Electrospinning of polystyrene fibers functionalized with inclusion complex of 1-methylcyclopropene and α-cyclodextrin

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ABSTRACT

1-Methylcyclopropene (1-MCP) shows high efficacy in inhibiting ethylene-induced physiological responses in various produce. Continuous 1-MCP treatment is expected to exhibit better outcome than the conventional post-harvest single treatment. The current research is devoted to developing 1-MCP-functionalized packaging materials for pre-harvest and in-transit 1-MCP treatments. Incorporation of 1-MCP into polymer fibers was achieved by electrospinning of polymer solutions in which the inclusion complex between 1-MCP and α-cyclodextrin (α-CD) was suspended. Polystyrene (PS) was used as the polymer and the electrospinning process parameters, i.e., PS concentration and α-CD loading, were investigated. A mixture solvent between chloroform and toluene was employed. The physicochemical properties of the electrospinning (ES) solutions, i.e., viscosity, conductivity, and surface tension, were determined. The average fiber diameter was proportional to approximately the one-sixth power of the capillary number of ES solution. The fibers were also subjected to X-ray diffractometry and electron microscopy. Finally, the ES solution containing 20.0 wt% PS suspended with 50 wt% 1-MCP/α-CD complex with respect to PS were electrospun. The complex-functionalized PS fiber was characterized to retain over 80% of the initial 1-MCP. The release profiles of 1-MCP from the complex and its functionalized fiber were studied with a home-made dynamic vapor sorption system in which the relative humidity was ramped at a constant rate at the constant temperatures of 30, 40, and 50°C. 1-MCP release was suppressed by the suspension of the complex on electrospun fiber and as well the coating of the complex with PS.

Keywords: Electrospinning; 1-methylcyclopropene; α-cyclodextrin; relative humidity; release

INTRODUCTION

1-Methylcyclopropene (1-MCP), consisting of a three-membered ring with a methyl group at C1 position, is a phytohormone antagonist which efficiently delays ethylene-induced color change, ripening, and senescence in various produce. Inclusion complexation with α-cyclodextrin (α-CD) stabilizes and transforms the highly reactive and explosive gaseous 1-MCP into powder form. At present, inclusion complexation with α-CD still remains the sole means for stabilization and application of 1-MCP. Based on the satisfactory results with the currently-in-use one-time post-harvest treatment, continuous application of 1-MCP on target produce is expected to provide a more promising outcome.

CDs are cyclic oligosaccharides with a truncated molecular structure made up of α(1–4)-glycosidic-bonded glucopyranose units. The macro-ring of CD has a relatively hydrophobic inner cavity as opposed to the hydrophilic exterior [1]. CDs are able to include a wide variety of guest molecules that are less polar than water into their cavities to form inclusion complexes [2]. Inclusion complexation by CDs as the host molecule can provide stabilizing and solubilizing effects on the guest molecules [3,4]. Uyar et al. [5] have successfully produced CD-functionalized poly(methyl methacrylate) nanofibers by electrospinning. CD molecules were found to locate on the fiber mats, which indirectly indicated the conservation of functionality of the CD as an encapsulant. Uyar et al. [6] showed that the inclusion complexes of menthol and native CDs can be incorporated in polystyrene fibers by electrospinning.

The current research was aimed at developing 1-MCP-functionalized packaging materials for pre-harvest and in-transit 1-MCP treatments of produce. We attempted to incorporate 1-MCP into polystyrene fibers by electrospinning polystyrene solutions in which the inclusion complex between 1-MCP and α-CD was suspended. Electrospinning refers to the method by which fine fibers are spun from a polymer solution electrically charged at high voltage. In this study, polystyrene (PS) was used and the electrospinning process parameters, i.e., PS concentration and α-CD loading, were investigated. The physicochemical properties of the electrospinning (ES) solutions, i.e., conductivity, viscosity, and surface tension, were determined. We
have also characterized the electrospun fibers by X-ray diffractometry (XRD) and scanning electron microscopy (SEM). The fiber functionalized with 1-MCP/α-CD complex was produced and subjected to release experiments under humidity ramping atmosphere at constant temperatures.

**MATERIALS & METHODS**

**Materials** Polystyrene (PS) (MW1C,D, Mw ~ 400,000) was purchased from Toyo Styrene Co., Ltd. (Tokyo, Japan). 3-Chloro-2-methylpropene (98%) and lithium diisopropylamide (30 wt% suspension in mineral oil) were from Sigma-Aldrich Japan K. K. (Tokyo, Japan). A 100 μL/L isobutylene standard gas was purchased from Sumitomo Seika Chemicals Co., Ltd. (Osaka, Japan). Tetrabutylammonium chloride (TBAC) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). 3-Chloro-2-methylpropene (98%) and lithium diisopropylamide (30 wt% suspension in mineral oil) were from Sigma-Aldrich Japan K. K. (Tokyo, Japan). Distilled water was used throughout the entire experiment.

**Preparation of ES Solutions** A mixture solvent of chloroform and toluene at the weight ratio of 6:4 was used as the solvent of the electrospinning (ES) solutions. The ES solutions were prepared by adding PS and α-CD into the mixture solvent. The PS concentration was varied between 10 and 22.5 wt% with respect to PS, while the α-CD loading was varied from 0 to 100 wt% with respect to PS. α-CD instead of the 1-MCP/α-CD complex was used to predict the possible effect of complex loading on the characteristics of the electrospun fibers. TBAC was added at 1.5 wt% with respect to PS to improve the spinnability of the ES solutions.

**Measurements of Physicochemical Properties of ES solutions** The physicochemical properties of the ES solutions were determined at 25°C. The surface tension was determined with a Digital Tensiometer K10ST (KRÜSS GmbH, Hamburg, Germany) using a suspended platinum plate (PL 10, KRÜSS GmbH) with a wetting length of 40 mm. The viscosity was measured using a Brookfield DV-II+ Programmable Viscometer (Brookfield Engineering Laboratories, Inc., Middleboro, MA) equipped with an SC4-18 spindle. A total of 30 readings collected every 4 s over a measurement time of 2 min were averaged to give the viscosity of the solutions. The conductivity was measured with a CT-57101B conductivity cell (DKK-TOA Corporation, Tokyo, Japan) connected to a benchtop conductivity meter (CM-R25, DKK-TOA Corporation).

**Electrospinning** The electrospinning process was performed under ambient condition in horizontal position. The ES solutions were loaded into a 5-mL glass syringe mounted with an 18G stainless steel spinneret. The syringe was fixed horizontally onto a syringe pump (KDS-100, KD Scientific, Inc., Holliston, MA). The electrode of a high voltage power supply (HVU-30P100, MECC Co., Ltd., Fukuoka, Japan) was clamped to the spinneret to positively charge the ES solution. The electrospinning process parameters were set at: working distance between spinneret and target = 10 cm; flow rate of ES solution = 1 mL/min; and electrical potential = 10 kV. A grounded stationary stainless steel collector (12 cm × 10 cm) enveloped with an aluminum foil was used as the target for random fiber deposition. The electrospinning process was carried out in a ventilated glove box.

**Characterization of Electrospun Fibers** The fibers electrospun from the ES solutions of 20 wt% PS and varying α-CD loading were subjected to X-ray diffractometry (XRD). The fibers were electrospun onto standard glass sample holders. The XRD patterns of the fibers were recorded on a Rigaku RINT 2500 HF X-ray diffractometer (Rigaku, Tokyo, Japan) with Cu Ka radiation (λ = 0.154 nm) generated at 40 mA and 40 kV. The samples were scanned at 1°/min from 3° to 50° (2θ) by a sampling width of 0.05°. The divergence slit was set at 0.5° and the receiving slit was set at 0.6 mm. All the electrospun fibers were morphologically characterized by scanning electron microscopy (SEM) and their average fiber diameters were also determined. The fibers were adhered to a SEM stub using a double-sided adhesive carbon tape (Nisshin EM Corporation, Tokyo, Japan). Electron microscopy was performed with a JEOL JSM-6060 scanning electron microscope (JEOL Ltd., Tokyo, Japan) at an operating voltage of 2.0 kV and a spot size of 30. Before observation, the stubs were coated with a sputtered layer of Pt-Pd using a magnetron sputter coater (Model MS-15, Vacuum Device Inc., Tokyo, Japan).

**Preparation of 1-MCP/α-CD Complex** 1-MCP synthesis was carried out according to the procedure detailed in our previously reported work [7,8] based on the method reported by Sisler and Serek [9] with some minor modifications. The complexation reaction between 1-MCP and α-CD was performed also as described previously [7,8] at 15°C. The reaction was aided by agitation of the α-CD solution at 300 rpm for 7 h. At the end of the encapsulation process, the wet precipitate of the 1-MCP/α-CD complex was recovered by centrifugation (3000 rpm, 15 min) and then dried in-vacuo at ambient temperature for 24 h.
Release Experiment under Relative Humidity Ramping Conditions  Both the inclusion complex and the complex-functionalized fiber were subjected to release experiments. Approximately 30 mg of inclusion complex was filled into a flat bottomed aluminum sample pan (1 mm × 13 mm i.d.). The sample holder was place in a glass vial (78 mm × 15 mm i.d.), the temperature of which was maintained at 30, 40, or 50°C. The flow rate of moistened air through the vial was set at 100 mL/min with a response time of 0.25-0.5 min. The relative humidity (RH) was ramped from 10 to 90% at the rate of 0.33% RH/min over a release period of 4 h. A Shimadzu GC-14B gas chromatograph (Shimadzu Corp., Kyoto, Japan) was utilized to continuously monitor the release of 1-MCP from the inclusion complex. Effluent from the vial was sampled with a 5.0-mL sampling loop and introduced every 5 min into the gas chromatograph via a heated (70°C) stainless steel transfer line. The gas sample was separated in a glass column (2.1 m × 3.2 mm i.d.) packed with PEG-20M (20% on Chromosorb W 80/100 AW mesh, Shinwa Chemical Industries, Ltd., Kyoto, Japan) and subsequently detected using a flame ionization detector. The injection port and detector temperatures were set at 140 and 200°C, respectively. The column temperature was 130°C. Quantification of 1-MCP was accomplished using an external standard protocol [10]. The isobutylene gas standard (100 μL/L) was analyzed with the GC-14B equipped with a stainless steel column (1.5 m × 3 mm i.d.) packed with the same PEG-20M.

The complex-functionalized fiber were electrospun from the ES solution containing 20 wt% PS and 50 wt% inclusion complex. Roughly 90 mg of fiber containing an equivalent amount of ca 30 mg inclusion complex was spun onto a φ76 mm stainless steel mesh. The mesh, along with the fiber, was place in a plastic container (46.4 mm × 81.5 mm i.d.) through which moistened air was flowed at the flow rate of 100 mL/min. The response time was 2.0-2.5 min. Similarly, the effluent was analyzed with the GC-14B and the glass packed column. The release fluxes of 1-MCP from the inclusion complex and the complex-functionalized fiber were expressed by [μg/s·g-complex].

RESULTS & DISCUSSION

To spin the complex-suspended ES solutions, solvents in which the complex dissociates the least, if not totally undissociated, are preferable. In a preliminary study, five organic solvents, i.e., acetone, chloroform, dichloromethane, dimethylformamide, and toluene, were tested for selection of suitable solvents. Dissociation of 1-MCP/α-CD complex in the organic solvents descends in the order of dimethylformamide > acetone > dichloromethane > chloroform ≥ toluene. Over 96% of the complex remained intact in toluene and chloroform. While dichloromethane was able to retain over 82% of the complexed 1-MCP, more than 96% of the complex dissociated in acetone. Despite being one of the most extensively studied organic solvents for electrospinning, the complete dissociation of 1-MCP/α-CD complex in dimethylformamide rendered it unsuitable for use in this study. With the previously mentioned electrospinning process parameters, the mixture solvent between chloroform and toluene at the weight ratio of 6:4 was found to perform satisfactorily. In addition to the non-conducting nature of PS, both the organic solvents of choice are of low conductivity. TBAC was added at 1.5 wt% with respect to PS to improve the conductivity and thus, the spinnability of the ES solutions. Nonetheless, precaution has to be taken as the hygroscopicity of the cationic surfactant may adversely affect the stability of the complex in the electrospun fibers.

The physicochemical properties of the ES solutions and the average diameter of their respective resultant fibers were tabulated in Table 1. As shown in Figure 1, we found that the average fiber diameter was proportional to approximately the one-sixth power of the capillary number of ES solution. The correlation between the two parameters could be represented by:

\[ \frac{d_L}{d_n} = 0.003Ca^{0.17} \]  

where \( d/d_n \) [-] is the ratio of average fiber diameter to the inner diameter of the spinneret and \( Ca \) [-] is the capillary number of ES solution. The α-CD-free ES solutions with PS concentrations between 15.0 and 22.5 wt% were spinnable but bead-free fibers could only be obtained at concentrations above 17.5 wt% PS. With the increase of PS concentration, the viscosity of the ES solutions increases. Hence, the fiber diameter also increases because the solutions turn increasingly resistant against stretching. The average fiber diameter recorded an increase from 3.3 to 5.1 μm with the increase of PS concentration within the spinnable range. The effects of suspension of solid on the physicochemical properties of the ES solutions and the characteristics of the electrospun fibers were investigated by addition of α-CD in the ES solutions instead of the 1-MCP/α-CD complex because the laboratory-scale batch-wise synthesis of the complex yields only a
Table 1. Physicochemical properties of ES solutions and average diameter of the resultant electrospun fibers

<table>
<thead>
<tr>
<th>Composition of ES solutions [wt%]</th>
<th>Viscosity [mPa·s]</th>
<th>Conductivity [μS·cm⁻¹]</th>
<th>Surface tension [mN·m⁻¹]</th>
<th>Average fiber Diameter [μm]</th>
</tr>
</thead>
<tbody>
<tr>
<td>PS 10.0 0 1.5 TBAC*</td>
<td>94.4</td>
<td>0.07</td>
<td>28.0</td>
<td>–</td>
</tr>
<tr>
<td>12.5</td>
<td>201.5</td>
<td>0.09</td>
<td>28.1</td>
<td>–</td>
</tr>
<tr>
<td>15.0</td>
<td>404.1</td>
<td>0.10</td>
<td>28.3</td>
<td>3.3±1.5</td>
</tr>
<tr>
<td>17.5</td>
<td>807.4</td>
<td>0.11</td>
<td>28.0</td>
<td>3.5±1.1</td>
</tr>
<tr>
<td>20.0</td>
<td>1442</td>
<td>0.13</td>
<td>27.2</td>
<td>4.3±1.0</td>
</tr>
<tr>
<td>22.5</td>
<td>2709</td>
<td>0.14</td>
<td>26.5</td>
<td>5.1±1.2</td>
</tr>
<tr>
<td>10.0 100 1.5 TBAC*</td>
<td>152.9</td>
<td>0.02</td>
<td>28.2</td>
<td>–</td>
</tr>
<tr>
<td>12.5</td>
<td>293.5</td>
<td>0.06</td>
<td>28.6</td>
<td>1.5±1.4</td>
</tr>
<tr>
<td>15.0</td>
<td>684.2</td>
<td>0.07</td>
<td>28.9</td>
<td>3.3±1.8</td>
</tr>
<tr>
<td>17.5</td>
<td>1435</td>
<td>0.08</td>
<td>27.7</td>
<td>4.1±2.6</td>
</tr>
<tr>
<td>20.0</td>
<td>2962</td>
<td>0.08</td>
<td>26.0</td>
<td>4.4±2.2</td>
</tr>
<tr>
<td>22.5</td>
<td>6007</td>
<td>0.08</td>
<td>25.1</td>
<td>–</td>
</tr>
<tr>
<td>20.0 0 1.5 TBAC*</td>
<td>1442</td>
<td>0.13</td>
<td>27.2</td>
<td>4.3±1.0</td>
</tr>
<tr>
<td>20</td>
<td>1770</td>
<td>0.12</td>
<td>27.1</td>
<td>4.3±0.9</td>
</tr>
<tr>
<td>40</td>
<td>2019</td>
<td>0.11</td>
<td>27.1</td>
<td>4.4±1.3</td>
</tr>
<tr>
<td>60</td>
<td>2334</td>
<td>0.10</td>
<td>26.9</td>
<td>4.6±1.7</td>
</tr>
<tr>
<td>80</td>
<td>2701</td>
<td>0.09</td>
<td>26.3</td>
<td>3.8±2.3</td>
</tr>
<tr>
<td>100</td>
<td>2962</td>
<td>0.08</td>
<td>26.0</td>
<td>4.4±2.2</td>
</tr>
</tbody>
</table>

* With respect to polymer (PS)

Figure 1. Ratio of average fiber diameter to the inner diameter of the spinneret as a function of the capillary number of ES solutions at various PS concentrations, 0 wt% α-CD (○); various PS concentrations, 100 wt% α-CD (□); and 20 wt% PS, various α-CD loadings (△).

limited amount. With the addition of 100 wt% α-CD with respect to PS into the ES solutions, the spinnable PS concentration was found between 12.5 and 20.0 wt%. At 10.0 wt% PS, the ES solution was rather sprayed than spun, resulting in seriously beaded fibers, whereas at 22.5 wt% PS, electrospinning was not possible due to high viscosity of the solution. The shift of the spinnable range of PS concentration was presumably ascribable to the increase in viscosity resulted from α-CD suspension. The average fiber diameter increased from 1.5 to 4.4 μm with increasing PS concentration from 12.5 to 20.0 wt%. The suspension of α-CD at 100 wt% exerted only trivial influences on the surface tension and even shifted the lower limit of spinnable conductivity range to a lower value of 0.06 μS·cm⁻¹. Hence, both the properties could reasonably be excluded from being the main parameters dictating the spinnability of the studied ES solution systems. Contrary to the positive effect of CD on electrospinning that has been related to conductivity improvement of the ES solutions by Uyar et al. [11], the increase of α-CD demonstrated a dilution effect on the conductivity plausibly because of its insolubility in the ES solutions.
The fibers electrospun from the ES solutions of 20 wt% PS with varying α-CD loading had almost similar average fiber diameter (3.8-4.6 μm). However, the fiber diameter was more dispersed around the mean values as α-CD content increased, presumably suggesting the disturbance introduced to the ES solution jet by the suspended α-CD particles. The α-CD crystals were observed as distributed on the fiber mats while being coated by a thin layer of PS (Figure 2). X-ray diffractometry of the fibers showed progressively higher crystallinity in the fibers with higher α-CD loadings, ruling out the inhomogeneous suspension of the crystals in the ES solutions as visually prompted by the SEM micrographs (Figure 3).

**Figure 2.** SEM micrographs of fibers electrospun from α-CD-suspended ES solutions containing (a) 0, (b) 20, (c) 40, (d) 60, (e) 80, and (f) 100 wt% α-CD with respect to PS. The PS concentration of the solutions was 20.0 wt% and TBAC content was 1.5 wt% with respect to PS.

**Figure 3.** XRD patterns of fibers electrospun from ES solutions with varying α-CD loadings. The PS concentration of the solutions was 20.0 wt% and TBAC content was 1.5 wt% with respect to PS.

The complex-functionalized fiber was electrospun from the ES solution containing 20.0 wt% PS, 50 wt% complex, and 1.5 wt% TBAC. The inclusion complex had an average particle size of 10.4 μm. Quantification by gas chromatography confirmed that more than 80% of the 1-MCP was retained in the fiber. The fiber basically exhibited 1-MCP release profiles that differed from the complex crystals. The differences could be accounted for by the difference in packing manner of the crystals in the two samples. The fiber demonstrated a 1-MCP release profile responsive to storage humidity. At 30°C, the flux of 1-MCP release increased slowly until 50% RH, above which the release rate was accelerated exponentially (Figure 4). Release acceleration
was triggered at lower RHs at higher temperatures. At 40°C, the release was disturbed at 65% RH and subsequently retarded at RHs above 90%. At 50°C, the disturbance occurred at ca 55% RH while the release impediment was observed at 80% RH. 1-MCP release was suppressed by the suspension of the complex crystals on electrospun fiber and as well the coating of the complex with PS.

Figure 4. 1-MCP release profiles of (a) 1-MCP/α-CD complex and (b) its functionalized fiber under RH-ramping conditions at 30 (□), 40 (○), and 50°C (△).

CONCLUSION

The viscosity of ES solution was the major physicochemical property that affects the average diameter of the electrospun fibers. 1-MCP retention of over 80% in the complex-functionalized fiber suggests the feasibility of electrospinning for preparation of 1-MCP-functionalized packaging materials for continuous 1-MCP treatment of produce. The 1-MCP/α-CD complex demonstrated an improved 1-MCP release behavior by suspension of the complex crystals on electrospun fiber and desensitization of moisture responsivity.

REFERENCES