Preparation and Characterization of Novel Chitosan-Silica-Poly(vinyl alcohol) Composite Films for Controlled Release of 1-Naphthaleneacetic Acid

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Abstract:

1-Naphthaleneacetic acid (NAA) is widely used as a plant growth regulator. However, high concentration of NAA inhibits plant growth, contaminates in environment, and affects human health. Here, we report a novel chitosan-silica-poly(vinyl alcohol) composite films for controlled release of NAA. The effects of neutralization condition on the chitosan structure as well as the NAA adsorption capacity were also studied. It showed that neutralization with ethanol solution promoted NAA adsorption whereas using the solution containing NaOH delayed NAA adsorption. This was caused by the presence of cationic amine groups (-NH₃⁺) in chitosan structure after treatment with ethanol solution resulting in high attractive force for binding with negative charged NAA molecules. The chitosan-silica-poly(vinyl alcohol) composite films were fabricated, characterized using several techniques, and used for controlled release of NAA. The composite film could adsorb NAA 2.82 ± 0.07 mg g⁻¹ after 1 hour which is lower than that adsorbed by pure chitosan film (0.63 ± 0.12 mg g⁻¹). However, the composite and pure chitosan films could release NAA in the same range of 57-60% after immersion in water for over 13 days. The developed composite film offers advantages over pure chitosan film in terms of reusability and can be used as a NAA controlled-release material with less chitosan content.

1. Introduction

Food security is one of the most significant problems in our world at this time because of increasing population, decreasing arable land and the presence of ecological adversities. The most challenging is to provide inexpensive, sustainable, and nutritious food to the fast-growing world’s population. This leads to an introduction of agrochemical technologies including plant growth regulators (PGRs), such as auxins, gibberellins, cytokinins, abscisic acid, ethylene, brassinosteroids and jasmonates, to increase crop yield qualitatively and quantitatively. Auxin is one of widely used PGR because of its ability to stimulate differential growth in response to gravity or light stimuli. 1-Naphthalene acetic acid (NAA) is one of potent native auxin family that is cheap and widely used in Thailand, commonly applied to stimulate the rooting potential of plant cutting, to prevent fruit drop in orchards, and to use as a pesticide in fruit by spraying. To produce an appropriate biological effect, this compound must be applied at low concentrations of 20-40 mg L⁻¹.¹ The use at high concentration can lead to negative effects to plants, including oxidative stress resulting in cellular death.
and plant growth inhibition. Moreover, excess amount of NAA in agricultural uses can contaminate groundwater and soil resulting in uncontrollable adverse impacts on environment and human health. Therefore, controlled release system is highly desirable for increasing the utilization of NAA as well as reducing environmental pollutions.

In this study, a novel chitosan-silica-poly(vinyl alcohol) composite film was introduced for controlled release of 1-naphthaleneacetic acid. An appropriate neutralization solution for modification of chitosan structure was investigated. Then a suitable pH of the composite solution for films preparation were also explored. Physical and chemical properties of the synthetic composite films were studied using several techniques. Performance of the composite films in adsorption and release of NAA including reusability of the films were investigated.

2. Materials and Methods
2.1 Materials
Chitosan (CS) (degree of deacetylation 85%) was obtained from Seafresh Industry Public Company Limited. Poly(vinyl alcohol) (PVA) (polymerization degree in the range of 1500~1800) was purchased from FUJIFILM Wako Chemical Corporation. Sodium metasilicate pentahydrate (Na$_2$SiO$_3$·5H$_2$O, ≥95.0%) was obtained from Aldrich Chemical Corporation. Acetic acid glacial was purchased from Carlo Erba Reagents Company. All chemical reagents were used as received without further purification.

2.2 Preparation of chitosan-silica-poly(vinyl alcohol) solution
In a typical procedure, 30 ml of 2% w/v of chitosan in acetic acid was prepared and added into 50 ml of 2% w/v PVA solution followed by stirring for 10 min to obtain PVACS solution. After that, 10 mL of sodium metasilicate solution was slowly added to the PVACS solution and stirred to obtain a homogeneous solution with 0.6% w/w SiO$_2$. The pH values of PVACS solutions were adjusted to 3.6, 4.5, 5.5 and 6.6 by addition of NH$_4$OH solution. The composite films were prepared by pouring mixed solutions with different pH (3.6, 4.5, 5.5 and 6.6) into 9×9 cm$^2$ aluminum mold. After drying at 70°C for 2 h, PVACS films were obtained. The dried films prepared from PVACS solutions at pH 3.6, 4.5, 5.5 and 6.6 were denoted as PVACS 3.6, PVACS 4.5, PVACS 5.5 and PVACS 6.6, respectively.

2.3 Film neutralization
The dried film was neutralized with three different solutions; aqueous sodium hydroxide (NaOH), NaOH/ethanol, and ethanol (EtOH) solutions. For the first method, PVACS films were immersed in 2% w/v NaOH solution for 30 min and then washed with deionized water for three times. The films neutralized with this method were denoted as PVACS-NaOH. For the second method, PVACS films were immersed in NaOH/ethanol solution for 30 min and then washed with 80% v/v ethanol solution for three times. The films neutralized with this method were denoted as PVACS-NaOH/EtOH. For the third method, PVACS films were immersed in 80% v/v ethanol solution for 10 min for four times. The films neutralized with this method were denoted as PVACS-EtOH.

2.4 PVACS film characterizations
Chemical functionalities of the films were investigated by using Fourier transform infrared spectroscopy (FT-IR, Thermo Scientific, Nicolet 6700) in the wavenumbers ranging from 400 to 4000 cm$^{-1}$. Surface chemical analysis was carried out using X-ray photoelectron spectroscopy (XPS) at BL5.2 in the Synchrotron Light Research Institute, Thailand. The XPS spectra were calibrated using the C 1s signal at 284.4 eV.
2.5 NAA adsorption and controlled release studies

NAA adsorption experiments were carried out by immersion of 1×1 cm² of the PVACS films in 5 mL of 10⁻⁴ M NAA solution at room temperature. After adsorption, the NAA content remaining in the solution was analyzed using UV-Vis-NIR spectroscopy at a wavelength of 282 nm. Then the NAA solution was replaced by fresh 5 mL of 10⁻⁴ M NAA solution. Numbers of adsorption cycles were carried out and the remained NAA contents were calculated to obtain adsorption capacity of the films. The amount of NAA adsorbed on composite films were calculated by the following equation:

\[ q_e = \frac{(C_0 - C_e)V}{w} \]  

where \( q_e \) is the amount of NAA adsorbed onto the unit amount of the films (mg g⁻¹), \( C_0 \) is the initial concentration of NAA (mg L⁻¹), \( C_e \) is the final or equilibrium concentration of NAA (mg L⁻¹), \( V \) is the volume of NAA solution (L) and \( W \) is the weight of dried composite films (g).

2.6 Reusability studies

Reusability of CS and PVACS 3.6 films were studied using UV-Vis-NIR spectroscopy technique. Two samples of 2×1 cm² films were immersed in 5 mL of 10⁻⁴ M NAA solution at room temperature. After adsorption, the NAA content remaining in the solution was analyzed at a wavelength of 282 nm. Then PVACS films were dried for 24 h at room temperature. The dried films were washed with deionized water until less than 10% of NAA content remaining in solution was obtained. Then the films were used for re-adsorption and removal of NAA for several cycles. The NAA adsorption capacity of each cycle were calculated and used for evaluation of film reusability.

3. Results & Discussion

3.1 Effect of neutralization solution on chitosan film structure.

The effect of neutralization solutions on chitosan (CS) film structures was studied using FT-IR spectroscopy technique as shown in Fig 1. The spectrum of chitosan film before neutralization (Fig 1a) showed broad bands observed between 3550 and 3200 cm⁻¹ which are associated with the O-H stretching obtained from the intermolecular and intramolecular hydrogen bonds. The main peaks were observed at 1630 cm⁻¹ and 1534 cm⁻¹ corresponded to amide I and amide II, respectively. The peak at 1402 cm⁻¹ indicated the presence of carboxylic acid and the peaks at 1063 and 1017 cm⁻¹ referred to C-O stretching in chitosan structures. After treatment with NaOH and NaOH/EtOH solutions (Fig 1b-1c), new peaks were found at 1578 and 1481 cm⁻¹ attributed to stretching of N-H from amine groups and symmetric stretching of carboxylate ions, respectively. Noticeably, the spectrum of the CS film after treatment with EtOH solution (Fig 1d) showed the peak at 1551 cm⁻¹ which is attributed to -NH₃⁺ symmetric deformation. This suggested that neutralization the CS film with only ethanol solution could induce the generation of -NH₃⁺ groups in the chitosan structure.

All CS films were further characterized using XPS technique in N 1s region as shown in Fig 2. The CS film before treatment (Fig 2a) showed major N 1s contributions near 399.3 eV and at 400.3 eV corresponded to non-protonated amine and amide groups (N-C=O), respectively. These two main peaks were also observed in the films after neutralization with all solutions. Additional peak was found at 401.3 eV after treatment with only EtOH solution (Fig 2d). This peak was assigned to amino groups in the ammonium form (NH₃⁺). This result indicated that neutralization with EtOH solution led to the
presence of protonated chitosan structure to reveal a positive charged ammonium form (NH$_3^+$), which is important for strong binding with negative charged NAA molecules. This was confirmed by NAA adsorption studies of the treated films (data not shown). It showed that the film treated with EtOH showed maximum NAA adsorption capacity at 3.45 mg g$^{-1}$ after an hour of immersion time. While the films treated with NaOH and NaOH/EtOH solutions showed less than 0.10 mg g$^{-1}$ of NAA adsorption capacity. Therefore, EtOH solution was selected as a neutralizing solution for further study.

**Fig 1.** FT-IR spectra of the (a) chitosan film, chitosan film after neutralization with (b) NaOH, (c) NaOH/EtOH, and (d) EtOH solutions.

**Fig 2.** XPS spectra of (a) chitosan film, chitosan films after neutralization with (b) NaOH, (c) NaOH/EtOH, and (d) EtOH solutions.

### 3.2 Effect of pH on PVA/CS/SiO$_2$ composite film structures.

The effect of pH of the prepared PVA/CS/SiO$_2$ composite solutions on the structures of composite films after neutralized with EtOH solution was studied using XPS technique in N 1s region as shown in Fig 3. The spectrum of pure CS film after treatment with EtOH was shown in Fig 3a for comparison. It composed of the peaks at 399.3 eV, 400.3 eV, and 401.3 eV, which attributed to non-protonated amine (-NH), amide groups (N-C=O), and amino groups in the ammonium form (NH$_3^+$), respectively. These peaks were also observed in all composite films prepared at different pH values (Fig 3b-3e). As pH of the prepared solutions increased, the ratio between NH$_3^+/\text{NH}$ decreased from 0.24 to 0.07 for the films prepared at pH 3.6 to pH 6.6, respectively. Among the composite films, the highest NH$_3^+/\text{NH}$ ratio was found in the film prepared at pH 3.6. This is likely caused by the most appropriate solubility of the chitosan structure in acidic condition at pH 3.6 leading to the presence of large amount of ammonium forms (NH$_3^+$).
3.3 Adsorption and release of NAA using the composite films

NAA adsorption of the composite films prepared at different pH values was investigated as shown in Fig 4. Fig 4a showed that all samples could steadily adsorb NAA as time increased. The adsorption time was fixed at an hour to minimize film degradation. It showed that as the pH increased from 3.6, 4.5, 5.5, and 6.6 the NAA adsorption capacities decreased as 3.45, 2.82, 2.52, 2.31 and 2.01 mg g\(^{-1}\), respectively (Fig 4b). The reason of this phenomenon is due to decreasing in active sites (\(\text{NH}_3^+\)) of the films for NAA adsorption as increasing in pH of the prepared composite solutions. This also caused reduction of adsorption capacity of the composite films. The maximum NAA adsorption capacity was obtained from CS film (3.45 mg g\(^{-1}\)). Therefore, release behavior of this sample was further studied and compared with that of PVACS 3.6 film.

Cumulative release profiles of the chitosan film (CS) and composited films prepared in pH 3.6 as a function of immersion time were investigated as presented in Fig 5. The NAA was gradually released in water and reached 60\% and 57\% after 13 days (312 h) from CS and PVACS 3.6 films, respectively. We noticed that the composite film with lower chitosan content (60\% CS) than CS film could slowly release NAA in the same range of that from CS film. This could be the effect of (i) chemical charge balance in the composite film environment provided by -\(\text{NH}_3^+\), -OH, and partial hydrophobic property, and (ii) film morphology which presented as a rigid and compact structure. This leads to reducing bursting effect and prolonging release time of NAA.
Fig 5. Cumulative release profiles of the chitosan film (CS) and composited films prepared in pH 3.6 as a function of immersion time and after hour of adsorption time.

3.4 Reusability of PVACS film

Number of cycle for adsorption-release of NAA using the both CS and composite films was used to consider reusability of the films. Fig 6 showed that, at the 1st re-cycling, CS and PVACS 3.6 films could re-adsorb NAA 85.9% and 97.45%, respectively. At the 2nd re-cycling, adsorption capacities obviously decreased by 25% for both films. This might be caused by missing or weakening of the vacant adsorption sites in the film structures. However, the composite film still showed higher adsorption capacity (70.67%) than that of CS film (62.52%) by 8.15% after the 2nd re-cycling the film.

Fig 6. Reusability of the chitosan (CS) (black line) and PVACS 3.6 (red line) films with repeated adsorption and desorption cycles.

4. Conclusion

We have shown that a novel chitosan-silica-poly(vinyl alcohol) composite films could be synthesized for controlled release of NAA. A suitable pH of the composite solution for film preparation was found at 3.6 because it could largely promote the presence of adsorption sites for NAA. The results showed that the composite films after neutralization with ethanol solution could effectively adsorb NAA 2.82 ± 0.07 mg g⁻¹ which is slightly lower than that adsorbed by pure chitosan film by 18.26%. However, the composite film offers more advantages over the CS film in terms of (i) slow release property to provide 57% NAA over 13 days, which is in the same range of that using the pure chitosan film, and (ii) reusability of the film.

Acknowledgements

The authors would like to thank Thailand Advanced Institute of Science and Technology and Tokyo Institute of Technology (TAIST-Tokyo Tech) for funding, Kasetsart University for suggestions, and Synchrotron Light Research Institute for technical help in using XPS. This work was supported by the National Nanotechnology Center (NANOTEC) (P 1651720), NSTDA.

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