

2,6-Dichloro-4-nitroaniline –loaded Electrospun Cellulose Acetate Fiber Mats and Their Release Characteristics

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Mats of cellulose acetate (CA) nanofibers containing 2,6-dichloro-4-nitroaniline (DCNA) were successfully fabricated by electrospinning from the neat CA solution (17% w/v in 2:1 acetone/dimethylacetamide) containing DCNA in various amounts (i.e. 5-15 wt. % based on the weight of CA). The morphological appearance of both the neat and the DCNA-loaded electrospun CA fibers were smooth and the incorporation of DCNA in the neat CA solution did not affect the morphology of the resulting fibers. The average diameters of the neat and the DCNA-loaded electrospun CA fibers ranged between 241-320 nm. The integrity of the as-loaded DCNA in the DCNA-loaded CA fiber mats was intact as verified by the ¹H-nuclear magnetic resonance spectroscopic method. The amount of water retention, the amount of weight loss, and release characteristics of the DCNA-loaded CA fiber mats and the DCNA-loaded as-cast films in distilled water at 30°C were studied. The release characteristics were investigated by the total immersion method. The DCNA-loaded CA fiber mats exhibited greater amount of water retention, weight loss, and DCNA released than the DCNA-loaded as-cast films.

Keywords: Drug delivery, Electrospinning, Cellulose Acetate, 2,6-Dichloro-4-nitroaniline

INTRODUCTION

Electrospinning is an interesting method for producing ultrafine fibers with diameters in the range of sub-micrometers down to nanometers. The principle of this technique is the application of high electrical forces as the means for fiber formation (Doshi et al. 1995). Ultrafine electrospun fibers have been proposed as ideal materials in areas such as

filters, composite reinforcements, scaffolds for cell cultures, and carriers for delivery of drugs, proteins and DNA. One of the advantages of the electrospun fiber mats over cast films as carriers for delivery of chemical agents is the highly porous structure of the electrospun fiber mats which exhibit much greater surface area that assumingly could allow drugs molecules to diffuse out from the matrix more

conveniently (Kenawy et al. 2002, Taepaiboon et al. 2006).

Cellulose acetate (CA) was successfully electrospun in the presence of curcumin, the herbal substance, and it was found that the total amounts of curcumin released from the electrospun fiber mats were greater than those released from the corresponding films (Suwantong et al. 2007). Most of the reports that relate to the use of electrospun fibrous systems as carriers for delivery of chemical agents have focused on biomedical and pharmaceutical applications. Therefore, it is of our interest to explore the use of these electrospun fiber mats as carriers in delivery system for chemical substances in agriculture field e.g., fertilizer, pesticides, and fungicide. In related, but not specific to the use of electrospun fibrous systems as carriers, Singh et al. (2008) investigated release characteristics of thiram fungicide from starch and poly(methacrylic acid)-based hydrogels, while Bottcher et al. (1999) studied the release of various types of biocides, i.e., benzoic, sorbic, and boric acids, from silica and hydroxypropylcellulose films.

The model chemical agent of our interest is 2,6-dichloro-4-nitroaniline (DCNA). The chemical structure of DCNA is shown in figure 1. DCNA is one of the fungicides that are widely used for postharvest control of *Rhizopus* rot on sweet potato roots, fruits of cherries, nectarines, and peaches (Wells 1972). DCNA is also used to control of foliage diseases on a number of vegetable crops caused by *Botrytis* spp. and *Sclerotinia* spp. (Beckman et al. 1965). Therefore, it is interesting here to investigate the release characteristics of DCNA from CA matrix. Electrospinning of CA has been successfully carried out from 12.5-20 wt. % CA in a mixed solvent of 2:1 v/v acetone/dimethylacetamide (Liu et al. 2002). Electrospun CA fiber mats were used as carriers for transdermal or topical delivery of various types of vitamins (Taepaiboon et al.

2007) and anti-inflammatory drugs (Tungprapa et al. 2007).

In this present contribution, DCNA was loaded into CA solution which was later fabricated into the electrospun fibers by electrospinning. Morphology, water retention and weight loss behavior of the neat and the DCNA-loaded electrospun CA fiber mats were studied. The chemical integrity of DCNA in the DCNA-loaded electrospun CA fiber mats was also investigated. Finally, the release characteristics of DCNA from the DCNA-loaded electrospun CA fiber mats were investigated and compared with the corresponding solution-cast films.

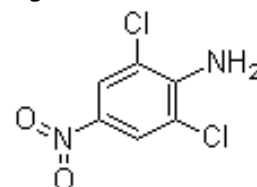


Figure 1. Chemical structure of 2,6-dichloro-4-nitroaniline

EXPERIMENTAL

Materials

Cellulose acetate (CA; $M_w \approx 30,000$ Da; acetyl content = 39.7 wt. %; degree of acetyl substitution ≈ 2.4) and 2,6-dichloro-4-nitroaniline (DCNA) were purchased from Sigma-Aldrich (Switzerland). Acetone (Carlo Erba, Italy), *N,N*-dimethylacetamide [DMAc, Labscan (Asia), Thailand] were of analytical reagent grade and used without further purification.

Preparation of neat and DCNA-loaded CA fiber mats and films

A weight amount of CA powder was dissolved in 2:1 acetone/DMAc to prepare the base CA solution at a fixed concentration of 17% w/v. DCNA-loaded CA solutions were prepared by dissolving DCNA powder in the amounts of 5, 10, and 15 wt.% (based on the weight of the CA powder) in the base CA

solution. In the electrospinning, each of the spinning solutions was contained in a 10 mL syringe, with a 20-gauge stainless steel needle (OD = 0.91 mm) being used as the nozzle. A rotating drum was used as a collector at a rotational speed of about 50 rpm. A Gamma High-Voltage Research ES30P-5W power supply was used to generate a high DC potential. The electric field was fixed at 15 kV/15 cm. The polarity of the emitting electrode was positive. The collection time was about 10 h. The feed rate of the solutions was controlled to about 1 mL·h⁻¹, by means of a syringe pump. DCNA-loaded CA films were also prepared for comparative studies by solvent-casting technique from the same solutions used to prepare the electrospun fiber mats. The thicknesses of both the electrospun fiber mats and the as-cast films were controlled between 40-50 μm.

Characterization of neat and DCNA-loaded CA fiber mats and films

A JEOL JSM-5200 scanning electron microscope (SEM) was used to examine the morphological appearance of the neat and the DCNA-loaded electrospun CA fiber mats and the corresponding as-cast films. Diameters of the individual fiber segments were measured directly from SEM images using SemAfore 4.0 software. The results for each sample were reported as an average value calculated from at least 50 measurements. Statistical analysis was carried out by the one-way analysis of variance (one-way ANOVA) and Scheffe's post hoc test in SPSS. The statistical difference between set of data was considered when $p < 0.05$. A Varian Unity Inova ¹H-nuclear magnetic resonance spectrometer (¹H-NMR) was used to investigate the chemical integrity of DCNA in the DCNA-loaded CA fiber mats, using deuterated dimethylsulfoxide (DMSO-*d*₆) as

the solvent. The water retention and weight loss behavior of both the neat and the DCNA-loaded CA fiber mats and the corresponding as-cast films were determined after submersion in distilled water at 30°C for 7 days according to the following equations:

$$\text{Water retention (\%)} = \frac{M - M_d}{M_d} \times 100,$$

(1)
and

$$\text{Weight loss (\%)} = \frac{M_i - M_d - M_r}{M_i - M_r} \times 100,$$

(2)

where M is the weight of each sample after submersion in distilled water for 7 days, M_d is the weight of the sample after submersion in distilled water for 7 days in its dry state, M_i is the initial weight of the sample in its dry state, and M_r is the weight of DCNA that was released from the sample.

Actual DCNA content

The actual amounts of DCNA in the DCNA-loaded CA fiber mats and the corresponding as-cast films were determined. Each specimen (square shape of 2 cm × 2 cm) was dissolved in 4 mL of 2:1 acetone/DMAc. After that, 0.5 mL of the solution was added into 9.5 mL of DMAc and the actual amounts of DCNA were quantified by a Shimadzu UV-1601 UV-vis spectrophotometer at the wavelength of 368 nm (λ_{max}). The actual amounts of DCNA in the DCNA-loaded CA fiber mat and film specimens were back calculated from the obtained data against a predetermined calibration curve for DCNA.

DCNA-release assay

The release characteristics of DCNA from the DCNA-loaded CA fiber mat and film specimens were investigated. Each specimen (square shape of 2 cm × 2 cm) was submerged in 40 mL of distilled water at 30°C. At a specified submersion period ranging between 4 and 60 days, 2.0 mL of the

solution was withdrawn and an equal amount of the fresh distilled water was refilled. The withdrawn solution was added into 8.0 mL of DMAc and the amounts of DCNA in the sample solutions were quantified by the UV-vis spectrophotometer at the wavelength of 368 nm. The obtained data was calculated against the predetermined calibration curve to determine the cumulative amounts of DCNA released from the specimens at each immersion time point. The experiments were carried out in triplicate.

RESULTS AND DISCUSSION

Morphology of neat and DCNA-loaded electrospun CA fiber mats

The as-prepared 17% w/v CA solution in 2:1 acetone/DMAc was electrospun under the electrostatic field strength of 15 kV/15 cm. Selected SEM images of the electrospun fibers from these solutions are shown in figure 2. Clearly, cross-sectional round fibers with smooth surface were obtained. No presence of DCNA aggregates was observed on the surface of these fibers, indicating that DCNA was incorporated well within the fibers. For the neat CA fibers, the average diameter of the individual fiber segments was 241 ± 56 nm, which is smaller than that reported previously by Suwantong et al. (2007) because in this work, the as-prepared 17% w/v CA solution in 2:1 acetone/DMAc was electrospun under the electrostatic field strength of 15 kV/ 15 cm which was lower than the electrostatic field strength of 17.5 kV/15 cm reported by Suwantong et al. (2007). The lower electrostatic field strength should be resulted in smaller size of the obtained fibers. For the fibers that had been prepared from the CA solutions containing 5, 10, and 15 wt.% of DCNA, the average diameters of the individual fiber segments were 320 ± 45 , 307 ± 51 , and 315 ± 48 nm, respectively. Evidently, the average diameters

of the obtained fibers were increased with the incorporation of DCNA in the base CA solution. For a given spinning condition, the viscosity of the solution is the important factor controlling the size of the electrospun fibers. In this work, even though the viscosities of the solutions were not be determined, it could be possible that the increasing in the size of the fibers upon incorporation of DCNA should be due to the increasing of viscosity of the solution. An increasing of viscosity of solution indicated the increasing of viscoelastic force which enabled the charge jet to withstand a stretching force and therefore resulting in larger diameter of fibers (Deitzel et al. 2001). However, there were no significant different (at $p < 0.05$) in the average diameter of the obtained fibers as increasing the amount of DCNA. This result suggested that the viscosity of the solution might not be different much as increasing the amount of DCNA.

For comparison, both the neat and the DCNA-loaded CA solutions were also fabricated into films by solvent-casting technique. The surface morphology of the as-cast films is not shown. It was found that the surface of the as-cast films was smooth, indicating that DCNA was incorporated well within the films, as in the case of the DCNA-loaded CA fiber mats.

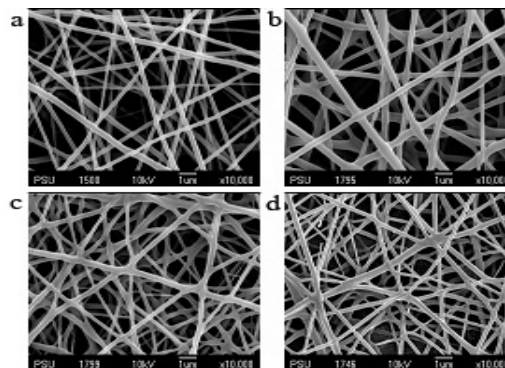


Figure 2. Selected scanning electron micrographs of a) neat CA fiber mat b) 5% DCNA-loaded CA fiber mat c) 10% DCNA-loaded CA fiber mat and d) 15% DCNA-loaded CA fiber mat.

Chemical integrity of DCNA in DCNA-loaded CA fiber mats

Due to the application of a high electrical potential to the DCNA-loaded CA solutions during the electrospinning process, it is questionable whether the chemical integrity of DCNA would be sustained after such a treatment. To examine that, the DCNA-loaded CA fiber mat was dissolved in DMSO- d_6 and the resulting solution was investigated by $^1\text{H-NMR}$. Solutions of both the neat CA fiber mat and DCNA in DMSO- d_6 were used as references. Figure 3 shows $^1\text{H-NMR}$ spectra of the electrospun CA mat, the as-received DCNA powder, and the DCNA-loaded CA fiber mat which contained 15% DCNA. Evidently, the chemical integrity of the as-loaded DCNA was sustained after the electrospinning process, as the peaks corresponding to both CA and DCNA were observed in the $^1\text{H-NMR}$ spectrum of the 15% DCNA-loaded CA fiber mat.

Water retention and weight loss behavior of neat and DCNA-loaded CA fiber mats and films

The neat and the DCNA-loaded CA fiber mats and corresponding as-cast films were characterized to determine their water retention and weight loss behavior after submersion in distilled water at 30°C for 7 days. Figure 4 shows the percentage of water retention and weight loss of the neat and the DCNA-loaded CA fiber mats, and the as-cast CA films, respectively. The amount of water retention for the neat and the DCNA-loaded CA fiber mats was in a range of about 679 to 687%, while these values for the as-cast CA films was in a range of about 122 to 137% (See Figure 4a). The weight loss for the neat and the DCNA-loaded CA fiber mats was in a range of about 51 to 59%, while these values for the as-cast CA films was in a range of about 29 to 35% (See Figure 4b).

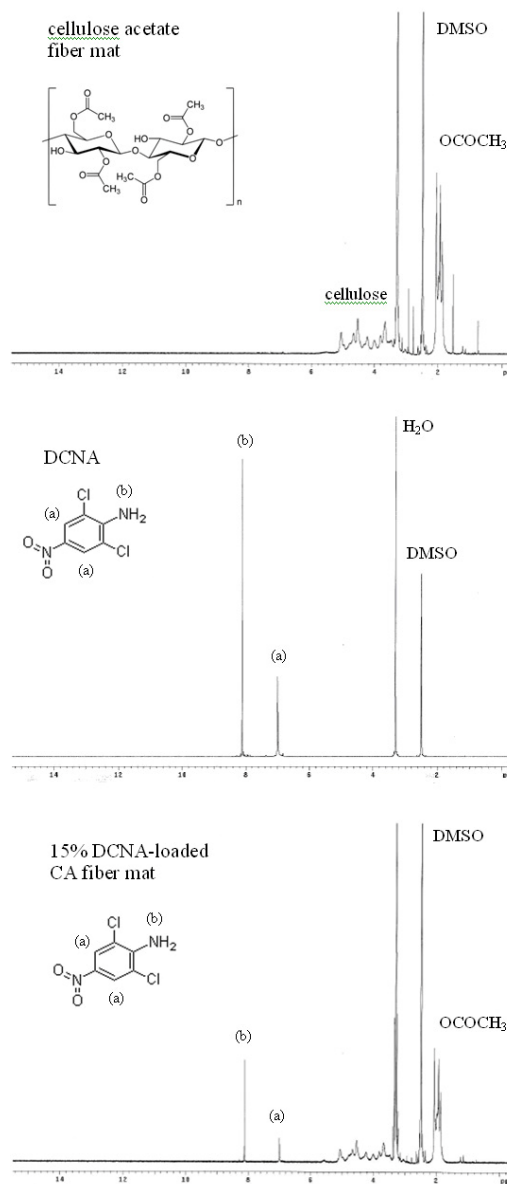


Figure 3. ^1H nuclear magnetic resonance spectra of CA fiber mats, DCNA powder, and 15% wt. DCNA-loaded CA fiber mats after being dissolved in DMSO- d_6 .

Evidently, both water retention and weight loss values for the fiber mat specimens were greater than those of the corresponding films, most likely due to the highly porous nature, therefore much greater surface area, of the fiber mats in comparison

to that of the films (Taepaiboon et al. 2006, Suwanton et al. 2007). However, for given types of samples, variation in the amount of DCNA content did not have much influence on the obtained property values. Suwanton et al. (2007) also reported the greater values of the degree of swelling and the weight loss of the electrospun CA fiber mats than the corresponding films. Both the water retention and the weight loss values of these samples are discussed along with the results on the release characteristics of DCNA from these samples in the following section.

Release of DCNA from DCNA-loaded CA fiber mats and films

Prior to investigate the release characteristics, the actual amounts of the DCNA within these samples need to be determined. The actual amounts of DCNA in the 5, 10, and 15 wt.% DCNA-loaded CA fiber mats were about 80.7 ± 5.1 , 81.2 ± 4.5 , and 84.9 ± 3.8 wt.%, respectively. For the DCNA-loaded CA films, the actual amounts of DCNA in the 5, 10, and 15 wt.% DCNA-loaded CA films were about 82.6 ± 3.4 , 83.5 ± 3.9 , and 86.1 ± 4.3 wt.%, respectively. These values are reported as the actual amounts of DCNA based on the initial amounts of DCNA loaded. The deviation from the ideal value of 100% for these samples could be due to the inhomogeneous distribution of DCNA in different areas of the samples, even though the specimens were carefully prepared. These actual amounts of DCNA were used as the bases in the calculation of the cumulative amounts of DCNA released from these samples.

The release characteristics of DCNA from the DCNA-loaded CA fiber mats and as-cast CA films were carried out by the total immersion method. The samples were immersed in distilled water at 30°C. The cumulative amounts of DCNA released from the DCNA-loaded CA fiber mats and films are

shown in figure 5. which reported as the percentage of the weight of DCNA released divided by the actual weight of DCNA in the specimens. Both the DCNA-loaded CA fiber mat and film specimens showed a gradual increase in the amounts of DCNA released from these specimens. For the 5, 10, and 15 wt.% DCNA-loaded CA fiber mats, the maximum released amounts of DCNA were about 91.8, 95.1, and 92.0% (i.e., weight of DCNA released $\times 100$ /weight of initial DCNA), respectively.

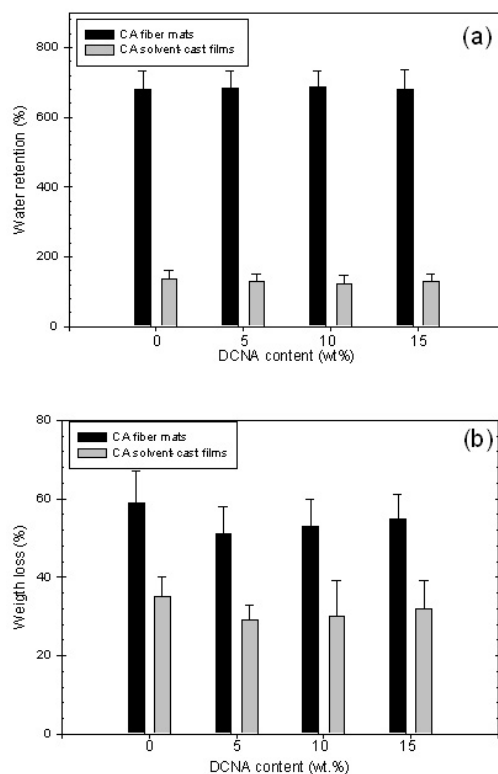


Figure 4. (a) Water retention and (b) weight loss of neat and DCNA-loaded CA fiber mats and corresponding solvent-cast CA films ($n = 3$).

Comparatively, lower values of DCNA released were observed in the cases of the corresponding films, in which, the maximum released amounts of DCNA were only about 68.1, 69.4, and 73.1%, respectively. Obviously, all of the DCNA-loaded CA fiber mats exhibited greater cumulative released amounts of DCNA than those of the corresponding films. The rate and the

amount of substance release in media depend on a number of factors, e.g. the amount of weight loss of a matrix (Zeng et al. 2005), the degree of swelling or the amount of water retention of a matrix, solubility in media and molecular weight of substance released (Rujiravanit et al. 2003). The fact that the amounts of DCNA released from the DCNA-loaded CA fiber mats were greater than those from the DCNA-loaded CA films could be due to the observed greater amounts of water retention and weight loss of all of the DCNA-loaded CA fiber mats over those of the DCNA-loaded CA films and also the highly porous nature of the fiber mats that contributed to greater surface area over that of the films.

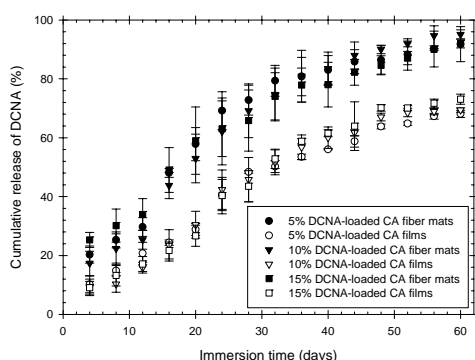


Figure 5. Cumulative release profiles of DCNA from DCNA-loaded CA fiber mats and corresponding solvent-cast CA films ($n = 3$).

CONCLUSIONS

In the present contribution, DCNA-loaded electrospun CA fiber mats were successfully fabricated by electrospinning from the neat CA solutions containing DCNA in the amounts of 5, 10, and 15 wt.% (based on the weight of CA). The surfaces of these fibers were smooth with no evidence of aggregates of DCNA being observed. The average diameters of both the neat and the DCNA-loaded CA fibers were in the range of 241 to 320 nm. The chemical integrity of the as-loaded DCNA in the DCNA-loaded CA fiber

mats was still intact despite being subjected to a high electrical potential during the fabrication into fibers. Upon submersion in distilled water, the water retention and the weight loss behavior of both the neat and the DCNA-loaded CA fiber mats were greater than those of the corresponding films. Finally, the release characteristics of DCNA from the DCNA-loaded CA fiber mats and films were investigated by the total immersion method in distilled water at 30°C for 60 days. It was found that the amounts of DCNA that released from the fiber mats (i.e. about 92-95%) were greater than those from the films counterparts (i.e. about 68-73%).

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REFERENCES

- Beckman, K.M., and Parsons, J.E. (1965). Fungicidal control of sclerotinia wilt in green beans, *Plant Dis. Rep.*, 49. 357.
- Bottcher, H., Jagota, C., Trepte, J., Kallies, K.H., and Haufe, H. (1999). Sol-gel composite films with controlled release of biocides, *J. Control. Release*, 60. 57.
- Deitzel, J.M., Kleinmeyer, J., Harris, D., and Beck Tan, N.C. (2001). The effect of processing variables on the morphology of electrospun nanofibers and textiles, *Polymer*, 42. 261.
- Doshi, J., and Reneker, D.H. (1995). Electrospinning process and applications of electrospun fibers, *J. Electrostat.*, 35. 151.
- Kenawy, E.R., Bowlin, G.L., Mansfield, K., Layman, J., Simpson, D.G., Sanders, E.H., and Wnek, G.E. (2002). New Drug Delivery

- System: Control Release of Tetracycline Hydrochloride as a Model Drug from Electrospun Fibers of Poly(lactic acid) and Poly(ethylene vinyl acetate), *J. Control. Release*, 81(1-2). 57.
- Liu, H., and Hsieh, Y.L. (2002). Ultrafine fibrous cellulose membranes from electrospinning of cellulose acetate, *J. Polym. Sci.-Polymer Physics*, 40. 2119.
- Rujiravanit, R., Kruaykitanon, S., Jamieson, A.M., and Tokura, S. (2003). Preparation of crosslinked chitosan/silk fibroin blend films for drug delivery system, *Macromol. Biosci.*, 3. 604.
- Singh, B., Sharma, D.K., and Gupta, A. (2008). In vitro release dynamics of thiram fungicide from starch and poly(methacrylic acid)-based hydrogels, *J. Hazard. Mater.*, 154. 278.
- Suwantong, O., Opanasopit, P., Ruktanonchai, U., and Supaphol, P. (2007). Electrospun Cellulose Acetate Fiber Mats Containing Curcumin and Release Characteristic of the Herbal Substance, *Polymer*, 48. 7546.
- Taepaiboon, P., Rungsardthong, U., and Supaphol, P. (2006). Drug-Loaded Electrospun Mats of Poly(vinyl alcohol) Fibres and Their Release Characteristics of Four Model Drugs, *Nanotechnology*, 17. 2317.
- Taepaiboon, P., Rungsardthong, U., and Supaphol, P. (2007). Vitamin-Loaded Electrospun Cellulose Acetate Nanofiber Mats as Transdermal and Dermal Therapeutic Agents for Vitamin A Acid and Vitamin E, *Eur. J. Pharm. Biopharm.*, 67. 387.
- Tungprapa, S., Jangchud, I., and Supaphol, P. (2007). Release Characteristics of Four Model Drugs from Drug-Loaded Electrospun Cellulose Acetate Fiber Mats, *Polymer*, 48. 5030.
- Wells, J.M. (1972). Heated wax-emulsions with benomyl and 2,6-dichloro-4-nitroaniline for control of postharvest decay of peaches and nectarines, *Phytopathology*, 62. 129.
- Zeng, J., Aigner, A., Czubayko, F., Kissel, T., Wendorff, J.H., and Greiner, A. (2005). Poly(vinyl alcohol) nanofibers by electrospinning as a protein delivery system and the retardation of enzyme release by additional polymer coatings, *Biomacromolecules*, 6. 1484.
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