

A Study of Riboflavin Release from Chito-Oligosaccharide/ Poly (Vinyl Alcohol) Electrospun Nanofibrous Structures

**Panu Danwanichakul, Duangkamol Danwanichakul,
Apichaya Duangjitchotchuang and Premkamon Montienthong**
Department of Chemical Engineering, Faculty of Engineering, Thammasat University,
Khlong Luang, Pathumthani, Thailand, 12120
E-mail: dpanu@engr.tu.ac.th

Abstract

A study of riboflavin (vitamin B₂) release from the nanofiber mats obtained from electrospinning process was investigated. The spinning solution was prepared from 3% chito-oligosaccharide or water soluble chitosan, 13% PVA, and 0.004% riboflavin. Prior to applying the nanofiber mats, the structures underwent crosslinking either with GA vapor or TPP solution. The structures after soaking in TPP solution showed some degree of swelling, whereas, the structures after GA were still the same, the form of nanofibers with many beads. The release media chosen in this study were deionized water, NaCl solution, HCl solution at pH 1.5 and the solution of NaCl and HCl at pH 1.5. The average values of the release rate obtained in deionized water, NaCl solution, HCl solution, and NaCl and HCl solution for GA crosslinked mats were 0.1276, 0.1655, 0.1619, and 0.14811/h respectively. For TPP crosslinked mats, they were 0.0986, 0.1484, 0.1070, and 0.1206 1/h, respectively. This implies that GA crosslinked mats release riboflavin more rapidly than TPP crosslinked mats. For both types of crosslinking, the release in NaCl solution was the fastest while the release in deionized water was the slowest. The mats seemed to withstand the dissolution in acidic solution better than in the salt solution.

Keywords: chitosan, poly(vinyl alcohol), tripolyphosphate, glutaraldehyde, drug release, riboflavin

1. Introduction

Chitosan (CS) is a high-molecular-weight polysaccharide, which is obtained by deacetylation of chitin derived from crab and shrimp shells. Chitosan has many good properties including biodegradability, antimicrobial activity and biocompatibility. Therefore, it has been used in many applications including fabrication, agriculture, diet foods, cosmetics, and pharmaceutical purposes. The latter have been investigated extensively for its potential use

in drug delivery systems such as chitosan microspheres loaded with centchroman [1] and chitosan films for controlled release of riboflavin [2].

Nanofibers have been a focus for being used in drug delivery because of their high ratio of surface area to volume. The construction of nanofibers by electrospinning technique has been studied widely for a decade because of its easiness and reliability. However, chitosan alone cannot be electrospun to obtain nanofibers. To

form chitosan nanofibers by this method, another polymer, which is easy to fabricate, biocompatible and not toxic, has to be mixed with chitosan. Poly (vinyl alcohol) or PVA is one of such polymers and their blend system has been successfully electrospun [3]. Previously, water soluble chitosan or chito-oligosaccharide (COS) has been investigated in the electrospinning process [4]. It is more suitable for medical applications than the system of high-molecular-weight chitosan in acetic acid solution.

To use nanofibrous structures for a drug release, they have to be able to withstand the dissolution in water. For example, glutaraldehyde was used as a crosslinking agent for gelatin/PVA nanofibers for controlled release of raspberry ketone [5]. Since both COS and PVA are water soluble, crosslinking of the nanofiber mat is required. In our earlier study, it was found that the non-crosslinked nanofibrous structures of COS and PVA disappeared in water in only 15 seconds.

In this work, we prepared cross-linked nanofibrous structures using tripolyphosphate (TPP) or glutaraldehyde vapor (GA) as a crosslinking agent. Riboflavin (vitamin B₂) was chosen as a model drug which has been used in several clinical and therapeutic situations. The mats were then put in various release media which were de-ionized water, sodium chloride solution, acid solution at pH 1.5 and sodium chloride solution at pH 1.5.

2. Experimental

2.1 Raw Material

Chitosan (COS) with degree of deacetylation of 96.4% and molecular weight of 275,000 Da was synthesized in our laboratory according to the method of Allan and Peyron [6]. The high-molecular-weight chitosan was bought from A.N. Lab, Ltd, Thailand and it was depolymerized by nitrous acid to yield water-soluble chitosan (COS). Riboflavin (MW= 376.38) was

obtained from Fluka. Poly(vinyl alcohol) with molecular weight of 30,000-70,000 Da was obtained from Merck. Sodium tripolyphosphate (TPP) and Glutaraldehyde (GA) were obtained from Ajax Finechem. Sodium chloride and hydrochloric acid were from Merck.

2.2 Solution preparation

The spinning solutions were prepared by using 3.0% w/v chitosan solution, 13.0% w/v poly(vinyl alcohol) and 0.004% w/v riboflavin. First, riboflavin was dissolved in distilled water and then it was mixed with chitosan and poly(vinyl alcohol) together. The solution was thoroughly mixed then left unstirred until the air bubbles disappeared.

2.3 Nanofiber fabrication

1 ml of the spinning solution was poured into a syringe. The applied nozzle-ground voltage difference was set at 21 kV and the distance between the needle-aluminum foil was fixed at 9 cm. The time for electrospinning was 1 h. The nanofiber mat was collected on a piece of aluminum foil (3x3 cm²).

2.4 Preparation of crosslinked nanofibers

GA Cross-linked nanofiber mats were prepared by leaving the mats in contact with GA vapor evaporating from 12.5% w/v GA solution in a closed system. The cross-linking time was 5 min, 30 min or 1 h.

TPP Cross-linked nanofiber mats were prepared by immersing the mats in 20 ml of 0.5% w/v TPP solution at 4°C. The cross-linking time was 1 min, 15 min or 30 min. The cross-linked nanofiber mats were then washed with distilled water and put on a glass plate, and finally dried in an oven at 37°C for 48 h.

2.5 Morphology observation

The morphology of cross-linked nanofibers was examined with scanning electron microscopy (SEM). Prior to observation, samples were mounted on a metal stub, using double-sided adhesive tape, and coated by gold under vacuum.

2.6 Drug release studies

Cross-linked nanofiber mats were soaked in glass bottles containing 20 ml of different release media. The chosen release media for drug release studies were water, 0.9% w/v sodium chloride solution, HCl solution at pH 1.5 and sodium chloride in acidic solution at pH 1.5.

At a chosen time, a sample of 3 ml solution was withdrawn and the drug content was examined with the UV-visible spectrophotometer (SHIMADZU, UV-1201) at 444 nm. An equal volume of the same release medium was added to maintain the constant volume.

3. Results and Discussions

3.1 Morphology of COS/PVA Nanofibrous structures crosslinked with GA

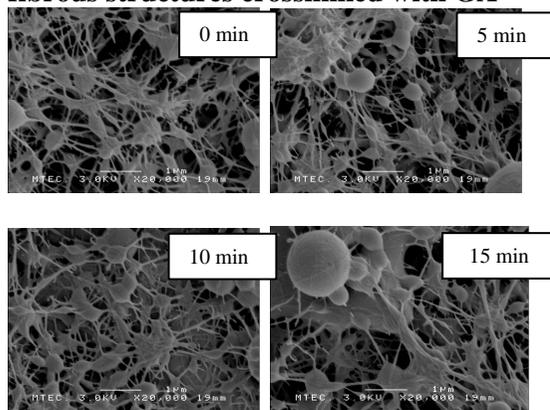


Fig. 1 SEM images of GA-crosslinked samples.

Figure 1 shows the nanofibrous structures cross-linked with GA for various crosslinking times. The electrospun structure is bead-on-string nanofibers, containing many beads with various sizes. The structures could not be differentiated with respect to the effect of cross-linking time. It is possible that bead generation was from the incorporated riboflavin since beads could be generated due to a short relaxation time after fiber stretching in electrical force field. Low molecular weight is responsible for a short relaxation time. Even though many studies attempted to suggest the optimal process conditions to generate

nanofibers without beads for various materials chosen to incorporate drugs, drugs themselves when embodied into the material would unavoidably cause beads on nanofibers [7].

Glutaraldehyde used as a cross-linking agent would react with the hydroxyl groups of poly (vinyl alcohol) chains and both reactive functional groups of chitosan which are hydroxyl-methyl groups and amine group [1], therefore, stronger structures would be obtained.

3.2 Morphology of COS/PVA Nanofibrous structures crosslinked with TPP solution

Figure 2 shows the nanofibrous structures undergoing crosslinking with TPP solution. It is obviously seen that the structure was swollen with water during the soaking in TPP solution. Besides, part of the nanofibers might be dissolved slightly during cross-linking and washing. Swelling made a nanofibrous mat look like a porous film.

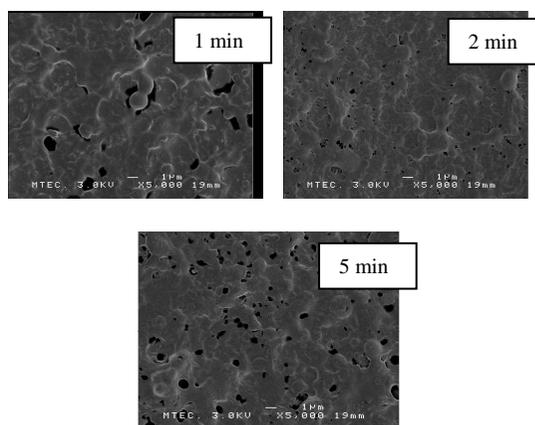


Fig. 2 SEM images of samples crosslinked with TPP solution at various crosslinking times.

Tripolyphosphate (TPP) contains three groups of anionic phosphate groups. It was reported that the crosslinking occurred from the electrostatic interaction between phosphate and protonated chitosan [2]. The strength of the film was greatly enhanced.

However, in this case, it is possible that PVA may be disentangled from crosslinked chitosan chains and dissolved in water.

3.3 Riboflavin release from COS/PVA crosslinked nanofiber mats

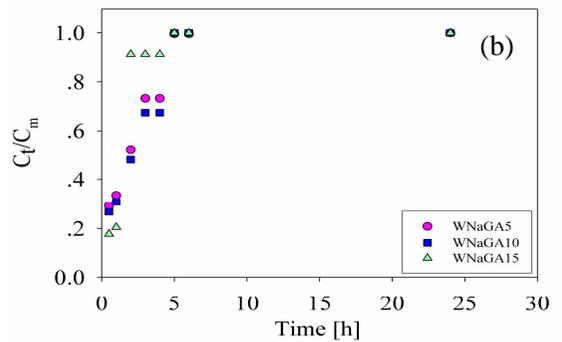
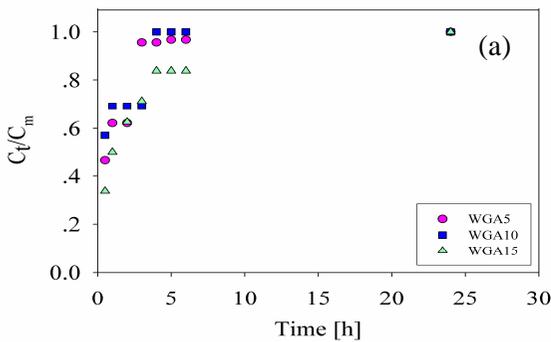
In Figures 3 and 4, the cumulative release was shown for the case of using GA vapor and TPP solution, respectively. It could be seen that the effect of crosslinking time specified in this study is not obvious. Basically, increasing the crosslinking time should increase the mechanical strength of the nanostructures and also hinder the release of riboflavin. The crosslinking times of 5, 10 and 15 minutes for GA and 1, 2, and 5 minutes for TPP solution are too close to differentiate the effect.

However, the mechanism of drug release could be studied by using the following equation,

$$\frac{C_t}{C_m} = kt^n, \tag{1}$$

where C_t is the cumulative concentration of riboflavin released into the media up to time t , C_m is the maximum cumulative concentration at long time, k is a parameter related to the rate of release of the drug that was incorporated in nanofibers, and n is an exponent referring to the mechanism with which the release can be described.

There are two methods of drug delivery, one of which is called the reservoir-type, where the concentrated drug is covered with polymer. The other is called the matrix-type, where the drug is mixed with the polymer matrix. This is the type that we discuss here. In this work riboflavin ($C_{17}H_{20}N_4O_6$) was incorporated in the polymer matrix of PVA/COS. Riboflavin contains four hydroxyl groups which may form hydrogen bonding with hydroxyl groups of PVA and hydroxyl methyl group of chitosan. The release of the drug depends both on the diffusion of the drug from the surface of the fibers and the possible dissolution of polymer matrix. As shown in both figures, for some samples the release reached maximum within 5 hours.



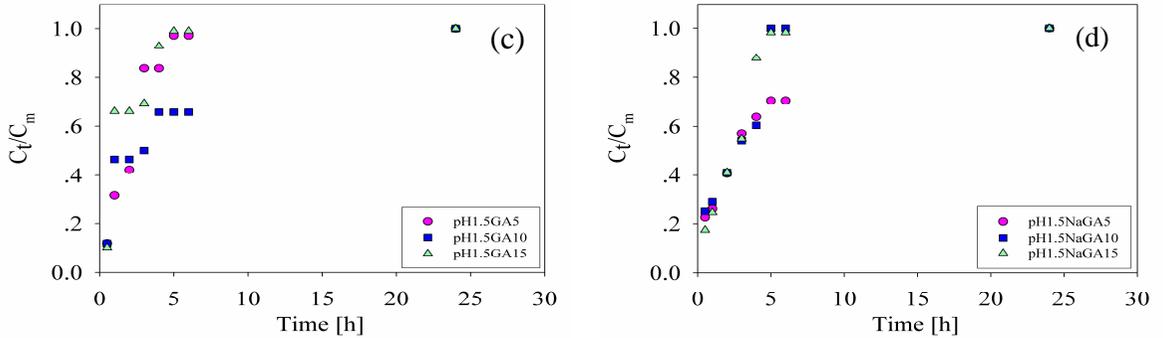


Fig. 3 Plots between $C_t/C_{m_{max}}$ and the release time of riboflavin in different media from the cross-linked nanofibers with GA 5 min, 10 min and 15 min: (a) water; (b) NaCl solution (c) HCl solution at pH 1.5; (d) NaCl in acidic solution at pH 1.5

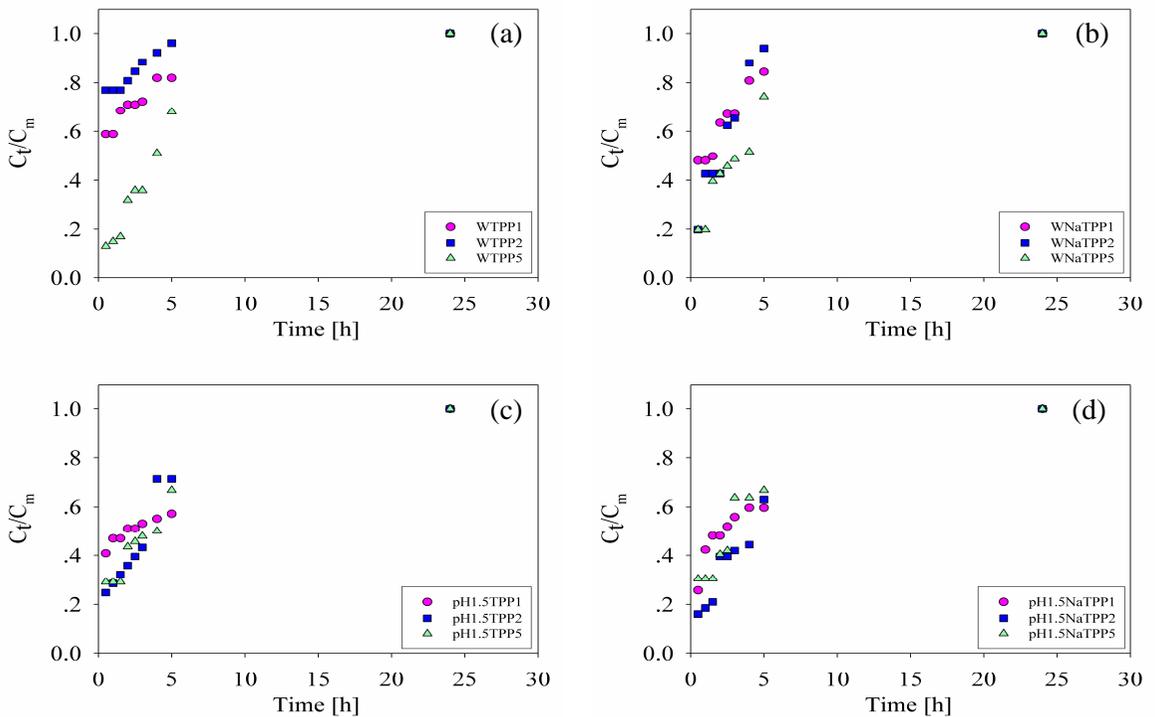


Fig. 4 Plots between $C_t/C_{m_{max}}$ and the release time of riboflavin in different media from the cross-linked nanofibers with TPP for 1 min, 2 min and 5 min: (a) water; (b) NaCl solution (c) HCl solution at pH 1.5; (d) NaCl in acidic solution at pH 1.5.

Using Equation (1), the parameters, k and n , could be obtained. The results are shown in Tables 1 and 2 for GA crosslinked nanofibers and TPP crosslinked nanofibers, respectively. It was seen again that the effect of crosslinking time on the parameter k and n cannot be explained. Theoretically,

the mechanism of diffusion was studied based on the relative rate of diffusion of substance and the relaxation of the polymer matrix [8]. The parameter n equal to 0.5 refers to Fickian diffusion where the diffusion is more rapid than the relaxation. On the other hand, when n is equal to 1.0,

the relaxation is more rapid than the diffusion. In addition, if n is between 0.5 and 1.0, the case is non-Fickian diffusion, where both the effect of diffusion and relaxation is comparable.

Table 1 The release parameters for GA crosslinked nanofiber mats are shown.

| Release media | Crosslinking time (min) | GA vapor | | |
|-------------------|-------------------------|----------|------|-------|
| | | k | n | R^2 |
| Deionized water | 5 | 0.62 | 0.22 | 0.75 |
| | 10 | 0.69 | 0.16 | 0.72 |
| | 15 | 0.49 | 0.28 | 0.89 |
| NaCl solution | 5 | 0.41 | 0.38 | 0.83 |
| | 10 | 0.38 | 0.41 | 0.83 |
| | 15 | 0.36 | 0.51 | 0.65 |
| HCl solution | 5 | 0.30 | 0.56 | 0.76 |
| | 10 | 0.29 | 0.47 | 0.76 |
| | 15 | 0.37 | 0.49 | 0.58 |
| NaCl+HCl solution | 5 | 0.31 | 0.43 | 0.93 |
| | 10 | 0.34 | 0.44 | 0.83 |
| | 15 | 0.30 | 0.53 | 0.82 |

The values of n for most cases in Table 1 and some samples in Table 2 are close to 0.5 similar to what was observed for the release of vitamin A and E from electrosun cellulose acetate nanofiber mats [9]. Therefore, the release of riboflavin from GA-crosslinked COS/PVA nanofibrous structures could be explained by Fickian diffusion, where the diffusion is more rapid than the relaxation of polymers. The dissolution of polymer seemed to be effectively prevented by crosslinking.

To determine the release rate, equation (1) is differentiated respect to time, so

Table 2 The release parameters for TPP crosslinked nanofiber mats are shown.

| Release media | Crosslinking time (min) | TPP solution | | |
|---------------|-------------------------|--------------|------|-------|
| | | k | n | R^2 |
| Deionized | 1 | 0.63 | 0.15 | 0.95 |

| | | | | |
|-------------------|---|------|------|------|
| water | 2 | 0.79 | 0.09 | 0.84 |
| | 5 | 0.17 | 0.75 | 0.91 |
| NaCl solution | 1 | 0.55 | 0.29 | 0.91 |
| | 2 | 0.37 | 0.42 | 0.79 |
| | 5 | 0.28 | 0.46 | 0.89 |
| HCl solution | 1 | 0.45 | 0.14 | 0.97 |
| | 2 | 0.29 | 0.48 | 0.85 |
| | 5 | 0.32 | 0.37 | 0.82 |
| NaCl+HCl solution | 1 | 0.38 | 0.32 | 0.94 |
| | 2 | 0.22 | 0.52 | 0.92 |
| | 5 | 0.34 | 0.37 | 0.86 |

$$\frac{d}{dt} \left(\frac{C_t}{C_m} \right) = kn t^{n-1} \quad (2)$$

For simplicity, the comparison are made at time 1 hr, so the rate is dependent on the product kn .

The average values of product kn obtained for deionized water, NaCl solution, HCl solution, and NaCl+HCl solution for GA crosslinked mats were 0.1276, 0.1655, 0.1619, and 0.1481 1/h respectively. For TPP crosslinked mats, they were 0.0986, 0.1484, 0.1070, and 0.1206 1/h, respectively. This implies that GA crosslinked mats release riboflavin more rapidly than TPP crosslinked mats.

The release mechanism may involve dissolution of non-crosslinked polymer chains which is probably expedited when hydrolyzed with the acid. However, we have not checked how much degree of dissolution occurred in our system.

The release in NaCl solution was found to be the most rapid due to the osmotic pressure driving water into the nanofibrous structures [2]. The release in deionized water was the slowest since there was no effect of other ions in the solutions. It seemed that hydrolysis with the acid caused the release to be more than with water. For GA crosslinked mats, the salt could help retard the release under the acidic condition, probably due to the screening effect of the salt. For TPP

crosslinked mats, protons from the acid could increase the degree of protonation of chitosan chains, thereby increasing probability to react further with remaining TPP inside the mats. However, this effect competed with the dissolution of PVA in the acidic condition so the overall result was that the release in HCl solution was slightly greater than in the release in water. For the release in NaCl+HCl solution, there would be the competition between continuing crosslinking with the aid of the acid HCl and the swelling with water by osmotic effect from NaCl.

4. Conclusion

In this study, we found that electrospinning by using the solution with 3% COS, 13% PVA and 0.004% riboflavin gave many beads on nanofibers. Prior to applying the nanofiber mats, the structures underwent crosslinking process either with GA vapor or TPP solution. The structures after immersing in TPP solution showed some degree of swelling. The riboflavin releases from the nanofiber mats obtained were conducted in deionized water, NaCl solution, HCl solution and NaCl+HCl solution. It was found that for both types of crosslinking, the release in NaCl solution was the fastest and the release in deionized water was the slowest. These show that the presence of other ions in solution influences the release. For our case, the ions expedite the releases probably by changing the structures of the mats. Comparing two types of crosslinking, crosslinking with TPP seemed to retard the release better than with GA. This is probably attributable to the effective electrostatic interactions. Therefore, it is of interest to explore the crosslinking with both methods, i.e. slight crosslinking with GA to prevent immediate swelling when the mat is in contact with water and then crosslink with TPP. This is an ongoing investigation.

5. Acknowledgement

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6. References

- [1] Gupta K. C., and Jabrail F. H., Effects of Degree of Deacetylation and Cross-Linking on Physical Characteristics, Swelling and Release Behavior of Chitosan Microspheres, *Carbohydrate Polymers*, Vol. 66, pp. 43-54, 2006.
- [2] Shu X.Z., and Zhu K.G., The Influence of Multivalent Phosphate Structure on the Properties of Ionically Cross-Linked Chitosan Films for Controlled Drug Release *J. Pharm. Biopharm.*, Vol. 54, pp. 235-243, 2002.
- [3] Yong-Tang J., and Jian G., Fabrication and Characterization of Poly(Vinyl Alcohol)/ Chitosan Blend Nanofibers Produced by Electrospinning Method, *Carbohydrate Polymer*, Vol. 6, pp. 403-409, 2007.
- [4] Suthamnoi O., Danwanichakul P., Koombhongse P., Loykulnant S., and Chaikumpollert O., Electrospinning of Chito-Oligosaccharide/ Poly(Vinyl Alcohol) Dissolved in Water, *International Journal of Electrospun Nanofibers and Applications*, Vol. 2, No. 2, pp. 135-150, 2008.
- [5] Yang D., Li Y., and Nie J., Preparation of Gelatin/ PVA Nanofibers and Their Potential Application in Controlled Release of Drugs, *Carbohydrate Polymers*, Vol. 69, pp. 538-543, 2007.
- [6] G. Allan, and M. Peyron, Molecular Weight Manipulation of Chitosan I: Kinetics of Depolymerization by Nitrous Acid, *Carbohydrate Research*, Vol. 277, pp. 257-272, 1995.
- [7] Zhang C., Yuan X., Wu L., Han Y., and Sheng J., Study on Morphology of Electrospun Poly(Vinyl Alcohol)

- Mats, *European Polymer Journal*, Vol. 41, pp. 423-432, 2005.
- [8] Crank J., *The Mathematics of Diffusion*, Clarendon Press, London, 1995.
- [9] Taepaiboon P., Rungsardthong U., and Supaphol P., Vitamin-Loaded Electrospun Cellulose Acetate Nanofiber Mats as Transdermal and Dermal Therapeutic Agents of Vitamin A acid and Vitamin E., *European Journal of Pharmaceutics and Biopharmaceutics*, Vol. 67, pp. 387-397, 2007.