Electrospinning of Pullulan/Linalool-Cyclodextrin Inclusion Complex Nanofibers for Face Mask Functionalization

Kai Yokoo
Course: FSAD 4010

College of Human Ecology
Department of Fiber Science and Apparel Design
Cornell University

December 18th, 2021

Introduction

In recent years, the development of nanofibrous structures have been used universally in medical and tissue engineering, commercial products, and nonwoven textiles (2). With the introduction of facemasks into everyday life as a result of the COVID-19 pandemic, there has been an uptick in necessity for masks with comfortability and protectivity. This experiment was conducted using an aromatic essential oil, linalool, and worked to form successful inclusion complexes made up of a combination of cyclodextrin, pullulan, and linalool. The goal is to create a nanofiber with similar bioactive properties as linalool, which can then be beneficially incorporated into the design of facial masks.

Pullulan, a microbial polysaccharide, was used as a polymeric carrier matrix during electrospinning process. It is a linear polymer chain structure consisting of maltotriose units, which are numbered glucose units connected by glycosidic bonds (Figure 1). Widely used for its adhesive properties and solubility enhancement, pullulan was implemented into electrospinning for its positive benefits. It resulted in less defective web structures and more control over the viscosity and surface tension of the electrospinning system (1).

Cyclodextrins (CD) are used extensively for its ability to make inclusion complexes. Its ring-shaped structure along with its hydrophobic interior creates a host that can interact with water insoluble molecules. The circular structure builds up a “cavity,” which encapsulates the intended hydrophobic molecules and in the case of linalool, can help fixate the volatility of a specific molecule (Figure 1). Its compatibility with other materials, being able to improve solubility, reactivity, and degradation, makes cyclodextrin quintessential for pursuing the electrospinning process (3).

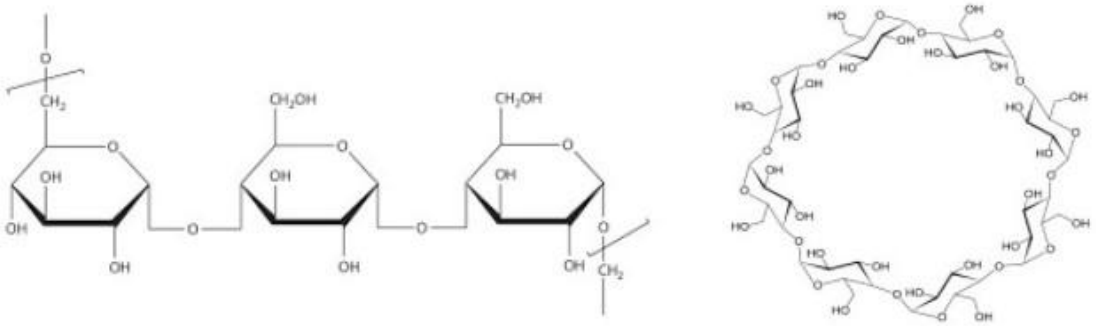

Figure 1. Left: Structure of Pullulan. Right: Structure of γ-Cyclodextrin (CD)

The linalool/γ-Cyclodextrin/pullulan nanofibrous material was created using the electrospinning process. Electrospinning is an electrohydrodynamic process where a high voltage power source pulls fibers from a spinneret. There are key components to our set-up of electrospinning: adding the homogenous polymer solution to the syringe, adjusting the flow rate through the syringe pump, applying a high voltage, and creating a collection method for resulting analysis (2). Electrospinning begins when the voltage applied causes a cone-shaped deformation on the tip of the syringe holding the solution. When the electric charge surpasses the surface tension of the solution, the solution is drawn out into nanofibers with diameters reaching as small as nanometers. The benefits of electrospinning as opposed to other spinning methods is based on its fiber diameter uniformity, high porosity, and its high surface area (4).

In this experiment, an essential oil, linalool, is being incorporated into the nanofibers. Linalool, typically used for its fragrance and antimicrobial properties, is the central focus of this experiment as its physical properties are what we are aiming to integrate directly into the nanofibers (5). However, since linalool is a volatile substance, the creation of inclusion complexes with γ-Cyclodextrin (CD) helps decrease the volatility while preserving its other characteristics.

Experimental Method

The experiment consisted of three different components. First, the solution was created by mixing measured amounts of linalool, pullulan, and γ-Cyclodextrin (CD). There were two

different samples that were mixed, one was a control experiment, and the other was a sample experiment. Then, these solutions went through the electrospinning process, where the various electrospinning conditions were additionally recorded. The samples were placed under a digital optical microscope, where the visual image analysis of the nanofibers helped determine if the sample needed to be re-spun. Additional tests were performed in order to characterize the sample further: X-ray diffraction (XRD), Thermogravimetric analysis (TGA), and Fourier transform infrared spectroscopy (FTIR).

In the first step, two samples were made, the control experiment and the sample experiment. The control experiment had a linalool concentration of 11.3% (w/w) and a pullulan concentration of 20% (w/v) and was dissolved in distilled water. The sample experiment, with a 2:1 molar ratio of linalool and γ-Cyclodextrin (CD), had a linalool concentration of 11.3% (w/w), a pullulan concentration of 20% (w/v), and a γ-Cyclodextrin (CD) concentration of 23% (w/v). Here, γ-cyclodextrin was initially dissolved in distilled water, then linalool was added and stirred overnight to form inclusion complexes Afterwards, pullulan was added to the white solution of inclusion complexes and both solutions were loaded into electrospinning set-up to be electrospun into nanofibers. .

The solutions for the control experiment and sample experiment were carefully transferred to syringes, making sure there were minimal air bubbles. Then, they were fitted with a 27 Gauge needle and securely placed in the syringe pump. The electrospinning collector was set-up with a thin layer of aluminum foil wrapped around it and secured with a glass microscope slide on the side. The glass slide was removed for optical microscope analysis after a short period of electrospinning. The conditions for the electrospinning process are listed in Table 1 below.

Table 1. Chart of the initial and environmental conditions for the electrospinning of the control and sample experiment.

Sample	**Control Experiment (pullulan/linalool)**	**Sample Experiment (pullulan/linalool-γCD-IC)**
Flow Rate (mL/hr)	1.0	0.9

Voltage (kV)	12.5	16
Relative Humidity (%)	41.2%	41.2%
Temperature (℃)	20	20

Upon further analysis of the collected sample for the sample experiment containing the pullulan/linalool-γCD-IC, the volume of nanofibers that were collected was deemed insufficient. This was due to the fact that the solution was dissolved in only 1 mL of water, so the thickness of the produced nanofibers was not enough to do further extensive analysis on. As a result of this, the sample was re-done but the solution was dissolved in 2 mL of water instead of 1 mL of water. In order to keep the same 2:1 molar ratio between linalool and γ-cyclodextrin, the amount of linalool, pullulan, and γ-Cyclodextrin was doubled. The procedure for preparing the solution and carrying out the electrospinning process was the same. A 2 mL syringe and a 27 Gauge needle was used to set-up the experiment, and the initial conditions for the electrospinning are represented by Table 2 below.

Table 2. Chart of the initial and environmental conditions for the electrospinning of the re-done sample experiment (pullulan/linalool-γCD-IC).

Sample	**Sample Experiment (pullulan/linalool-γCD-IC)**
Flow Rate (mL/hr)	0.8
Voltage (kV)	16
Relative Humidity (%)	57%
Temperature (℃)	21

After removing the glass slide from the collector and taking visual images from the optical microscope, the re-done sample experiment was the correct thickness to be able to

continue conducting the various analysis techniques. Both the control experiment and the re-done sample experiment were analyzed via X-ray diffraction (XRD) analysis, Thermogravimetric analysis (TGA), and the Fourier transform infrared spectroscopy (FTIR) analysis.

Results and Discussion

The sample experiment containing pullulan/linalool-γCD-IC had different physical properties compared to the control experiment containing pullulan/linalool. While both solutions appeared homogeneous, the sample experiment initially had a milky white physical appearance and a viscous quality to it. This viscosity is directly resulted from the inclusion complexes formed in the sample experiment, as the significantly larger molecules make the solution more difficult to move.

In Figure 2, glass slides with the previously collected nanofibers from both experiments were put under an optical microscope where photographs were taken directly from the microscopic lens.

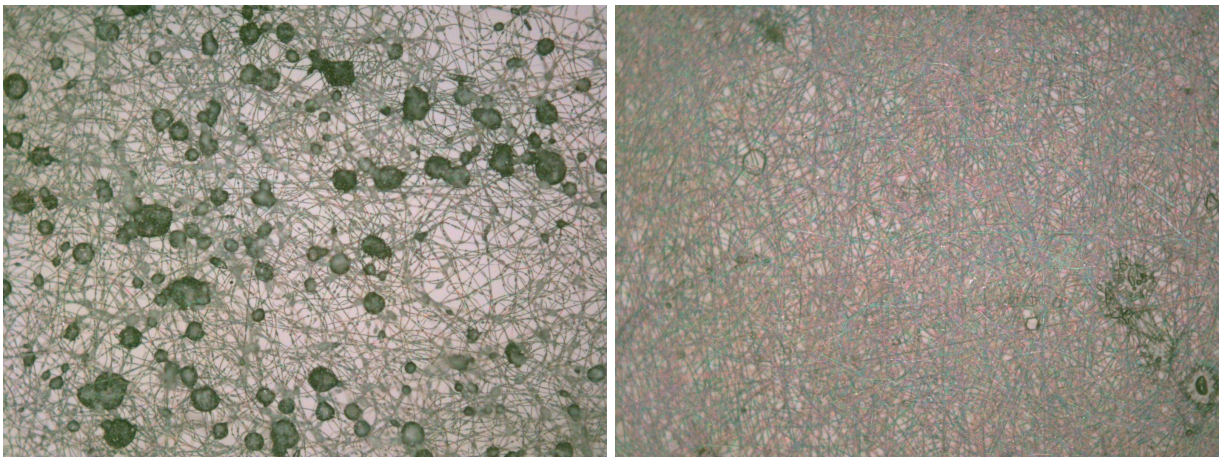

Figure 2. Left: Optical microscope images of the pullulan/linalool-γCD-IC sample experiment. Right: Optical microscope images of the pullulan/linalool control experiment.

From the microscope images, there's a distinct difference between the beads present in the control experiment and the sample experiment. The optical image of the pullulan/linalool-γCD-IC nanofibers contains a high proportion of beads in comparison to the pullulan/linalool fibers. The presence of beads indicates the crystals of inclusion complexes. .

There are also some splashed areas on glass slide for the inclusion complex included sample. The optical microscopy results from the re-done sample experiment is depicted below.

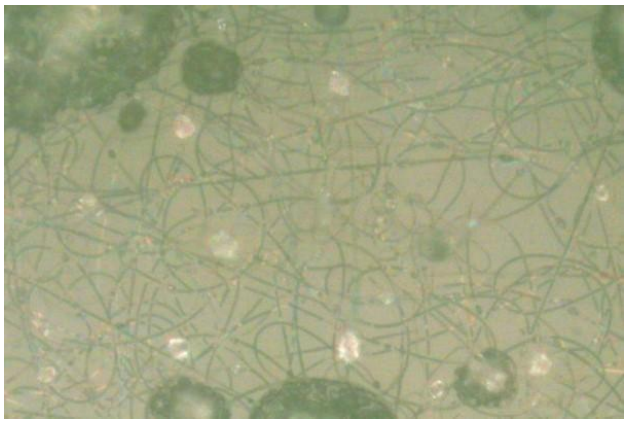

Figure 3. Optical microscope images taken of the re-done sample experiment containing pullulan/linalool-γCD-IC nanofibers dissolved in 2 mL of water instead of 1 mL.

Although the optical microscope image is not as clear, a physical analysis of the resulting sample after it had been fully electrospun showed a thicker, more functional nanofiber. Although there are some beads and splashes present in the glass slide optical microscopy analysis, the overall homogeneity of the sample was more consistent. Additionally, having more layers of electrospun nanofibers means that when it is cut apart for further analysis, it is able to come off the aluminum foil, and the fibers don't lose their structural integrity when doing so. Accordingly, both samples of electrospun nanofibers were ready for more analytical tests.

Both samples were further analyzed through Fourier transform infrared spectroscopy (FTIR) analysis. They were loaded into the spectrometer, where a force gauge was launched, and the necessary measurements were recorded in the form of a graph.

Thermogravimetric analysis (TGA) was then performed on the two samples separately. Each sample was weighed using the Cahn 29 Automatic Electrobalance and was loaded onto the instrument, immediately being zeroed. The samples were loaded onto the furnace, and the proceeding measurements were recorded. The X-ray diffraction (XRD) analysis was performed in a similar manner.

For further investigation, the Fourier transform infrared spectroscopy (FTIR) analysis was used to determine the presence of linalool in the electrospun nanofibers. The results from this test, represented by Figure 4, are illustrated through the graph below.

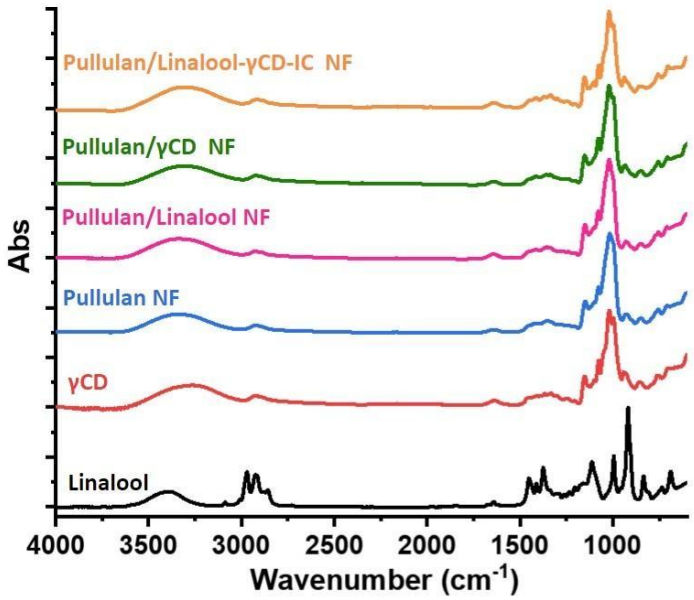

Figure 4. Fourier Transform Infrared Spectroscopy (FTIR) graphical representation of the observed wavenumber (in 1/cm) with the resulting absorption of various combinations of pullulan, linalool, and γ-Cyclodextrin. Credits to Asli Celebioglu.

Fourier transform infrared spectroscopy (FTIR) analysis helps quantify the presence of specific molecules in the electrospun nanofibers. It uses an infrared spectrometer to determine the wavelengths of a specific material, and from this estimation, it can be compared with other materials to determine the presence or absence of certain molecules. Each component of this experiment was analyzed individually and in association with other components. This analysis is shown in Figure 4. In order to determine the presence of linalool, magnified graphs of the regions of linalool where the wavelength reaches maximum peaks were created in Figure 5.

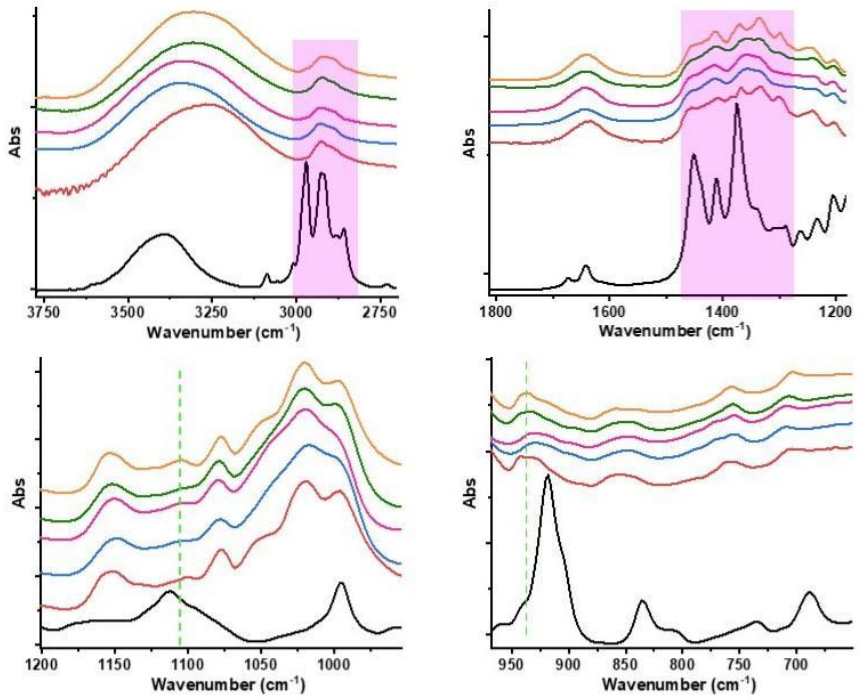

Figure 5. Magnified Fourier transform infrared spectroscopy (FTIR) graphical representation of the observed wavenumber (in 1/cm) of pullulan, linalool, and γ-Cyclodextrin. The top graphs show the highlighted regions that are going to be magnified in the graphs below. The bottom graphs contain a vertical dotted green line that indicates an absorption peak of linalool. Credits to Asli Celebioglu.

In Figure 5, a magnification of different parts of Figure 4 are done. In these magnifications, it is easier to see the distinctive peaks of linalool absorption. The top graphs highlight a specific region, and the bottom graphs magnify that specific zone. When there is a distinctive peak in a curve that matches another curve, it means that there is a presence of similar molecules. The vertical dotted green line in the bottom graphs show that in inclusion complexes including pullulan, there is an existence of linalool. In the overall experiment, the presence of linalool in the nanofibers is especially important considering the volatile nature of the chemical.

In Figure 6, the result from the thermogravimetric analysis (TGA) is shown below. Thermogravimetric analysis (TGA) is used to measure the degradation and the weight is lost in a material between a multitude of temperatures.

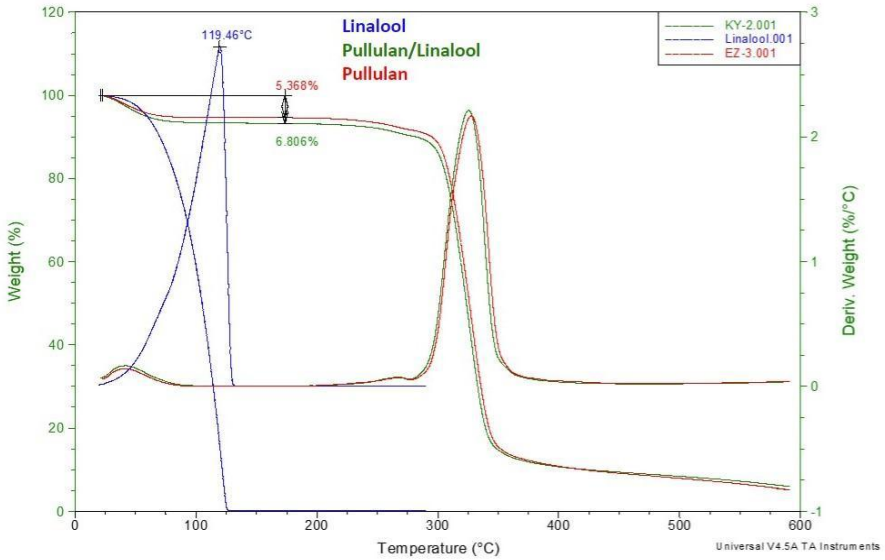

Figure 6. Thermogravimetric Analysis (TGA) thermograms shows the degradation of linalool, pullulan/linalool, and pullulan by weight percentage with respect to temperature (°C). Credits to Asli Celebioglu.

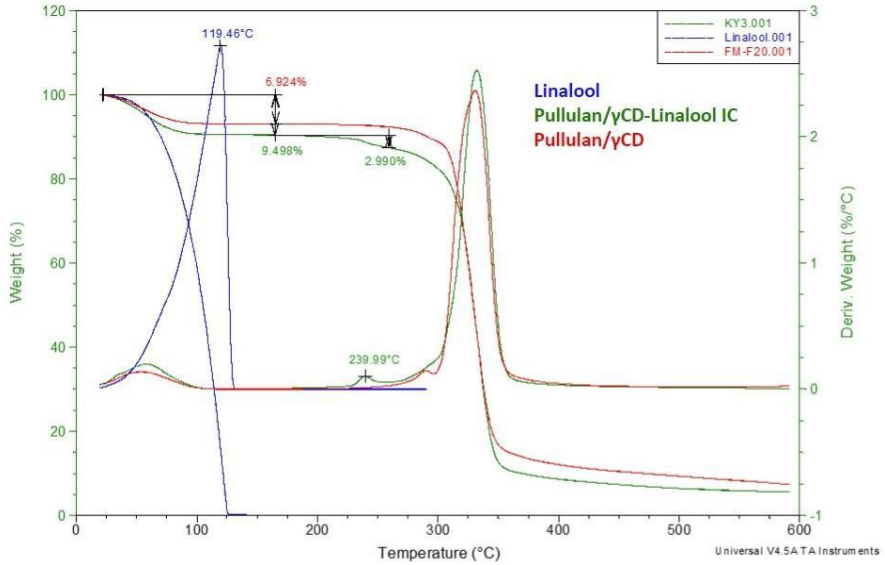

Figure 7. Derivative TGA thermograms Graph shows the degradation of linalool, pullulan/γCD, and pullulan/γCD-Linalool IC by weight percentage with respect to temperature (°C). Credits to Asli Celebioglu.

Figure 6 shows the degradation of linalool, pullulan/linalool, and pullulan by the remaining weight percentage. Linalool, being a volatile substance by nature, degrades almost immediately. When linalool is incorporated into the solution, whether with pullulan or γ-Cyclodextrin, the presence of the essential oil affects its temperature stability and degradation susceptibility. Moreover, the magnitude to which the molecule affects temperature stability shows how strong the bond between the subsequent molecules is. Since the weight percentage between the pullulan/linalool curve and the pullulan curve are so similar, differing by only 1.438%, a conclusion can be drawn that the bond between pullulan and linalool is considerably weak in comparison to other bonds.

In Figure 7, it shows the thermal degradation of samples containing γ-Cyclodextrin. In our overall experiment, a goal was to create inclusion complexes between γ-Cyclodextrin and linalool. An indication of the successful creation of the inclusion complexes is to compare curves when linalool was present and when it was not. When comparing the pullulan/γ-Cyclodextrin curve and the pullulan/linalool-γCD-IC curve, the weight percentage difference is more significant than Figure 6, as the difference is roughly 2.990%. The inclusion complex between γ-Cyclodextrin and linalool produces a large molecule, as the γ-Cyclodextrin contains a “cavity” in its molecular structure that bonds with a neighboring linalool molecule. Thus, the relatively high drop in weight percentage reflects the bond breakage in these molecules and confirms that there were successful inclusion complexes between γ-Cyclodextrin and linalool.

The results from the X-ray diffraction analysis are shown in Figure 8 below. The X-ray diffraction analysis (XRD) was done to indicate a change in crystallinity in the fiber sample.

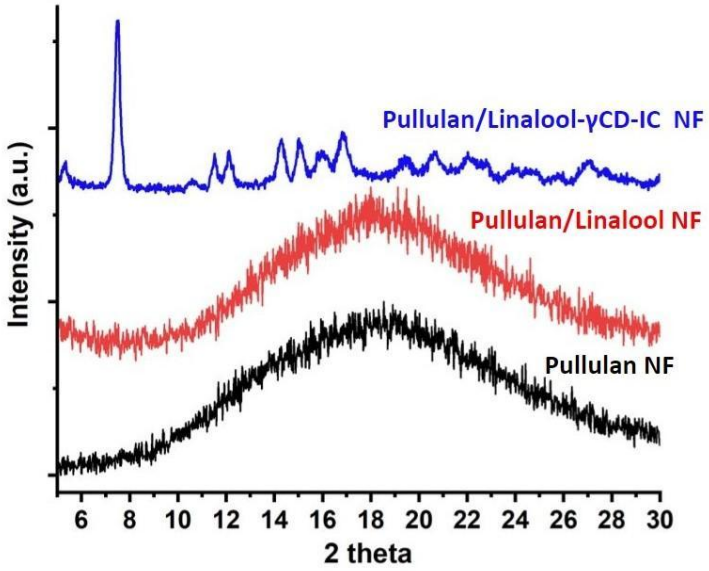

Figure 8. X-Ray diffraction (XRD) graphs depict the relative emission intensity of amorphous and crystalline regions to the distance, measured in 2θ between 10 degrees and 50 degrees. Credits to Asli Celebioglu.

In Figure 8, the crystallinity of pullulan is represented by a relatively amorphous curve. This curve is similar to the pullulan/linalool compound in terms of the general visual shape and appearance. When XRD analysis is done, its main purpose is to record significant crystallinity changes. These physical changes are represented by a curve that looks visually different, meaning that the intensity of the amorphous and crystalline regions of the solid have proportionally varied. The pullulan/linalool-γCD-IC curve being completely different from both the other curves indicates that in fact, the inclusion complexes successfully changed the crystalline structure of the solution.

In conclusion, the sample experiment containing pullulan/linalool-γCD-IC showed success in the electrospinning process. Further characterizations can evaluate the potential of our nanofibers to eventually be used for the functionalization of the face mask.

References

1. Asli Celebioglu, Tamer Uyar, Electrohydrodynamic encapsulation of eugenol-cyclodextrin complexes in pullulan nanofibers, Food Hydrocolloids, Volume 111, 2021, 106264, ISSN 0268-005X, https://doi.org/10.1016/j.foodhyd.2020.106264.

2. Greiner, A. and Wendorff, J. (2007), Electrospinning: A Fascinating Method for the Preparation of Ultrathin Fibers. Angewandte Chemie International Edition, 46: 5670-5703. https://doi.org/10.1002/anie.200604646

3. E.M.Martin Del Valle, Cyclodextrins and their uses: a review, Process Biochemistry, Volume 39, Issue 9, 2004, Pages 1033-1046, ISSN 1359-5113, https://doi.org/10.1016/S0032-9592(03)00258-9.

4. Electrospinning and Electrospun Nanofibers: Methods, Materials, and Applications, Jiajia Xue, Tong Wu, Yunqian Dai, and Younan Xia, Chemical Reviews 2019 *119* (8), 5298-5415, DOI: 10.1021/acs.chemrev.8b00593

5. Rather AH, Wani TU, Khan RS, Pant B, Park M, Sheikh FA. Prospects of Polymeric Nanofibers Loaded with Essential Oils for Biomedical and Food-Packaging Applications. *Int J Mol Sci*. 2021;22(8):4017. Published 2021 Apr 13. doi:10.3390/ijms22084017