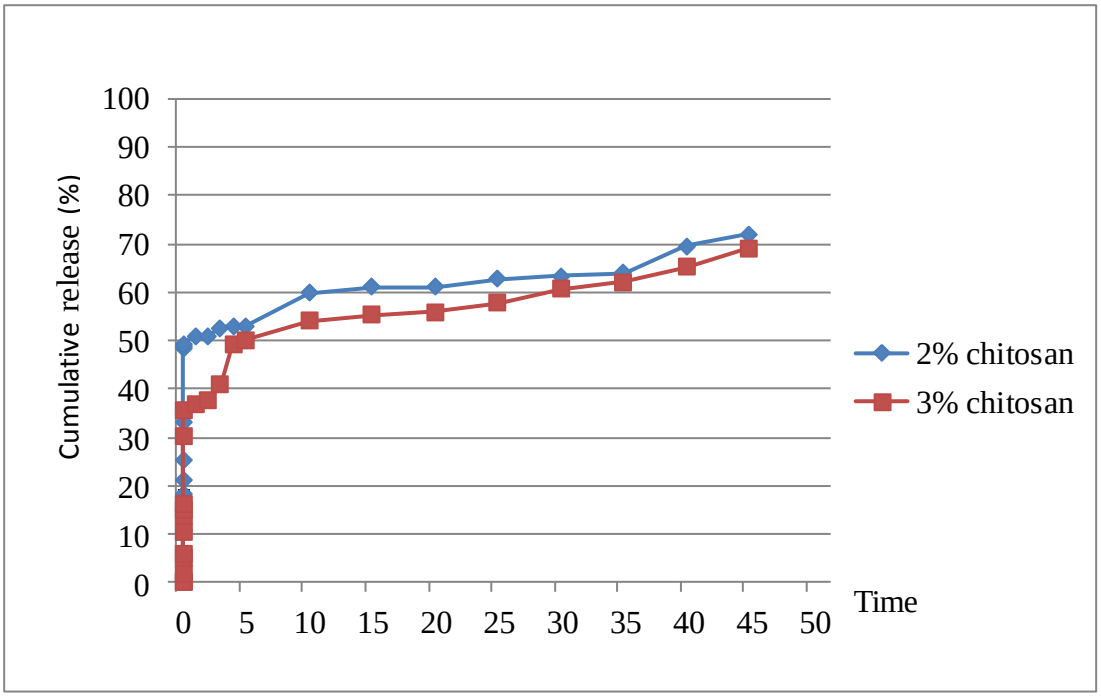


Figure 4.7 The effect of chitosan concentration on release of 8-HQ from 30% 8-HQ loaded chitosan films.

The release of 8-HQ was found to prolong when the concentration of chitosan increased. For example, when the concentration of 8-HQ was fixed and the concentration of chitosan was increased from 2 to 3% w/v, the release of 8-HQ was slightly decreased from 72.2 to 69.1% in 45 days. The phenomenon could be explained by the fact that increasing chitosan concentration made the film more strength, and reduced the pore size of polymer network.

4.6.2 Effect of the amount of 8-HQ in loaded chitosan films

The effect of amount of 8-HQ in loaded chitosan films on the release of 8-HQ. When the concentration of 8-HQ increased, the release profile of 8-HQ at fixing concentration of chitosan (3%w/v), was 77.1, 69.1%w/v within 45 days with the concentrations of 8-HQ 20 and 30%, respectively (Figure 4.8). The prolongation of the release profile from the chitosan films with increasing 8-HQ concentration could be explained that 8-HQ loaded into chitosan films was encapsulated with polymer when increased 8-HQ concentration, the period of release profile was thus longer than that at low concentration of 8-HQ.

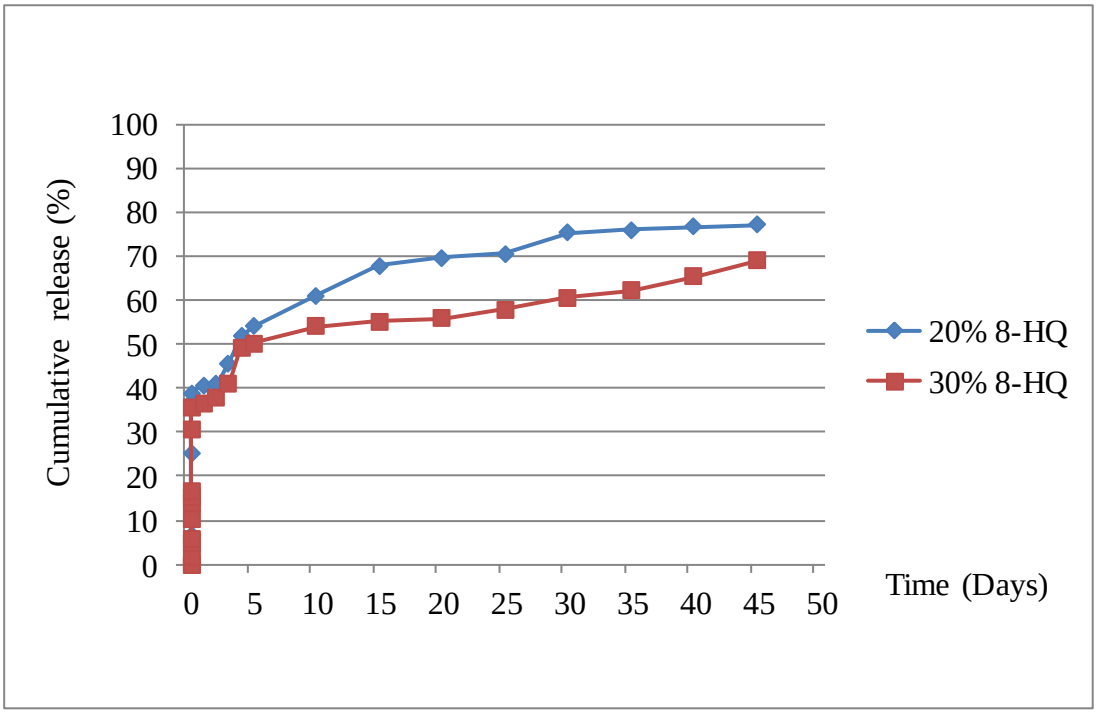


Figure 4.8 Effect of 8-HQ concentration on the release of 8-HQ loaded 3% chitosan films.

4.6.3 Effect of crosslinker

The comparison of the release rate of 8-HQ in tripolyphosphate (TPP) crosslink and non-crosslink chitosan film exhibited that the crosslink chitosan film could prolong the release of 8-HQ more than non-crosslink chitosan film with fixing 8-HQ concentration (30%), chitosan concentration (3%) and crosslink time (60 min). The release of 8-HQ was 56.0 and 69.1% within 45 days with crosslinking chitosan film and non-crosslink chitosan film, respectively (Figure 4.9). This could be explained that TPP crosslink chitosan film was more stable than non-crosslink film because the rigid form of chitosan structure with physical entanglement of cross-linked structure make chitosan film stronger.

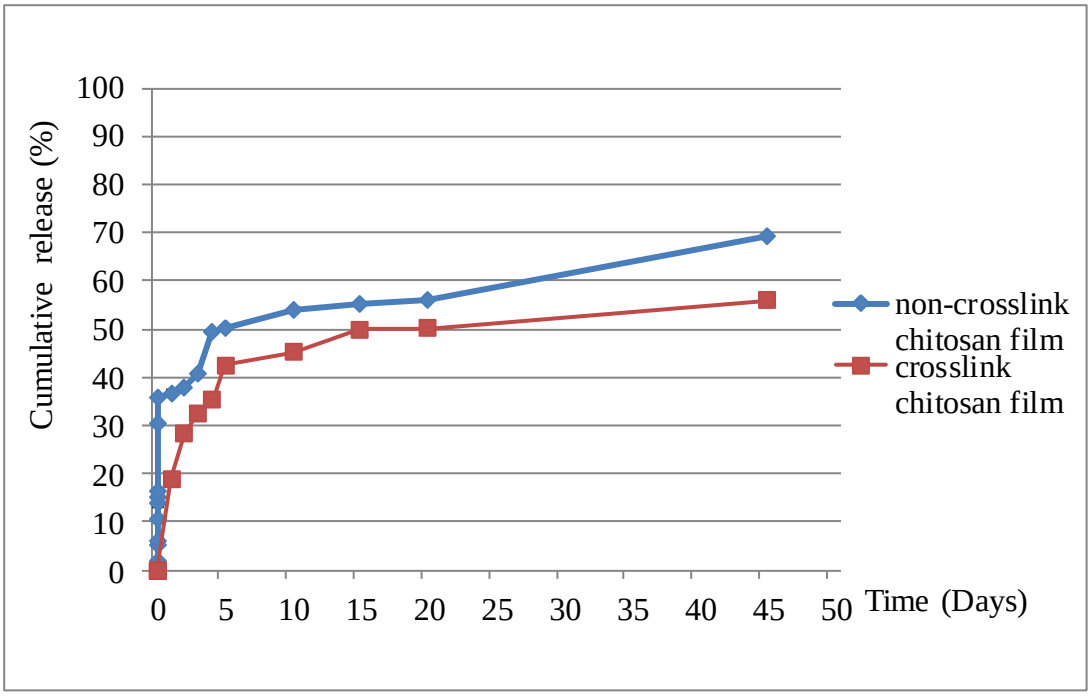


Figure 4.9 Effect of TPP crosslink chitosan film on the release of 30% 8-HQ loaded 3% chitosan films.

4.6.4 Effect of TPP concentration as crosslinker

The data reported in Figure 4.10 show the release of 8-HQ loaded chitosan-films with different TPP concentrations at 30 min. The release could be controlled with increasing TPP concentration. For example, if the concentration of 8-HQ and that of chitosan was fixed and the amount of TPP was increased from 3 and 5% w/v, the release of 8-HQ was slightly decreased from 63.1 and 57.9% within 45 days. This could be explained by the structure of films. At low concentration of TPP, 8-HQ could easily be released from films. The more TPP was added, the more strength of films was observed. The physical entanglement of crosslink chitosan-TPP had a profound effect on controlling the release of 8-HQ from films.

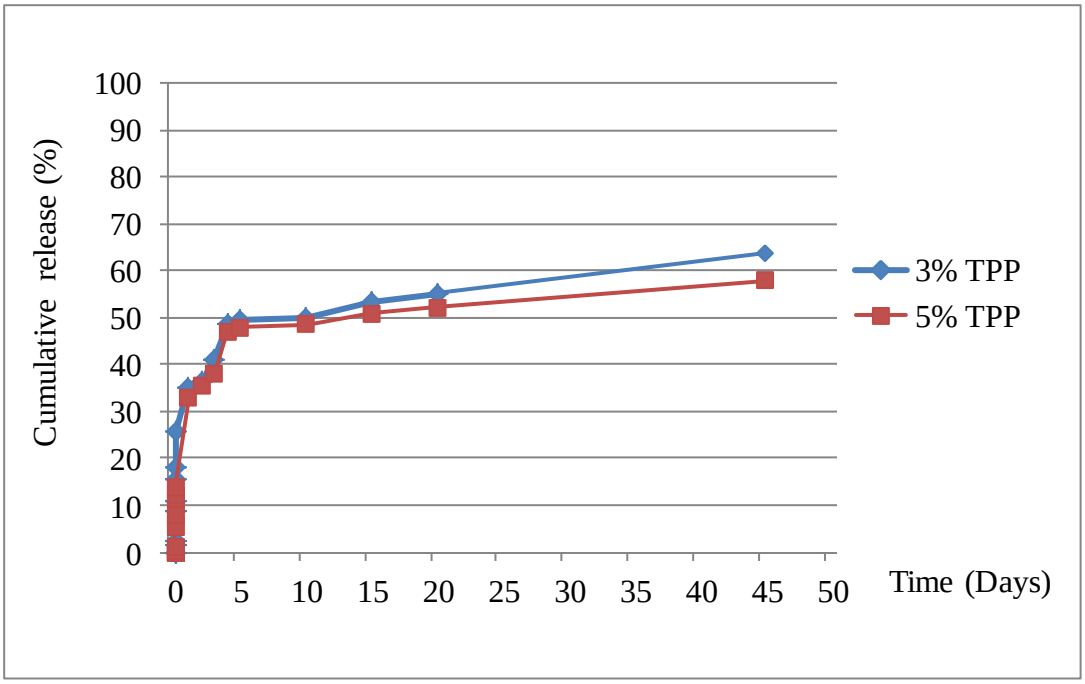


Figure 4.10 Effect of TPP concentration on the release of 30% 8-HQ loaded 3% chitosan films.

4.6.5 Effect of crosslink time

The time of crosslink was concerned the 8-HQ loading efficiency and release profile of films. The loss of 8-HQ in process of crosslink (time of contact with TPP solution) was be showed in Table 4.3. The results showed if the crosslink time was increased, the loss of 8-HQ in the chitosan film was increased by fixing 8-HQ concentration at 30% and chitosan concentration at 3% w/v the loss of 8-HQ from chitosan film was 4.5, 5.5 and 7.8% with 30, 60 and 180 min, respectively. This could be explained that 8-HQ release from chitosan film occurred during the period of crosslink film process.

Figure 4.11 shows the effect of crosslink time on the release of 8-HQ. At 30% 8-HQ, 5%w/v TPP, and 3% w/v chitosan, there was difference in releasing of 8-HQ at the beginning period (0-20 days). If the crosslink time was increased, the release of 8-HQ was prolonged. After 20 days, the release of 8-HQ was nearly the same: 57.89, 55.98 and 53.82% with crosslink time of 30, 60 and 180 min, respectively (Figure 4.11).

The prolonged release was found when increasing crosslink time. This could be explained that the structure of film was depended on degree of crosslink formed in the matrix, causing a slow release of 8-HQ. The crosslink time of 30 min was chosen to be an optimum crosslink time for maximum 8-HQ loading efficiency.

Table 4.3 % Loss of 8-HQ in crosslink solution

Crosslink time (min)	% 8-HQ loading		% Loss of 8-HQ in crosslink solution
	Theory	Experimental	
30	30.20	25.64± 0.35	4.47 ± 0.40
60	30.20	24.52±0.45	5.53± 0.55
180	30.20	22.38±0.42	7.76± 0.37

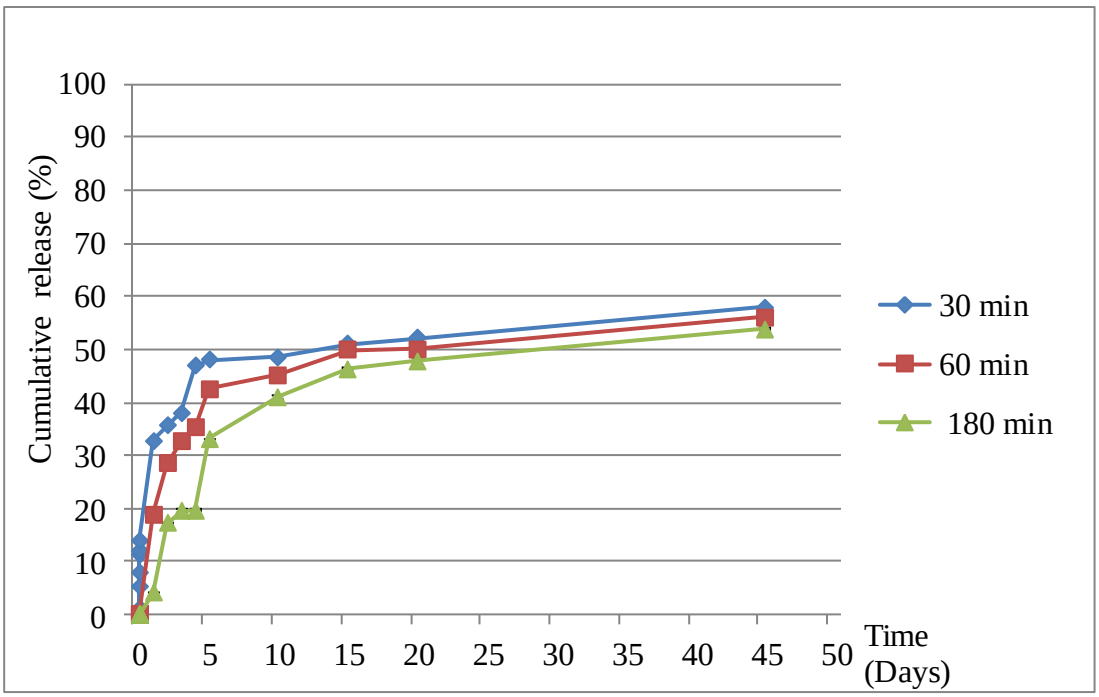


Figure 4.11 Effect of crosslink time on the release of 30% 8-HQ loaded 3% chitosan films.

4.7 Swelling studies

The swelling behavior of chitosan films was examined with different crosslink times. Using short crosslink time (30 min), a maximum degree of swelling

(559%w/w) was obtained, whereas the medium and long crosslink time (60 and 180 min) exhibited a maximum degree of swelling of 336 and 155% w/w, respectively (Figure 4.12).

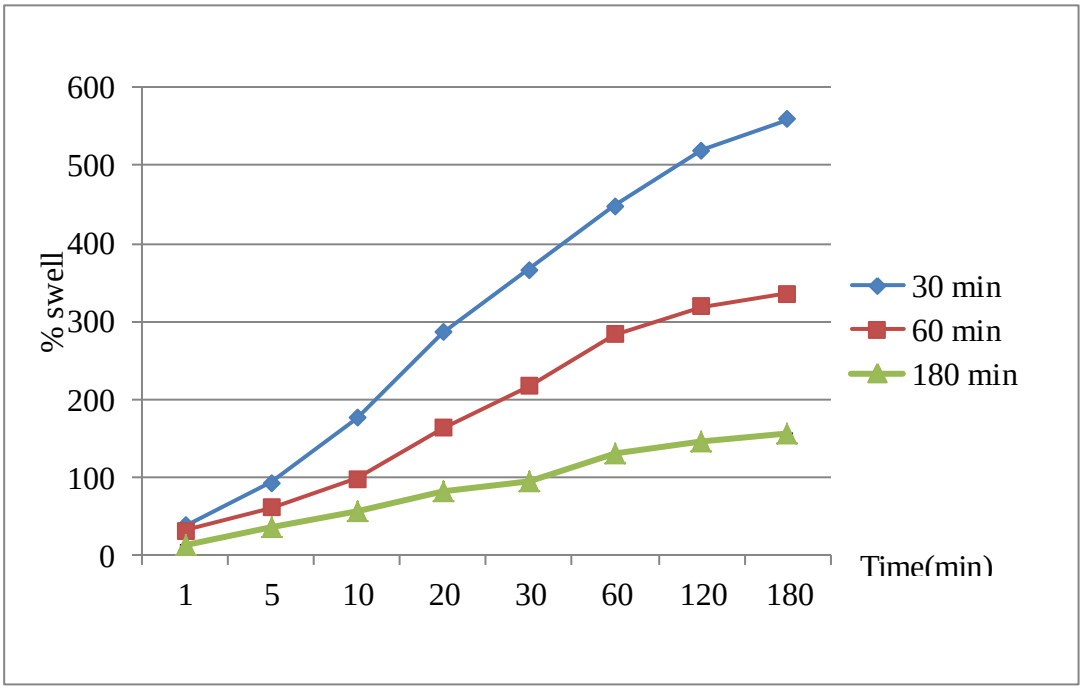


Figure 4.12 Effect of crosslink time in 5% TPP solution on 30% 8-HQ loaded 3% chitosan films.

These studies clearly indicated that the crosslink time controlled the degree of swelling through crosslinking. The film with 30 min of crosslink time was less stable because the structure of film had weak crosslinking between TPP and chitosan. Thus, water could penetrate inside the films and caused a great swelling degree.

4.8 Prolongation of rose vase life

4.8.1 The 1st experiment of prolongation of rose vase life

4.8.1.1 The effect of tap water, 10 and 20% w/v of sugar on rose vase life

The prolongation of rose vase life was comparatively investigated using tap water, 10 and 20% w/v of sugar. The results displayed that using tap water and 10% sugar could extend vase life of rose for 3 days, while using 20% sugar could extend vase life of rose for 6 days (Table 4.4 and Figure B-1).

4.8.1.2 The effect of 8-HQ in tap water and 20% sugar solution on rose vase life

From a previous experiment (topic 4.8.1.1), 20% sugar solution could extend rose vase life better than using 10% sugar solution and tap water. However, using sugar alone in preservative solutions promoted microbial growth. Another set of experiments was then set up to compare the use of 400 ppm of 8-HQ in tap water and that in 20% sugar. The results are presented in Figure B-2.

Using 400 ppm of 8-HQ in 20% sugar solution could extend rose vase life significantly for 8 days, whereas using 400 ppm of 8-HQ in tap water could extend rose vase life for 6 days (Table 4.4)

4.8.1.3 The effect of non-crosslink and TPP crosslink film on rose vase life

From topic 4.8.1.2, using 400 ppm of 8-HQ in 20% sugar solution could prolong rose vase life more than using 400 ppm of 8-HQ in tap water. This experiment aimed to comparative study focusing on reducing the concentration of 8-HQ from 400 to 200 ppm, and on the use of crosslink and non-crosslink films. The results are depicted in Figure B-3

The results displayed that using 200 ppm of 8-HQ could prolong rose vase life up to 10 days. This observation was similar to the situation using 400 ppm of 8-HQ in 20% sugar solution. In the case of using 200 ppm of 8-HQ loaded on non-crosslink chitosan film, rose vase life could be extended for 6 days (Table 4.4). Another independent experiments employing 400 ppm of 8-HQ loaded crosslink chitosan film did not exhibit significant outcome of rose vase life. Approximately 8 days of rose with end of vase-life were defined as the time that flowers showed symptoms of petal wilting or curling, stem bending ($\geq 90^{\circ}$) or breaking.

A main parameter for wilting of roses concerned with bacteria which caused vascular blockage. Thus, 8-HQ may reduce the block of xylem vessels. 49% of 8-HQ was detected in the solution containing loaded crosslink chitosan film at 10^{th} day, while that of 8-HQ loaded in non-crosslink film was 54% at 10^{th} day and approximate 35% in 6^{th} day.

4.8.1.4 The effect of crosslink time on rose vase life

This experiment examined the effect of crosslink time on rose vase life using 200 ppm of 8-HQ in 20% sugar solution. The results are presented in Figure B-4.

Three types of crosslink chitosan films were prepared using different crosslink times: 30, 60 and 180 min. Using 200 ppm of 8-HQ, the films using 30 and 60 min crosslink time exhibited similar results to prolong rose vase life for 10 days, while that obtained from 180 min crosslink time gave worse result (8 days prolongation) (Table 4.4).

The amount of 8-HQ in the solution at 10^{th} day was analyzed to be 49, 45 and 41% for crosslink chitosan films of crosslink time 30, 60 and 180 min, respectively.

Table 4.4 Effect of different holding solution on the vase life of rose for 1^{st} experiment.

Holding solution	Vase life (days)
Tap water	3
10% sugar	3
20% sugar	6
400 ppm 8-HQ in 20 % sugar	8
400 ppm 8-HQ in tap water	6
200 ppm 8-HQ loaded crosslink chitosan film in 20% sugar	10
200 ppm 8-HQ loaded non crosslink chitosan film in 20% sugar	6
200 ppm 8-HQ loaded crosslink chitosan film in 20% sugar (crosslink time 30 min)	10
200 ppm 8-HQ loaded non crosslink chitosan film in 20% sugar (crosslink time 60 min)	10
200 ppm 8-HQ loaded non crosslink chitosan film in 20% sugar (crosslink time 180 min)	8

The effect of different concentrations of 8-HQ, sugar solution and crosslink time of films on relative fresh weight are presented in Figure 4.13.

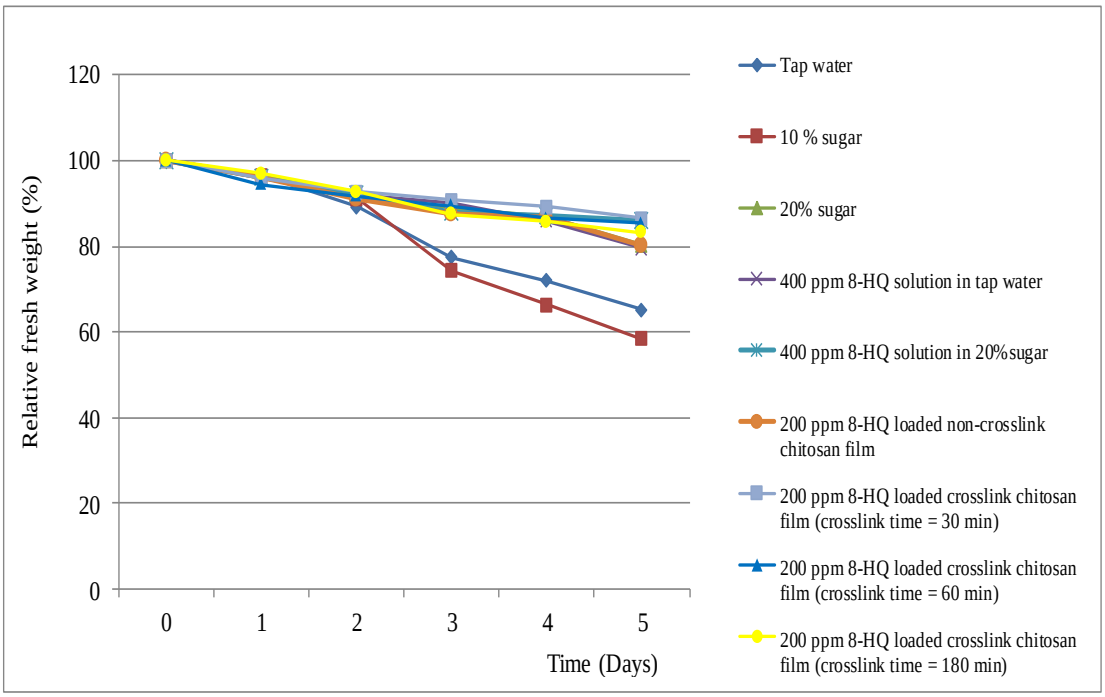


Figure 4.13 Effect of different concentrations of 8-HQ, sugar solution and crosslink time of films on relative fresh weight during first five days of rose vase life (1st experiment)

There was significant observation in relative fresh weight of rose in several solutions. The relative fresh weight of roses in 10% sugar was the lowest, whereas that in tap water was less than 20% sugar. The relative fresh weight of rose in 400 ppm 8-HQ in tap water was less than that in 20% sugar and in the solution containing 400 ppm 8-HQ in 20% sugar, but similar to that containing 200 ppm 8-HQ loaded non-crosslink chitosan film.

Compared with the solution containing 200 ppm of 8-HQ loaded crosslink chitosan film (30, 60 and 180 min), the relative fresh weight of rose was similar. This result could be explained that 8-HQ affected on the prolongation of rose vase life. 8-HQ could reduce the blockage of vessel due to 8-HQ forming complex with metal ion and 8-HQ as antibacterial agent [2].

4.8.2 The 2nd experiment of prolongation of rose vase-life

4.8.2.1 The effect of tap water, 10 and 20% w/v of sugar solution on rose vase life

The 2nd set of experiments was performed using the original solution from the 1st experiment to observe the prolongation of rose vase life. The results are presented in Figure B-5.

Using tap water and 10% sugar solution could prolong rose vase life for 3 days, while using 20% sugar of solution could extend the vase life of rose for 4 days (Table 4.5). However, the turbid white of sugar solution with bad smell was observed.

4.8.2.2 The effect of 8-HQ in tap water and 20% sugar solution on rose vase life

The 2nd set of experiments was performed using the original solution from the 1st experiment to observe the prolongation of rose vase life. The results are presented in Figure B-6.

Using 400 ppm of 8-HQ in 20% sugar could extend rose vase life for 4 days, while that in tap water could prolong rose vase life for 3 days (Table 4.5).

4.8.2.3 The effect of non-crosslink and TPP crosslink film on rose vase life

In this 2nd set of experiment, the use of 400 ppm of 8-HQ in 20% sugar (Figure B-6) could extend vase life more than that in tap water. In addition the reduction of the concentration of 8-HQ to 200 ppm and the effect of crosslink and non-crosslink chitosan films were examined. The results are presented in Figure B-7.

Using the original solution containing 200 ppm of 8-HQ loaded crosslink chitosan film could extend rose vase life for 7 days which was longer than using 400 ppm of 8-HQ in 20% sugar solution and 200 ppm of 8-HQ loaded non-crosslink chitosan film (4 and 5 days), respectively (Table 4.5).

4.8.2.4 The effect of crosslink time on rose vase life

The 2nd set of experiments examined the effect of crosslink time on rose vase life using the original solution from the 1st experiment to observe the prolongation of rose vase life. The results are presented in Figure B-8.

Table 4.5 Effect of different holding solution on the vase life of rose for 2nd experiment.

Holding solution	Vase life (days)
Tap water	3
10% sugar	3
20% sugar	4
400 ppm 8-HQ in 20 % sugar	4
400 ppm 8-HQ in tap water	3
200 ppm 8-HQ loaded crosslink chitosan film in 20% sugar	7
200 ppm 8-HQ loaded non crosslink chitosan film in 20% sugar	5
200 ppm 8-HQ loaded crosslink chitosan film in 20% sugar (crosslink time 30 min)	7
200 ppm 8-HQ loaded non crosslink chitosan film in 20% sugar (crosslink time 60 min)	7
200 ppm 8-HQ loaded non crosslink chitosan film in 20% sugar (crosslink time 180 min)	6

Using 200 ppm of 8-HQ loaded crosslink chitosan film (30 and 60 min) could extend rose vase life for 7 days, while using 200 ppm of 8-HQ loaded crosslink chitosan film (180 min) could prolong rose vase life for 6 days (Table 4.4). This result could be explained that the original solution could still be used because the amount of 8-HQ maintained in the solution. A total duration of rose vase life for two experiments was approximate 20 days. At the 20th day, the amount of 8-HQ present in the solution could be quantified as 52.16, 50.13 and 47.73% for chitosan films with crosslink time of 30, 60 and 180 min, respectively.

The 2nd experiments was performed to observe the effect of different concentrations of 8-HQ, sugar solution and crosslinking time of films on relative fresh weight. The results are presented in Figure 4.14.

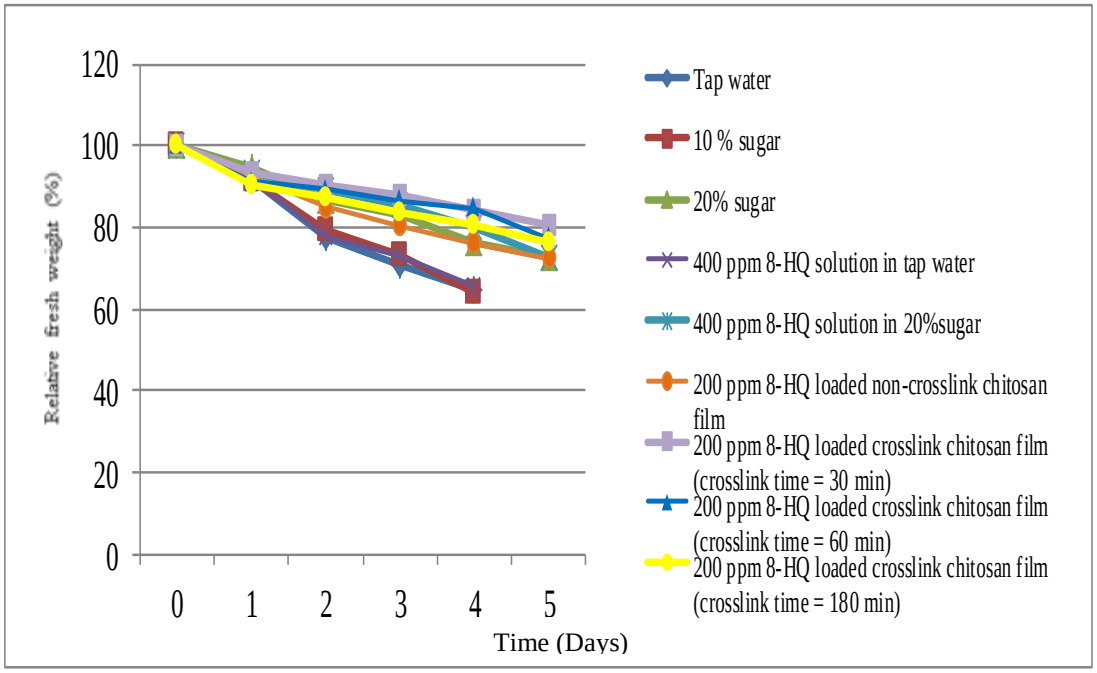


Figure 4.14 Effect of different concentrations of 8-HQ, sugar solution and crosslink time of films on relative fresh weight during first five days of rose vase life (2nd experiment)

Compared with tap water and 10% sugar, it was showed that relative fresh weight of rose was similar.

Compared with the solution containing 400 ppm of 8-HQ in tap water and 400 ppm of 8-HQ in 20% sugar solution, it was showed that the relative fresh weight of rose was similar, but decreased more than the solution containing 200 ppm of 8-HQ loaded chitosan crosslink film.

Compared with the solution containing 200 ppm of 8-HQ loaded chitosan crosslink film (30 and 60 min), it was showed that relative fresh weight of rose was similar but decreased less than the solution containing 200 ppm of 8-HQ loaded chitosan crosslink film (180 min).

This result could explain that the presence of8-HQ in solution affected to the prolongation of rose vase life since 8-HQ could reduce the blockage of vessel to ability to transport water in large quality into stem of rose.

In conclusion, optimum conditions for 200 ppm of 8-HQ loaded chitosan film with crosslink time 30 min combined with 20% sugar solution could prolong rose

vase life. The proper amount of 8-HQ in the solution to prolong rose vase life was found in the range of 45-50%.

A previous research [14] reported the use of 400 ppm of 8- HQS combined with 50 g/L sucrose could prolong rose vase life for 11 days. This present study employed only 200 ppm of 8-HQ loaded chitosan films combined 20% sugar could extend rose vase life for 10 days. The advantage for this finding was the reduced amount of 8-HQ and chitosan films was easy to use and reusable.

CHAPTER V

CONCLUSION

This study focused on the preparation and release profile of 8-HQ from chitosan film. The preparation of chitosan film was studied by varying the parameters such as concentration of chitosan, 8-HQ and TPP as crosslinker including crosslink time. The results can be concluded as below.

- When using high concentration of chitosan (3% w/v) and 8-HQ (30% w/w), it could be seen that the loading efficiency of 8-HQ in the polymer was higher compared with low concentration of chitosan (1 and 2% w/v) and 8-HQ (10 and 20% w/w). This could be the result of high concentration of chitosan that caused better rigidity of the film structure network and lower 8-HQ loss.

- For the result of the film crosslink, it could be found that 8-HQ could be trapped better in the crosslink chitosan film than non-crosslink film and also slower the release of 8-HQ from chitosan film. This came from better rigidity of the structure network due to physical entanglement. When using 5% TPP, it could be seen that the degree of crosslinking was higher than using 3% TPP and also slower the release of 8-HQ from chitosan film. For the effect of the crosslink time, it could be summarized that the optimum crosslink time was at 30 min due to maximum 8-HQ loading percentage compared with 60 and 180 min.

As a conclusion, the release control of 8-HQ by chitosan film using the selected parameters could prolong the vase life of rose up to 10 days. Moreover, the film solution could be reused to another rose that it could extend the vase life of another rose up to 7 days compared with using 20% of sugar solution. It can be summarized that the 8-HQ can inhibit the microorganism in the sugar solution. In addition, it can combine with metallic ions that could block the rose stem. Therefore, the 8-HQ film is recommended to place in the sugar solution in order to inhibit the microorganism and reduce the blockage of pipe stem.

The use of chitosan film as a support material can prolong the release of the 8-HQ in the sugar solution for over than 10 days. This could extend the vase life of the rose, which slower the withering of the rose. Moreover, the chitosan film solution can be reused for the next roses. It can be seen that the chitosan film can reduce the amount of 8-HQ by reutilization. This can also reduce the hazardous effect from the release of the toxic 8-HQ to the environment.

Future work

For the next study, the concentration of 8-HQ and crosslinking agents should be optimized, because some amount of 8-HQ was found in the sugar solution after the second rose withered. By the way, the possibility of the sugar loading on the chitosan film should be studied, since this could simplify the solution usage.

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APPENDICES

APPENDIX A

TABLE A-1 8-HQ loading efficiency of chitosan and alginate beads.

Formulation	% 8-HQ loading efficiency (%)							
	in bead				in crosslink solution			
	I	II	Average	SD	I	II	Average	SD
30% 8-HQ loaded in chitosan beads	2.13	1.98	2.06	0.11	27.46	28.08	27.77	0.44
30% 8-HQ loaded in alginate beads	2.87	2.36	2.62	0.36	27.13	26.47	26.80	0.47

TABLE A-2 8-HQ loading efficiency in chitosan films.

Entry	Concentration		% 8-HQ loading efficiency			
	Chitosan (%w/v)	8-HQ (%w/w)	I	II	Average	SD
1	1	30	15.34	14.86	15.10	0.34
2	2	30	22.46	21.92	22.19	0.38
3	3	30	28.07	28.75	28.41	0.48
4	3	10	7.12	7.58	7.35	0.33
5	3	20	15.43	16.01	15.72	0.41
6	3	30	28.08	28.74	28.41	0.47

TABLE A-3 Effect of different concentration of chitosan on the release of 8-HQ from 30% 8-HQ loaded chitosan films.

Time	Cumulative release (%)									
	2% chitosan					3% chitosan				
(Days)	I	II	III	Average	SD	I	II	III	Average	SD
0.001	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.003	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.007	10.13	11.43	10.77	10.78	0.65	1.76	1.90	1.82	1.83	0.07
0.01	19.05	18.38	17.87	18.43	0.59	4.96	5.24	4.80	5.00	0.22
0.014	21.13	21.30	21.38	21.27	0.13	5.50	5.81	6.12	5.81	0.31
0.02	25.01	25.09	25.61	25.24	0.33	10.30	10.33	10.67	10.43	0.21
0.04	32.89	33.15	33.09	33.04	0.14	13.27	13.54	14.20	13.67	0.48
0.08	36.03	35.45	35.98	35.82	0.32	15.06	14.64	15.79	15.16	0.58
0.13	49.03	48.26	48.07	48.45	0.51	16.71	15.85	16.76	16.44	0.51
0.17	48.98	49.07	49.02	49.02	0.05	30.11	30.47	30.92	30.50	0.41
0.21	49.78	49.18	48.89	49.28	0.45	35.87	36.21	35.38	35.82	0.42
1	51.23	51.00	50.96	51.06	0.15	36.01	36.73	37.51	36.75	0.75
2	50.93	51.13	51.27	51.11	0.17	37.99	38.23	37.42	37.88	0.42
3	52.86	52.45	52.98	52.76	0.28	41.22	40.98	40.47	40.89	0.38
4	53.02	53.12	53.01	53.05	0.06	49.90	49.24	49.16	49.43	0.41
5	53.22	53.11	53.01	53.11	0.11	50.12	50.08	50.41	50.20	0.18
10	60.01	59.71	59.65	59.79	0.19	54.22	54.05	54.09	54.12	0.09
15	60.98	61.16	61.07	61.07	0.09	55.01	55.21	55.67	55.30	0.34
20	61.01	61.23	61.09	61.11	0.11	55.99	55.42	56.26	55.89	0.43
25	62.86	62.37	62.95	62.73	0.31	57.72	57.57	58.26	57.85	0.36
30	63.51	63.44	63.41	63.45	0.05	60.26	60.64	61.43	60.78	0.60
35	64.21	64.01	63.98	64.07	0.13	62.31	61.93	62.45	62.23	0.27
40	69.45	69.56	70.01	69.67	0.30	65.12	65.01	65.89	65.34	0.48
45	72.08	71.98	72.39	72.15	0.21	69.11	69.08	69.08	69.09	0.02

TABLE A-4 Effect of different concentration of 8-HQ on the release of 8-HQ loaded 3% chitosan films.

Time	Cumulative release (%)									
	20% 8-HQ					30% 8-HQ				
(Days)	I	II	III	Average	SD	I	II	III	Average	SD
0.001	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.003	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.007	2.87	2.61	2.97	2.82	0.19	1.76	1.90	1.82	1.83	0.07
0.01	6.73	6.29	6.45	6.49	0.22	4.96	5.24	4.80	5.00	0.22
0.014	9.66	9.98	9.76	9.80	0.16	5.50	5.81	6.12	5.81	0.31
0.02	12.88	12.98	12.84	12.90	0.07	10.30	10.33	10.67	10.43	0.21
0.04	14.98	15.90	15.29	15.39	0.47	13.27	13.54	14.20	13.67	0.48
0.08	16.96	16.49	16.33	16.59	0.33	15.06	14.64	15.79	15.16	0.58
0.13	25.08	24.96	25.07	25.04	0.07	16.71	15.85	16.76	16.44	0.51
0.17	36.97	37.02	36.67	36.89	0.19	30.11	30.47	30.92	30.50	0.41
0.21	39.12	38.78	38.76	38.89	0.20	35.87	36.21	35.38	35.82	0.42
1	40.75	41.01	40.23	40.66	0.40	36.01	36.73	37.51	36.75	0.75
2	40.98	40.72	40.85	40.85	0.13	37.99	38.23	37.42	37.88	0.42
3	46.11	45.98	45.26	45.78	0.46	41.22	40.98	40.47	40.89	0.38
4	51.62	52.09	51.69	51.80	0.25	49.90	49.24	49.16	49.43	0.41
5	53.87	54.03	54.09	54.00	0.11	50.12	50.08	50.41	50.20	0.18
10	61.29	61.41	60.97	61.22	0.23	54.22	54.05	54.09	54.12	0.09
15	67.98	67.54	68.14	67.89	0.31	55.01	55.21	55.67	55.30	0.34
20	69.82	69.65	70.06	69.84	0.21	55.99	55.42	56.26	55.89	0.43
25	70.54	71.05	70.34	70.64	0.37	57.72	57.57	58.26	57.85	0.36
30	75.41	75.52	75.59	75.51	0.09	60.26	60.64	61.43	60.78	0.60
35	76.15	75.98	76.13	76.09	0.09	62.31	61.93	62.45	62.23	0.27
40	76.67	76.32	77.12	76.70	0.40	65.12	65.01	65.89	65.34	0.48
45	77.08	77.67	76.86	77.20	0.42	69.11	69.08	69.08	69.09	0.02

TABLE A-5 Effct of TPP crosslink chitosan film on the release of 30% 8-HQ loaded 3% chitosan films.

Time (Days)	Cumulative release (%)									
	non-crosslink chitosan					crosslink chitosan film				
	I	II	III	Average	SD	I	II	III	Average	SD
0.001	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.003	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.007	1.76	1.90	1.82	1.83	0.07	0.00	0.00	0.00	0.00	0.00
0.01	4.96	5.24	4.80	5.00	0.22	0.00	0.00	0.00	0.00	0.00
0.014	5.50	5.81	6.12	5.81	0.31	0.00	0.00	0.00	0.00	0.00
0.02	10.30	10.33	10.67	10.43	0.21	0.00	0.00	0.00	0.00	0.00
0.04	13.27	13.54	14.20	13.67	0.48	0.00	0.00	0.00	0.00	0.00
0.08	15.06	14.64	15.79	15.16	0.58	0.00	0.00	0.00	0.00	0.00
0.13	16.71	15.85	16.76	16.44	0.51	0.00	0.00	0.00	0.00	0.00
0.17	30.11	30.47	30.92	30.50	0.41	0.00	0.00	0.00	0.00	0.00
0.21	35.87	36.21	35.38	35.82	0.42	0.00	0.00	0.00	0.00	0.00
1	36.01	36.73	37.51	36.75	0.75	18.98	19.07	18.89	18.98	0.09
2	37.99	38.23	37.42	37.88	0.42	28.63	28.59	28.09	28.44	0.30
3	41.22	40.98	40.47	40.89	0.38	32.76	32.64	32.44	32.61	0.16
4	49.90	49.24	49.16	49.43	0.41	34.71	35.41	35.57	35.23	0.46
5	50.12	50.08	50.41	50.20	0.18	42.04	42.74	42.69	42.49	0.39
10	54.22	54.05	54.09	54.12	0.09	45.09	44.99	45.18	45.09	0.10
15	55.01	55.21	55.67	55.30	0.34	49.73	49.93	50.01	49.89	0.14
20	55.99	55.42	56.26	55.89	0.43	50.14	49.97	50.28	50.13	0.16
45	69.11	69.08	69.08	69.09	0.02	56.09	55.98	55.87	55.98	0.11

TABLE A-6 Effect of TPP concentration on the release of 30% 8-HQ loaded 3% chitosan films.

Time (Days)	Cumulative release (%)									
	3% TPP (as crosslinker)					5% TPP (as crosslinker)				
	I	II	III	Average	SD	I	II	III	Average	SD
0.001	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.003	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.007	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.010	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.014	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.04	1.46	1.56	1.32	1.45	0.12	0.29	0.45	0.42	0.39	0.09
0.08	2.56	2.34	2.00	2.30	0.28	1.21	1.32	1.10	1.21	0.11
0.13	8.07	8.61	9.01	8.56	0.47	5.54	5.47	5.02	5.34	0.28
0.17	10.83	10.70	10.64	10.72	0.10	7.68	7.90	8.16	7.91	0.24
0.21	15.32	16.01	15.68	15.67	0.35	11.38	11.87	10.75	11.33	0.56
0.25	18.03	17.67	17.97	17.89	0.19	11.96	12.23	11.76	11.98	0.24
0.33	25.36	26.06	25.48	25.63	0.37	13.56	13.96	13.79	13.77	0.20
1	34.59	35.09	34.98	34.89	0.26	33.11	32.61	32.76	32.83	0.26
2	36.17	36.59	35.76	36.17	0.42	36.02	35.67	35.31	35.67	0.36
3	41.03	41.24	41.07	41.11	0.11	38.19	37.87	38.18	38.08	0.18
4	49.12	48.86	48.06	48.68	0.55	47.06	46.98	46.75	46.93	0.16
5	49.27	49.83	49.55	49.55	0.28	48.16	47.89	48.02	48.02	0.14
10	50.07	50.49	49.79	50.12	0.35	48.37	48.48	48.73	48.53	0.18
15	53.21	53.79	53.34	53.45	0.30	50.99	51.02	50.86	50.96	0.09
20	55.08	54.93	55.30	55.10	0.19	52.05	52.34	52.09	52.16	0.16
45	63.89	63.74	63.77	63.80	0.08	57.75	58.02	57.89	57.89	0.14

TABLE A-7 % Loss of 8-HQ in crosslink solution.

Entry	Crosslink-time	% 8-HQ loading				% Loss of 8-HQ in crosslink solution			
	(min)	I	II	Average	SD	I	II	Average	SD
1	30	25.89	25.39	25.64	0.35	4.19	4.75	4.47	0.40
2	60	24.84	24.20	24.52	0.45	5.14	5.92	5.53	0.55
3	180	22.08	22.68	22.38	0.42	8.02	7.49	7.76	0.37

TABLE A-8 Effect of crosslinking time on the release of 30% 8-HQ loaded 3% chitosan films.

Time	Cumulative release (%)														
	crosslink time = 30 min					crosslink time = 60 min					crosslink time = 180 min				
(Days)	I	II	III	Average	SD	I	II	III	Average	SD	I	II	III	Average	SD
0.001	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.003	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.007	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.014	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.04	0.29	0.45	0.42	0.39	0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.08	1.21	1.32	1.10	1.21	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.13	5.54	5.47	5.02	5.34	0.28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.17	7.68	7.90	8.16	7.91	0.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.21	11.38	11.87	10.75	11.33	0.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.25	11.96	12.23	11.76	11.98	0.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.33	13.56	13.96	13.79	13.77	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
1	33.11	32.61	32.76	32.83	0.26	18.98	19.07	18.89	18.98	0.09	4.06	4.11	4.08	4.08	0.03
2	36.02	35.67	35.31	35.67	0.36	28.63	28.59	28.09	28.44	0.30	17.02	17.38	17.41	17.27	0.22
3	38.19	37.87	38.18	38.08	0.18	32.76	32.64	32.44	32.61	0.16	19.11	19.35	19.83	19.43	0.37
4	47.06	46.98	46.75	46.93	0.16	34.71	35.41	35.57	35.23	0.46	19.71	19.44	20.01	19.72	0.29
5	48.16	47.89	48.02	48.02	0.14	42.04	42.74	42.69	42.49	0.39	33.16	33.23	33.01	33.13	0.11
10	48.37	48.48	48.73	48.53	0.18	45.09	44.99	45.18	45.09	0.10	41.46	40.52	40.99	40.99	0.47
15	50.99	51.02	50.86	50.96	0.09	49.73	49.93	50.01	49.89	0.14	46.56	46.33	46.11	46.33	0.23
20	52.05	52.34	52.09	52.16	0.16	50.14	49.97	50.28	50.13	0.16	48.41	47.52	47.26	47.73	0.60
45	57.75	58.02	57.89	57.89	0.14	56.09	55.98	55.87	55.98	0.11	53.71	53.86	53.89	53.82	0.10

TABLE A-9 Effect of crosslinking time in 5% TPP solution on % swell of 30% 8-HQ loaded 3% chitosan films.

Time (min)	% Swell											
	crosslink time = 30 min				crosslink time = 60 min				crosslink time = 180 min			
	I	II	Average	SD	I	II	Average	SD	I	II	Average	SD
1	37.59	37.55	37.57	0.03	31.41	31.18	31.30	0.16	11.32	11.12	11.22	0.14
5	92.29	92.84	92.57	0.39	59.52	60.14	59.83	0.44	35.78	35.87	35.83	0.06
10	175.32	175.61	175.47	0.21	98.12	97.46	97.79	0.47	56.11	56.64	56.38	0.37
20	285.93	285.65	285.79	0.20	163.41	163.01	163.21	0.28	81.35	81.19	81.27	0.11
30	365.84	365.71	365.78	0.09	216.08	215.62	215.85	0.33	95.34	95.12	95.23	0.16
60	448.01	447.15	447.58	0.61	283.34	283.68	283.51	0.24	130.58	129.96	130.27	0.44
120	518.99	519.61	519.30	0.44	318.99	318.90	318.95	0.06	145.43	145.31	145.37	0.08
180	558.68	558.91	558.80	0.16	335.98	335.60	335.79	0.27	154.67	154.45	154.56	0.16

TABLE A-10 Effect of different concentration of 8-HQ, sugar solution and crosslink time of films on relative fresh weight during first five days of rose vase-life. (1st experiment of prolongation of vase life)

Condition	Item	% Relative fresh weight / Time (Day)				
		1	2	3	4	5
Tap water	I	97.05	88.99	77.98	71.71	65.12
	II	97.02	89.23	77.69	72.06	65.38
	VI	96.28	89.06	77.02	71.88	65.02
Average		96.62	89.18	77.43	71.94	65.17
SD		0.48	0.20	0.48	0.19	0.15
10% sugar	I	96.69	91.05	73.86	66.45	58.77
	II	96.49	91.68	74.27	66.34	57.97
	III	96.54	90.46	74.74	66.43	58.63
	VI	96.50	91.08	74.51	65.98	58.31
Average		96.56	91.07	74.35	66.30	58.42
SD		0.09	0.50	0.38	0.22	0.36
20% sugar	I	96.17	92.04	89.19	87.25	80.19
	II	95.88	92.10	89.42	86.98	80.42
	III	96.07	92.18	88.81	87.18	80.81
	VI	96.24	92.09	89.02	87.08	80.02
Average		96.09	92.10	89.11	87.12	80.36
SD		0.16	0.06	0.26	0.12	0.34
400 ppm 8-HQ solution in tap water	I	96.13	92.14	90.14	86.98	79.36
	II	96.17	91.26	90.26	85.78	79.50
	III	96.43	92.05	90.05	85.59	79.59
	VI	95.84	91.98	89.98	85.71	79.71
Average		96.14	91.86	90.11	86.02	79.54
SD		0.24	0.40	0.12	0.65	0.15
400 ppm 8-HQ solution in 20% sugar	I	96.38	92.11	87.94	87.00	86.32
	II	96.17	92.28	87.66	86.62	86.33
	III	96.05	92.70	87.97	87.41	85.78
	VI	95.54	92.01	88.28	87.06	85.89
Average		96.04	92.28	87.96	87.02	86.08
SD		0.36	0.30	0.25	0.32	0.29

Condition	Item	% Relative fresh weight / Time (Day)				
		1	2	3	4	5
200 ppm 8-HQ loaded non-crosslink chitosan film	I	95.77	90.72	86.94	87.01	80.21
	II	96.23	90.74	87.77	86.78	80.06
	III	96.02	91.21	87.87	86.94	80.18
	VI	96.16	91.34	87.98	87.11	80.69
Average		96.05	91.00	87.64	86.96	80.29
SD		0.20	0.32	0.47	0.14	0.28
200 ppm 8-HQ loaded crosslink chitosan film (crosslink time = 30 min)	I	95.83	92.75	90.97	88.90	86.78
	II	96.24	92.54	90.48	89.35	86.58
	III	95.80	92.67	90.63	89.12	86.03
	VI	96.28	92.87	90.78	89.38	86.46
Average		96.04	92.71	90.72	89.19	86.46
SD		0.26	0.14	0.21	0.22	0.32
200 ppm 8-HQ loaded crosslink chitosan film (crosslink time = 60 min)	I	95.91	92.91	90.41	87.40	85.83
	II	96.41	92.41	90.54	88.28	84.83
	III	96.68	92.68	91.03	87.67	85.53
	VI	96.64	92.64	91.22	87.99	85.14
Average		96.41	92.66	90.80	86.58	85.33
SD		0.35	0.20	0.39	0.38	0.44
200 ppm 8-HQ loaded crosslink chitosan film (crosslink time =180min)	I	96.65	92.45	87.41	86.01	83.22
	II	97.08	93.03	87.87	85.65	83.48
	III	96.89	92.72	87.34	85.40	83.01
	VI	96.98	92.45	87.55	85.96	83.41
Average		96.90	92.66	87.54	85.76	83.28
SD		0.18	0.28	0.24	0.29	0.21

TABLE A-11 Effect of different concentration of 8-HQ, sugar solution and crosslink time of films on relative fresh weight during first five days of rose vase-life. (2nd experiment of prolongation of vase life)

Condition	Item	% Relative fresh weight / Time (Day)				
		1	2	3	4	5
Tap water	I	92.01	78.12	70.19	64.13	-
	II	91.52	77.51	71.47	64.37	-
	III	91.70	77.45	70.98	65.08	-
	VI	91.84	78.07	70.87	64.87	-
Average		91.77	77.79	70.88	64.61	-
SD		0.21	0.36	0.53	0.44	-
10% sugar	I	92.46	79.05	73.13	64.22	-
	II	91.46	79.09	73.45	64.71	-
	III	91.52	79.34	73.89	64.27	-
	VI	92.29	79.25	73.44	64.00	-
Average		91.93	79.18	73.48	64.30	-
SD		0.52	0.14	0.31	0.30	-
20% sugar	I	94.27	86.77	83.15	76.13	72.31
	II	94.95	86.65	83.38	76.02	72.69
	III	94.76	86.43	83.11	76.24	73.06
	VI	94.65	86.23	83.02	76.02	72.81
Average		94.66	86.52	83.17	76.10	72.72
SD		0.29	0.24	0.15	0.11	0.31
400 ppm 8-HQ solution in tap water	I	92.75	77.98	65.98	60.54	-
	II	92.47	77.76	65.34	60.34	-
	III	93.30	78.67	65.62	60.98	-
	VI	92.69	78.16	65.59	60.09	-
Average		92.80	78.14	65.63	60.49	-
SD		0.35	0.39	0.26	0.38	-
400 ppm 8-HQ solution in 20% sugar	I	93.43	88.89	86.89	80.03	73.03
	II	93.56	89.20	85.20	79.87	72.87
	III	93.22	89.68	85.34	80.23	72.23
	VI	93.09	89.95	85.09	80.12	72.12
Average		93.33	89.43	85.63	80.06	72.56
SD		0.21	0.48	0.85	0.15	0.45

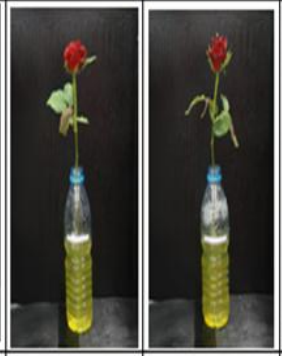
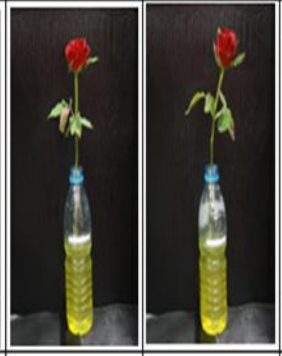
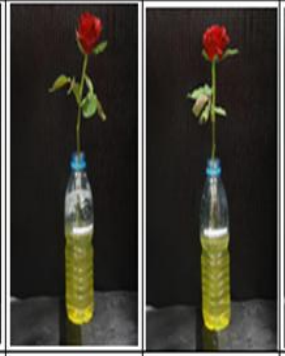
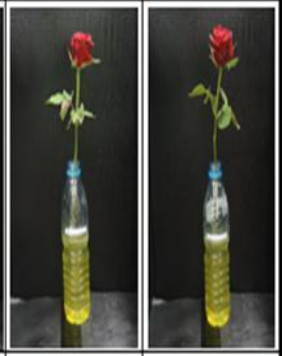
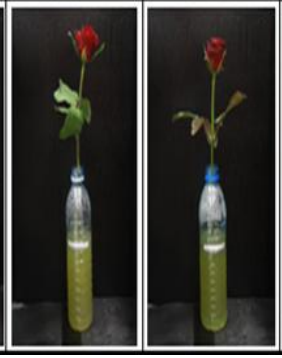
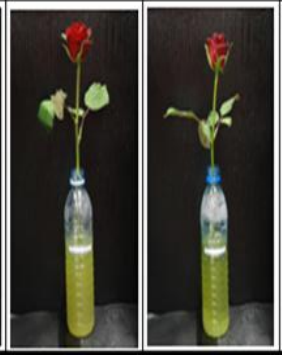
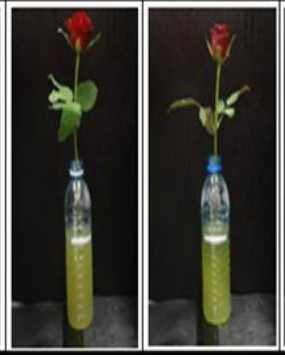
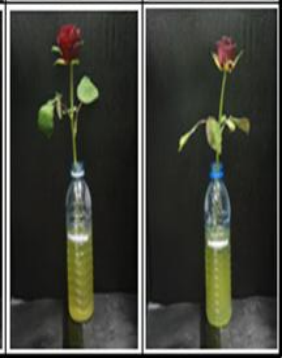
Condition	Item	% Relative fresh weight / Time (Day)				
		1	2	3	4	5
200 ppm 8-HQ loaded non-crosslink chitosan film	I	93.12	85.43	80.45	75.12	72.12
	II	92.89	85.19	81.09	76.54	72.68
	III	92.07	84.98	80.69	76.34	72.80
	VI	92.66	85.08	80.32	76.57	73.09
Average		92.69	85.17	80.64	76.14	72.67
SD		0.45	0.19	0.34	0.69	0.41
200 ppm 8-HQ loaded crosslink chitosan film (crosslink time = 30 min)	I	93.71	90.76	87.90	84.86	80.16
	II	93.34	90.09	88.13	84.12	80.34
	III	93.09	90.91	87.61	84.32	81.08
	VI	93.14	90.35	87.45	84.06	80.54
Average		93.32	90.53	87.77	84.34	80.53
SD		0.28	0.38	0.30	0.36	0.40
200 ppm 8-HQ loaded crosslink chitosan film (crosslink time =60 min)	I	90.89	89.12	86.56	84.16	77.89
	II	91.40	89.32	86.54	84.22	78.06
	III	91.34	89.58	86.11	85.96	77.23
	VI	91.56	90.12	87.01	85.32	77.13
Average		91.30	89.54	86.56	84.92	77.58
SD		0.29	0.43	0.37	0.88	0.47
200 ppm 8-HQ loaded crosslink chitosan film (crosslink time =180min)	I	90.56	87.98	83.87	80.17	76.08
	II	90.11	86.45	83.15	81.23	77.12
	III	90.34	87.69	84.09	80.90	76.45
	VI	90.80	86.99	83.11	81.16	76.15
Average		90.45	87.28	83.56	80.87	76.45
SD		0.30	0.69	0.50	0.48	0.47

APPENDIX B

Day / Holding solution	1		2		3		4	
Tap water								
10% sugar								
20%sugar								

Day / Holding solution	5		6		7	
Tap water						
10% sugar						
20%sugar						

Figure B-1 Rose vase life using tap water, 10 and 20% w/v

Day / Holding solution	1	2	3	4	5
400 ppm of 8-HQ in 20% sugar					
400 ppm of 8-HQ in tap water					

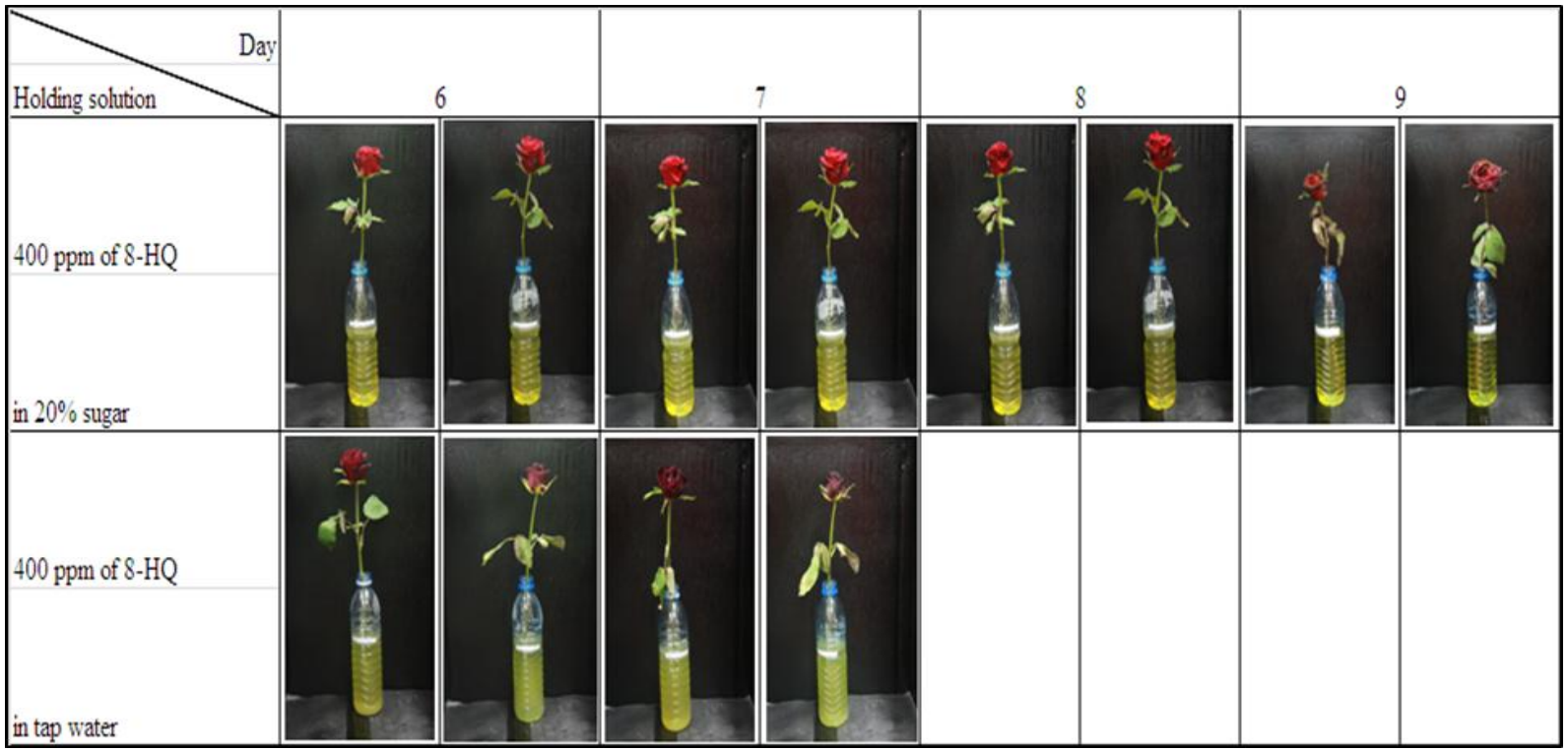


Figure B-2 Rose vase life experiments using 400 ppm of 8-HQ in 20% sugar and tap water.

Day / Holding solution	1	2	3	4	5
200 ppm 8-HQ loaded crosslink chitosan film										
200 ppm 8-HQ loaded non-crosslink chitosan film										

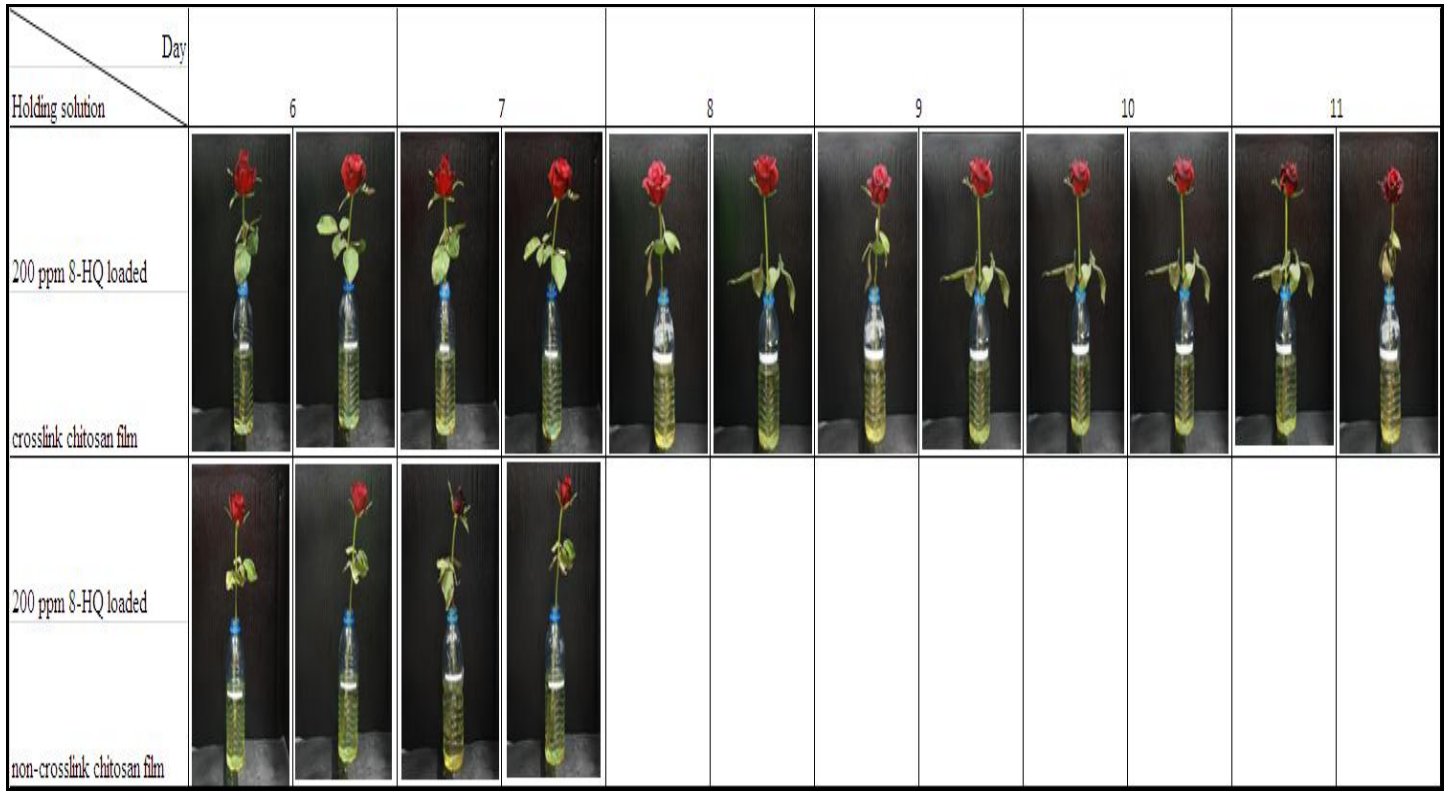


Figure B-3 Rose vase life experiments using 200 ppm of 8-HQ in loaded crosslink chitosan fims and non-crosslink chitosan films.

Holding solution \ Day	1		2		3		4		5	
crosslink time = 30 min										
crosslink time = 60 min										
crosslink time = 180 min										

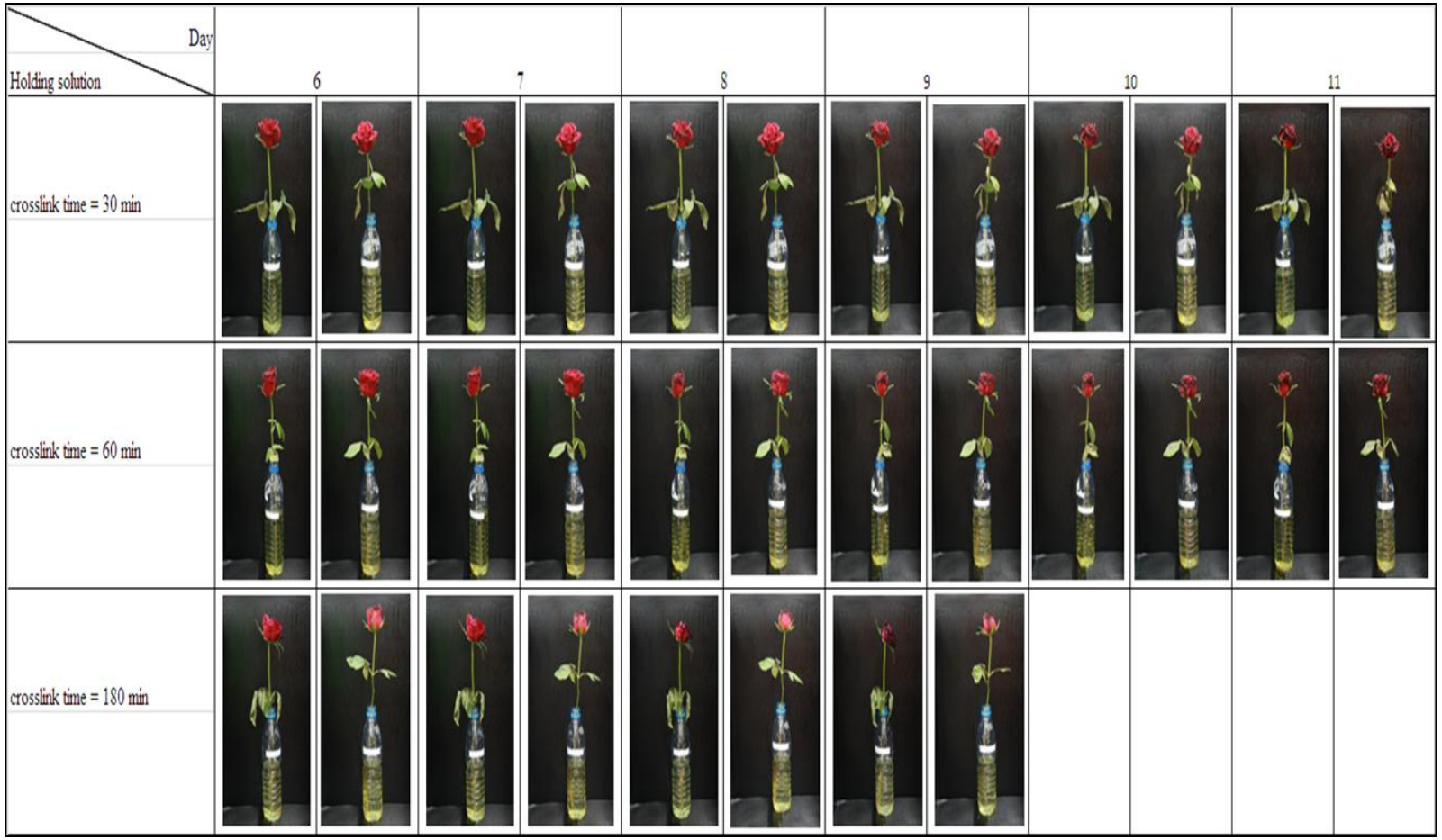


Figure B-4 Rose vase life experiments using 200 ppm of 8-HQ loaded chitosan film with crosslink time 30, 60 and 180 min.

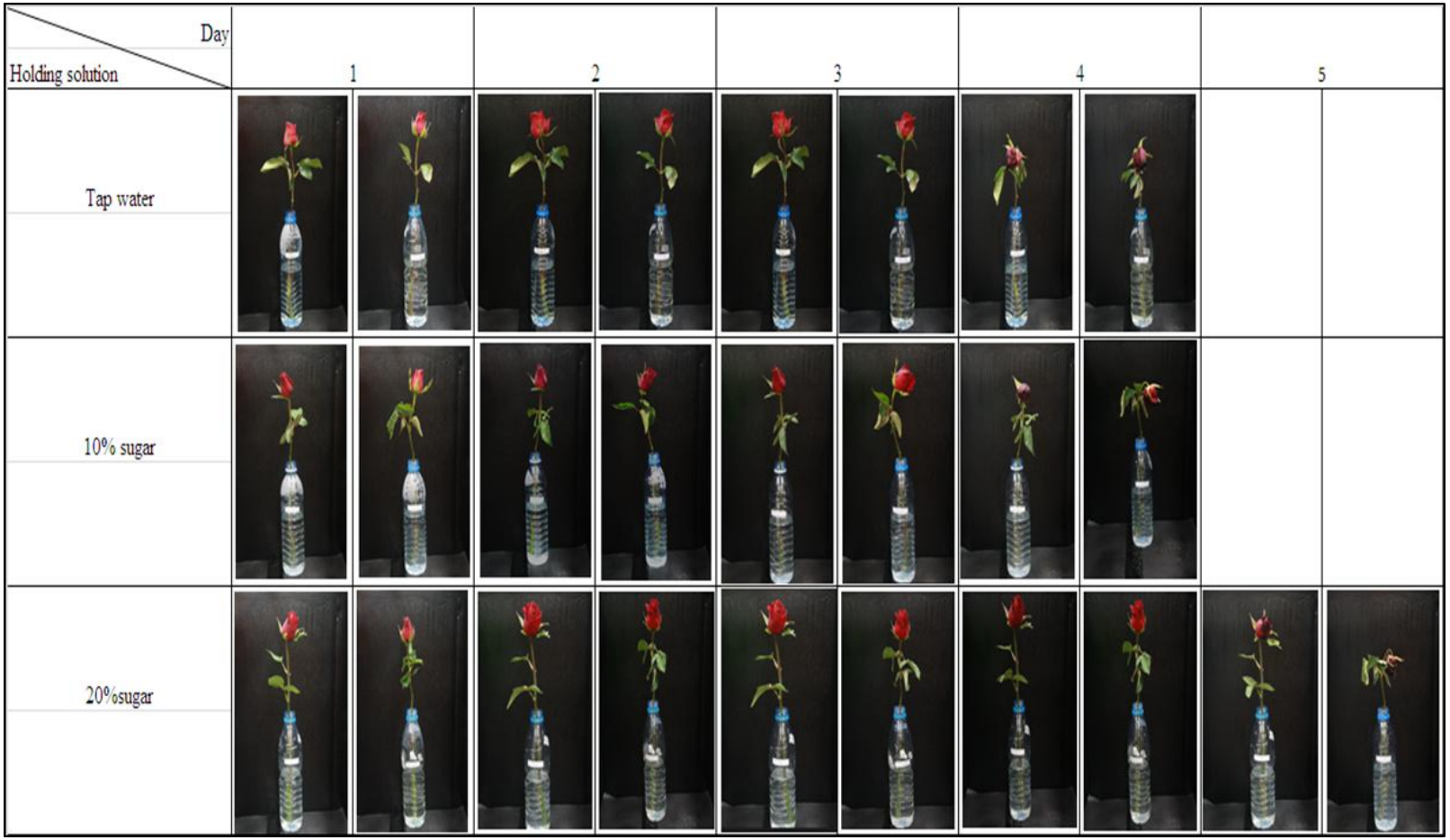
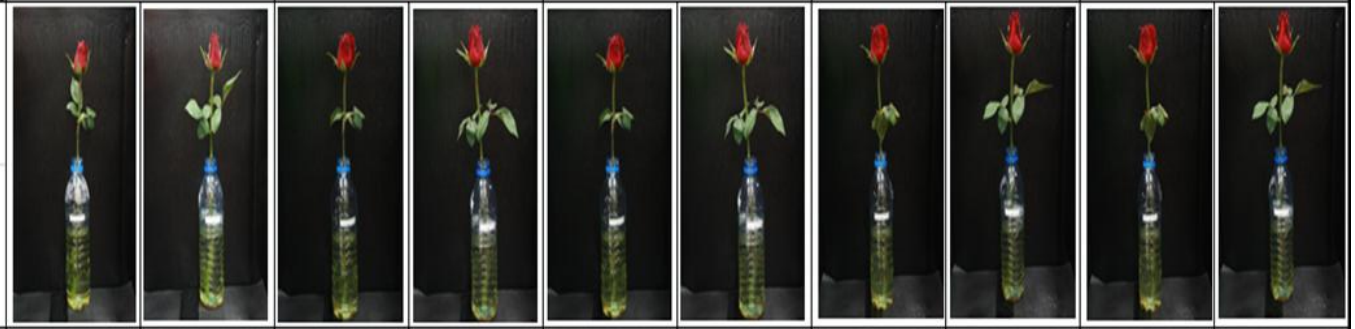
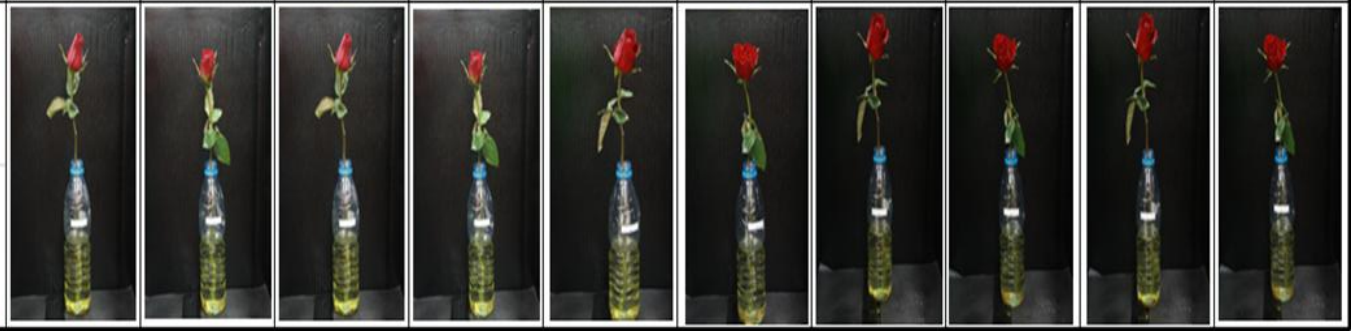


Figure B-5 The 2nd experiment of rose vase life prolongation using original tap water, 10 and 20%w/v of sugar solution.

Figure B-6 The 2nd experiment of rose vase life prolongation using original 400 ppm of 8-HQ in 20% sugar and tap water.

Day / Holding solution	1	2	3	4	5
200 ppm 8-HQ loaded crosslink chitosan film					
200 ppm 8-HQ loaded non-crosslink chitosan film					

Holding solution \ Day	6		7		8	
200 ppm 8-HQ loaded crosslink chitosan film						
200 ppm 8-HQ loaded non-crosslink chitosan film						

Figure B-7 The 2nd experiment of rose vase life prolongation using original 200 ppm of 8-HQ loaded crosslink and non-crosslink chitosan films.

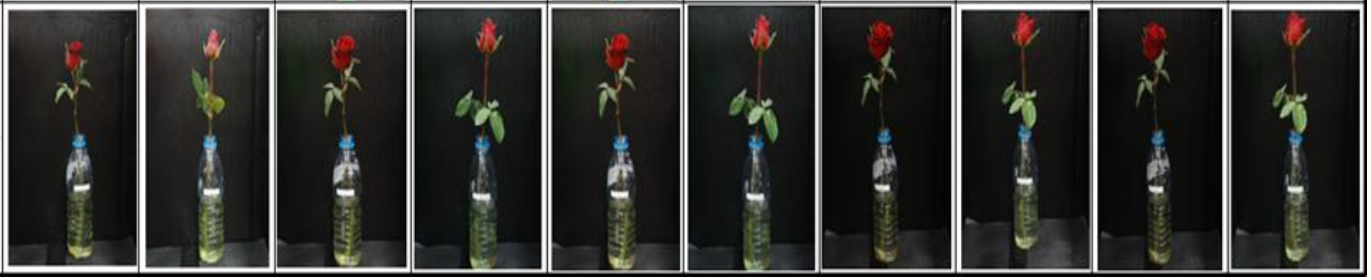
Day / Holding solution	1	2	3	4	5
crosslink time = 30 min					
crosslink time = 60 min					
crosslink time = 180 min					

Figure B-8 The 2nd experiment of rose vase life prolongation using original 200 ppm of 8-HQ loaded chitosan film with crosslink time of 30, 60 and 180 min.

VITA

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