

Analytical Study Using PLM, XRD, And ATR On The Morphology And Drug Release
Potential Of Film Casted, Cold Pressed, And Heat Pressed Poly (L-lactic acid) And
Curcumin

By

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Thesis

Submitted in partial fulfillment of the requirements for the Degree of Master of Science
in the Graduate Program of Biotechnology at Brown University

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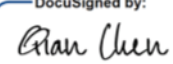
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Preface

The experiments outlined in this thesis were carried out as a two-year master program student in the Department of Biotechnology at Brown University from September 2021 to May 2023. The work discussed in this paper was directed and supported by Dr. Edith Mathiowitz in the Department of Pathology and Laboratory Medicine, as well as inspired by a former lab member Cameron Baptista's work on the characterization and quantification of the induced mesophases of poly-L-lactic acid.

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Born in a family without a father's care and financial support since 0 year old must be harsh for both the child and his or her mother, but unfortunately I am one of the instances. I have never experienced love from a real father during childhood. It was my mother who played two roles throughout these years and let me understand how to become a real man in life. She never gives up. No matter how difficult a situation is, she can always recognize the pin on the needle. She taught me to be patient with things,

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Abstract

This paper illustrated the analytical study using PLM, XRD and ATR for the morphology and drug release potential of film casted, first pressed and heat pressed poly-l-lactic acid with curcumin. PLM was used to analyze the anisotropy of my samples' optical properties so that we could determine the molecular order. XRD being a powerful nondestructive technique for characterizing crystalline materials was used to obtain information on structures, phases, crystal orientations, and other structural parameters, such as average grain size and crystallinity. ATR provided information related to the presence or absence of specific functional groups, as well as the chemical structure of polymer materials. PLM results indicated the existence of potential PLA/Curcumin interaction at molecular level when curcumin concentration was in between 1% and 4%, which hindered the formation of typical PLA spherulites. 5% acted as a potential threshold concentration of curcumin to allow the phase separation between curcumin and PLA. PLM also revealed the similarities between 100%PLA and 1%Cur_PLA at all heat pressing conditions. The curcumin color was so strong that it blocked the observation of birefringence phenomenon beyond 5% curcumin concentration heat pressed samples. XRD showed a decreasing PLA peak height from 100%PLA to 4%Cur_PLA_FC, indicating a continuous decreasing of crystallinity, which supported the PLM observations that secondary interaction at molecular level might have occurred. XRD also showed that 5% curcumin concentration brought back the PLA peak height, representing the onset of phase separation. From 7.5% curcumin to 50% curcumin concentration, there was minimum change in PLA peak heights, as well as continuous occurrence of multiple curcumin peaks, representing the completion of phase separation. XRD showed that first

press at room temperature could induce both liquid crystals and condis crystals at all curcumin concentrations. XRD showed that pressure and duration could induce different morphologies at room temperature. 10000 lbs. of pressure created the most consistent liquid crystals at both 5-minute and 10-minute first presses with both 100%PLA and 1%Cur_PLA. XRD showed that temperature difference during the heat press process may change the morphology of cur/PLA mixtures. 1%Cur_PLA had the most versatility during the heat press process. ATR of pure PLA film and FTIR of pure curcumin powder reveal the importance of wavenumber from 1550 to 1650, where curcumin within any sample of curcumin/PLA mixture located. Among all the samples, only 5%Cur_PLA_85C, 5%Cur_PLA_125C, and 30%Cur_PLA_FC was observed with drug release onto the pig cadaver skin at 37°C.

Background

Curcumin is a naturally occurring chemical compound found in the turmeric root, a plant belonging to the ginger family (Zingiberaceae). It is responsible for the distinctive yellow color of turmeric and has been used in traditional medicine for centuries (Lakshmi). Studies have suggested that curcumin has anti-inflammatory, antioxidant, and anticancer properties, among other potential health benefits (Kamal, Sahebkar, Basnet). It has been investigated for its potential to treat a variety of health conditions, including arthritis (Pourhabibi), Alzheimer's disease (Hamaguchi), and depression (Fusar). However, the oral bioavailability of curcumin is limited, meaning that the body does not absorb it very well (Anand). To address this issue, researchers have explored various methods to enhance the absorption of curcumin, such as combining it with other compounds or using specialized delivery systems (Rahimi).

PLA (polylactic acid) is a biodegradable and compostable thermoplastic made from renewable resources such as corn starch, sugarcane, or cassava (Vink). It is a popular material in 3D printing and packaging industries due to its eco-friendly properties. PLA is created through the polymerization of lactic acid, which is produced by fermenting cornstarch or other plant-based sources (Vink). The resulting plastic is lightweight, strong, and has a melting point of 174 degrees Celsius (Vink). One of the main advantages of PLA is its biodegradability, meaning that it can break down naturally into non-toxic substances in the environment, such as water and carbon dioxide, without leaving harmful residue behind (Kasirajan). This makes it a more sustainable alternative to traditional plastics made from fossil fuels, which can take hundreds of years to decompose and can cause pollution and harm to wildlife. PLA is also a versatile material

that can be used for a variety of applications, including food packaging, disposable utensils, 3D printing filaments, and medical implants (Mikula). However, PLA does have some limitations, including lower durability and heat resistance compared to traditional plastics (Ncube).

There has been research on using curcumin in combination with PLA for various applications. For example, some studies have explored the use of curcumin-PLA nanoparticles as a drug delivery system for cancer treatment (Watkins). Other studies have investigated the use of curcumin-PLA coatings for food packaging, as curcumin has been shown to have antimicrobial properties that can help extend the shelf life of food products (Mikula). There have also been papers discussing the change of thermal stability and mechanical strength after implementing curcumin into PLA (Kasirajan).

The purpose of my research is to further explore how curcumin concentration affects the PLA morphology, how different processing conditions of curcumin and PLA mixture (e.g., solvent cast, cold press with various pressures, heat press at close to glass transition temperature, or close to melting temperature under 20,000 lbs. of pressure) change the properties of films, and the possibility of developing curcumin as transdermal patches for drug release using pig cadaver skin using scientific analysis such as PLM, ATR, and X-ray Diffraction. In the paper, I made samples with the following conditions: film cast of pure PLA (noted as 100%PLA_FC), film cast with total weight of 1%, 2%, 3%, 4%, 5%, 7.5%, 10%, 15%, 30%, 50% curcumin in PLA (note as x%Cur_PLA_FC), heat press of 100%PLA and with total weight of 1%, 5%, 7.5%, 10%, 15% curcumin in PLA under 20,000 lbs. for 5 minutes at 64 (glass transition temperature), 85, and 125 (close to melting temperature) degrees Celsius (noted as x%cur_PLA_xx°C).

Materials and Methods

This section are the materials and methods for studying the conditions of the induction of mesophase for mixture of high molecular weight polylactic acid (PLA) and curcumin from turmeric extract, and the mimic of release of curcumin to human skin using pig cadaver skins. The overall sequence of this experiment is composed of film cast, first press (cold press), second press (at different temperatures while maintaining the pressure and time of pressing), XRD analysis, and ATR.

Curcumin, PLA, and DCM

The curcumin used was distributed by SIGMA-ALDRICH, Co. under the tag C1386-10G and Lot # SHBN9867. Its chemical function was $C_{21}H_{26}O_6$. It had a molecular weight of 368.38 g/mol, and a melting point at 175 degrees Celsius. It was soluble in 0.5M NaOH.

The Poly(L-lactide) with ester terminated we used was distributed by Durect Lactel (Part # B6002-2P, and Lot # A12-039a). It had an inherent viscosity range 0.9-1.2 dL/g in $CHCl_3$.

Solvent used was Dichloromethane (CH_2Cl_2) purchased from Sigma-Aldrich. It contained 40-150 ppm amylene as stabilizer. It had a density of 1.325 g/mL at 25°C, and a molecular weight of 84.93 g/mol.

Film cast

The weight percentages of curcumin in PLA sample we made are 1%, 2%, 3%, 4%, 5%, 7.5%, 10%, 15%, 30%, and 50%. The total weight of curcumin and PLA mixture was kept at 250mg. The below table is a quick datasheet for preparing the different concentrations.

	1%	2%	3%	4%	5%	7.5%	10%	15%	30%	50%
Curcumin weight (mg)	2.5	5	7.5	10	12.5	18.75	25	37.5	75	125
PLA weight (mg)	247.5	245	242.5	240	237.5	231.25	225	212.5	175	125

Table 1. Weight percentages of curcumin in PLA.

During the weight measurements of PLA, one may notice that the small PLA chunks have different sizes and weights, which would make it hard to obtain the expected total weight without being chopped into smaller pieces. In this scenario, one may need to use a balance sheet and an art knife to chop the PLA chunks into multiple much smaller pieces for a more precise measurement.

After mixing the exact weights of curcumin and PLA, we put the mixture into a 50mL disposable glass vial, and 2 mL of dichloromethane (DCM) was added to the vial using a glass pipet. Depending on the physical and chemical characteristics (i.e., solubility, co-solubility, molecular weight, etc.) of the material used, the duration of sonication and vortex can vary. For curcumin and PLA specifically, we used Aquasonic Model 150T as the sonicator and Talboys™ for vortex. The mixing of curcumin + PLA + DCM involves a two-step process for two times. We would firstly place the tightly capped vials onto the rack of the sonicator machine. Fill the water till it barely exceeds the liquid level in the vial. Filling too much water may lead to too much buoyance which would cause the vials to flip over and the not-yet-well-mixed drug and polymer to touch and stay at the cap, making it difficult to achieve the precise concentration for the film cast. The sonication should last at least 5 minutes for curcumin + PLA + DCM combination. After 5 minutes of sonication, we would take the vials out and wipe off the water on the glass surface (so that they are less slippery during vortex). Then we started to vortex the vials at 5000 rpm for another 5 minutes. If more than 3 vials of samples were to be vortexed, the

order of sonication would normally be: first 3 vials for a 5-min sonication, then second 3 vials for the next round of sonication while bringing the first 3 vials to the vortex, and so on so forth. After repeating the same sonication and vortex procedures once more, we then slowly poured the mixture onto the KIMAX® COVER Kimble glass petri dish with a diameter of 6 centimeters. It is noteworthy that pouring the liquid at the center and very gently and slightly tilt the petri dish to the directions where the liquid had not been covered gives a more homogenous film than simply pouring the liquid randomly onto the petri dish. Wait 24 hours for the film to dry completely before peeling it off from the petri dish.

First press

First press is also known as the cold press, which the casted films get pressed by Specac handpress machine under a certain pressure at room temperature. We prepared the pressable films by folding the circular films to half for 4 times. The folded films should appear like sharp equilateral triangles, which are well smaller than the size of the hole on the die sleeve. A complete set of die for our experiment was composed of a top pushing rod, a die sleeve, two die cores, and a support plate. After assembling them based on the lab manual, one folded film can be inserted to the hole on the die sleeve, followed by inserting the top pushing rod. By inserting the top pushing rod, the folded film will be naturally pushed further into the die sleeve. It is important to keep the same way of pushing the film with the rod while conducting a series of first presses. Some polymers especially those with low molecular weights frequently shatter or are non-foldable due to fragility. In this case, simply dump everything left from the film cast plate into the die sleeve and gently seal with the pushing rod, and first press is ready to roll. To begin the press, seal up the gas sealer valve and screw tightly the die fixator. For the first part of

my experiments, I controlled every sample pressed under 5000 lbs. of pressure for 5 minutes. For the second part of my experiments, I used combinations of 5k lbs./5 min, 5k lbs./10min, 10k lbs./5min, 10k lbs./10min, 15k lbs./5min, 15k lbs./10min, and 15k lbs./15 min for the cold press. After the press was done, untight the gas sealer valve, unscrew the die fixator, and take the pressed tablet out by removing the pushing rod and the die sleeve.

Second press

Second press is also known as the heat press. It involves heating a tablet at a constant temperature under a steady pressure for 5 minutes, followed by cooling the tablet at a fast and theoretically constant rate. The machine used for our heat presses was MITSUBISHI GOT₁₀₀₀ AUTO SERIES NE and CARVER® with PLATEN 1 and PLATEN 2. For my entire research on PLA and curcumin, I worked with 64, 85 and 125 degrees Celsius due to the thermal features of PLA, as well as the previous studies. It usually took 20-30 minutes for the machine to stabilize at the expected temperature, so we would turn the machine on, set the prospected temperature, and leave it for 30 minutes before returning to the basement. All my heat press samples came straight from the cold press. Inside the heat press machine, two platens were synchronously heated to the prospect temperature. This step made sure that both sides of a tablet specimen were evenly heated, rendering the polymers with optimum and controlled mobility at a certain temperature. After the thermal reading on the screen stayed constant, we carefully place the tablet which was placed at the center of two metal sample holder plates onto Platen number 2. By pressing both “close” buttons on the machine after closing the lid, Platen number 2 would slowly be pushed up till it could not move further. After releasing the close buttons, the two platens started to synchronously heat up the sample holder plates. We controlled the heating to 10 minutes, so that

the holder plates were heated both to the prospected temperature. Then, lift the pressure gauge, press once more a “close” button, and the platens would start to quickly generate pressure till the value we set. Make sure that the lid is completely closed. Otherwise, the screen would turn red and make some scary warnings, and Platen number 2 would drop automatically. After 5 minutes of pressurized heating, we click air and then water to allow a fast cooling with a combination of air and water till room temperature. This step generally took less than 10 minutes. Manually drop the pressure gauge, and press “open” button to disengage the two platens. Once Platen number 2 fully descended, we could take the holder plates out, and gently remove the heat pressed sample. Very frequently, at around 125 degrees Celsius, which is close to PLA’s melting temperature, the heat pressed samples almost stick tightly onto the holder plate (sometimes on one side, sometimes on both). So, removing the sample after 125 degrees Celsius treatment requires more patience and caution. Also, because the sample holder plates were not completely flat, so 64 degrees Celsius always output a curved shape, which might be unfriendly for PLM and XRD observations.

Polarized light microscope

Polarized light microscope, also known as PLM, uses a set of polarizer filters with one attached to eye lens and the other attached to object lens on a regular optical microscope to generate incident light with different polarized effects. By rotating the polarizer on the object lens, a special angle at which its polarization lines stay perpendicular to those on the eye lens, making the entire field of observation visually dark if there are no crystalline or semi-crystalline structures. Once intense light spots observed, one can confirm much more ordered structures within the sample. The PLM machine we used was SEBA™ LAXCO, which was connected to a

SEBA™ PRO computer screen. Normally, we observed the sample with 4X, 10X, 20X, and 40X magnifications, stacking with the eye lens with 20X magnification.

X-ray diffraction

X-ray diffraction (analysis), also known as the XRD, was used to determine the morphology and crystallographic structure of each sample. While using XRD, a material is exposed to incoming X-rays at multiple incident angles (2θ), and the intensity and scattering angles of the X-rays that escape the material are then measured. At the XRD room, 2D-XRD machine was used for our samples. After opening the XRD machine control software, we adjusted the X-ray generator voltage from 20 kV to 40kV, and current from 5 mA to 40mA. Then we clicked on 'Video' button to enable a visual of sample holder on the machine, where we were about to load the specimens. Click on the "Laser 1" button to enable the two laser dots that functioned as confirming the focal point of the X-ray beam. If the two laser dots did not align with each other perfectly, we would adjust the x, y, or z plane to have them align together. Normally, we only adjusted the z plane (the altitudes) after fixing the sample on the sample holder. All my film cast, first press, and heat press samples were carried by a piece of XRD glass slide during XRD measurements. In this way the background noise of the metal surface of the sample holder unit would be greatly limited. In the "Scan Setup" menu, Scan type was selected for "Coupled TwoTheta/Theta"; Scan mode: Stop; Time/Stop: 60.000, Steps:5; Total time: 300; 2Theta Start 20.0000; 2Theta Increment: 15.0000; 2Theta Stop: 80.0000; Theta Start: 10.0000; Theta Increment: 7.5000; Theta Stop: 40.0000. After a sample was adjusted to the perfect plane, close the hoods after hearing the "click" sound, then click the "Start" button, and XRD would start to run automatically.

After the measurement was finished, data was then saved as an XRD file. Open the file using XRD analysis software called DIFFRAC.SUITE EVA and convert the 2D scans into an intensity curve with 2Theta (Coupled Two Theta/Theta) wavelength as X-axis, and intensity as Y-axis by click “merge as single”. The final output image file was processed by background subtraction.

Attenuated total reflectance

Attenuated total reflectance, also known as ATR, was used to measure the surface composition of our samples. Perkin Elmer ATR/FTIR machine was used for the experiments. Spectrum IR was the software used for collecting data. Set the accumulation scan to 20 scans. Click “Background” and simultaneously screw down the green knob that represented the force gauge on the ATR machine till it reached 100. Load film cast or tablet samples onto the ATR machine after background scan was done, making sure that the entire ATR crystal was covered. Click “Scan” and simultaneously screw down the green knob till the force gauge reached 100 again. When the scanning completed, convert the intensity curve to absorption by clicking “Absorption”. Graphical analysis took place at another software called the Spectragryph. Normally, we treated every saved IR file with Advanced Baseline (to make sure every peak started from 0 on the Y-axis) and Normalization (set the highest peak at 1, and normalize other peaks based on the highest peak).

Pig cadaver skin release

To verify if PLA could be used as a potential carrier polymer for a stable release of curcumin, two separate pig cadaver skin release studies were conducted at two temperatures. The first one was more like a pre-experimental test, in order to determine if the second one was

experimentally necessary and viable. Two pieces of 12 cm in length, 3.5 cm in width, and 3 mm in thickness (with a thin layer of fat tissue) pig cadaver skin cut from a piece of freshly frozen pork meat with depilated skin bought from a food supplier platform called “Weee!” was used for both release studies.

a. -20 degrees Celsius Release Study

Six pieces of 2 cm long pig cadaver skin were averagely cut from the 12 cm skin. Skin pieces were sanitized with 75% ethanol and dried with Kimwipes. They were pressed gently till the entire surface appeared flat. 5%, 10%, 15%, 30%, 50% Curcumin_PLA film cast samples and 100% PLA film cast samples were prepared by cutting the samples into random small triangle shapes that could fit the center of each 2 cm pig cadaver skin. Place each triangle sample onto each pig cadaver skin, so that each skin held one film cast sample with a certain concentration of curcumin. Insert each pig cadaver skin into a 3x5 plastic pouch and place them under the edge of a stuffed box (to give some pressure to the films so that they remained contact with the skin after being frozen) at -20 degrees Celsius. ATR was taken on Day0 and Day5 for 5% Cur_PLA, and on Day0 and Day30 for the rest.

b. 37 degrees Celsius Release Study

12 cm pig cadaver skin was kept at its original shape. Sanitize the skin well with 75% ethanol, and dry with an air gun. Instead of cutting the films into random triangle shapes, this time I precisely cut all samples with a special 5mm punch into perfect circle shape with 5mm in diameter, except the 64- and 85-degrees Celsius heat pressed samples because of their rigidity. These two heat press conditions made the tablets too rigid to be punched off into the shape we expected, so I cut them with sanitized scissors into little

triangles (much smaller than the ones prepared for -20 degrees Celsius, similar size to the precisely punched samples).

Results

PLM

PLM was used to reveal the characteristics of film casts and tablets upon polarized light. Film casts and tablets from first press and second press with various percentage of curcumin in PLA were observed. The film cast of 100%PLA at 10X magnification showed a trend that four-leaf-clover-shaped spherulites tend to gather around into clusters and left some spaces of darkness that represent amorphous structures. 20X and 40X indicated the same phenomenon. The film cast of 1% curcumin in PLA (1%Cur_PLA_filmcast) showed a more closely gathered four-leaf-clover shaped spherulites compared to 2%, 3% and 4%Cur_PLA_filmcast (Figure 1i, 2i, 2iii, 2v). On the other hand, the clover shaped spherulites became much less and spread when the concentration of curcumin went beyond 2% (Figure 1 vs 2). The dominant composition transited from the clover spherulites to cuboid spherulites, and it seemed that the cuboid spherulites transited to needle-like/rod shape (with much longer and narrower body) starting from 5%cur (Figure 1 vs 2vii and 2viii vs 2x and 2xii), while beyond 10%cur the rod shaped spherulites tended to become more squared again. Beyond 2%cur, film casts showed a trend of having increasing amount of curcumin-orange/yellow color trapped near the cuboid spherulites. At 30% and 50%cur, the film casts appeared to be bulkily covered by some layers that were too thick to allow a descent penetration of PLM light source (Figure 2 xv-xviii). The first press

samples of curcumin + PLA were relatively much thicker than the film casts and second presses, so the PLM captures were visually darker and less transparent.

The 5k lbs. / 5 minutes first press tablets of 100%PLA, 1%Cur_PLA, 5%Cur_PLA, 7.5%Cur_PLA, 10%Cur_PLA, and 15%Cur_PLA were observed on PLM. 100%PLA showed a trace of semi-crystalline structure induced based on the birefringence color observed. Also, the layers of folded and pressed PLA film formed can be visually recognized. The 1%Cur_PLA and 5%Cur_PLA first press displayed dark-lined structures with a certain orientation, and the yellowish background color remained observable. Beyond 7.5%cur, the lined structure disappeared, the long dark lines became small dark spots, and the color of the entire tablet became much reddish visually. The dark spots showed similar structure to 10%Cur_PLA_FC.

PLM captures were also taken for heat pressed samples pressed at 64, 85 and 125 degrees Celsius with curcumin concentration of 1%, 5%, 10% and 15% (Table 2). Based on the images, when the temperatures went higher (closer to the melting temperature of PLA but not exceeding), the curcumin color and rod-shaped crystalline came back massively. When the heating temperature was close to (but not lower than) the glass transition temperature of PLA, not much of crystallization occurred.

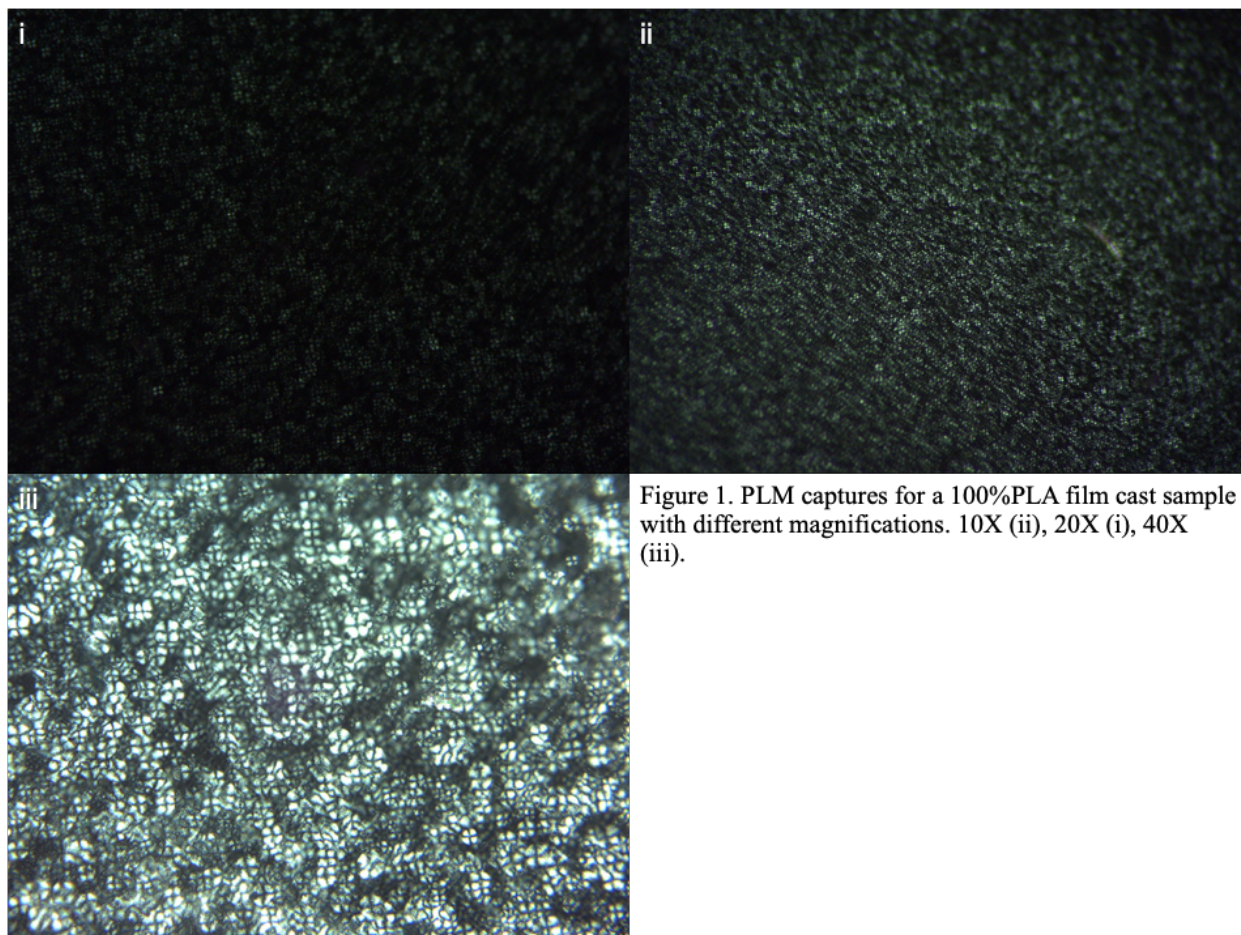


Figure 1. PLM captures for a 100%PLA film cast sample with different magnifications. 10X (ii), 20X (i), 40X (iii).

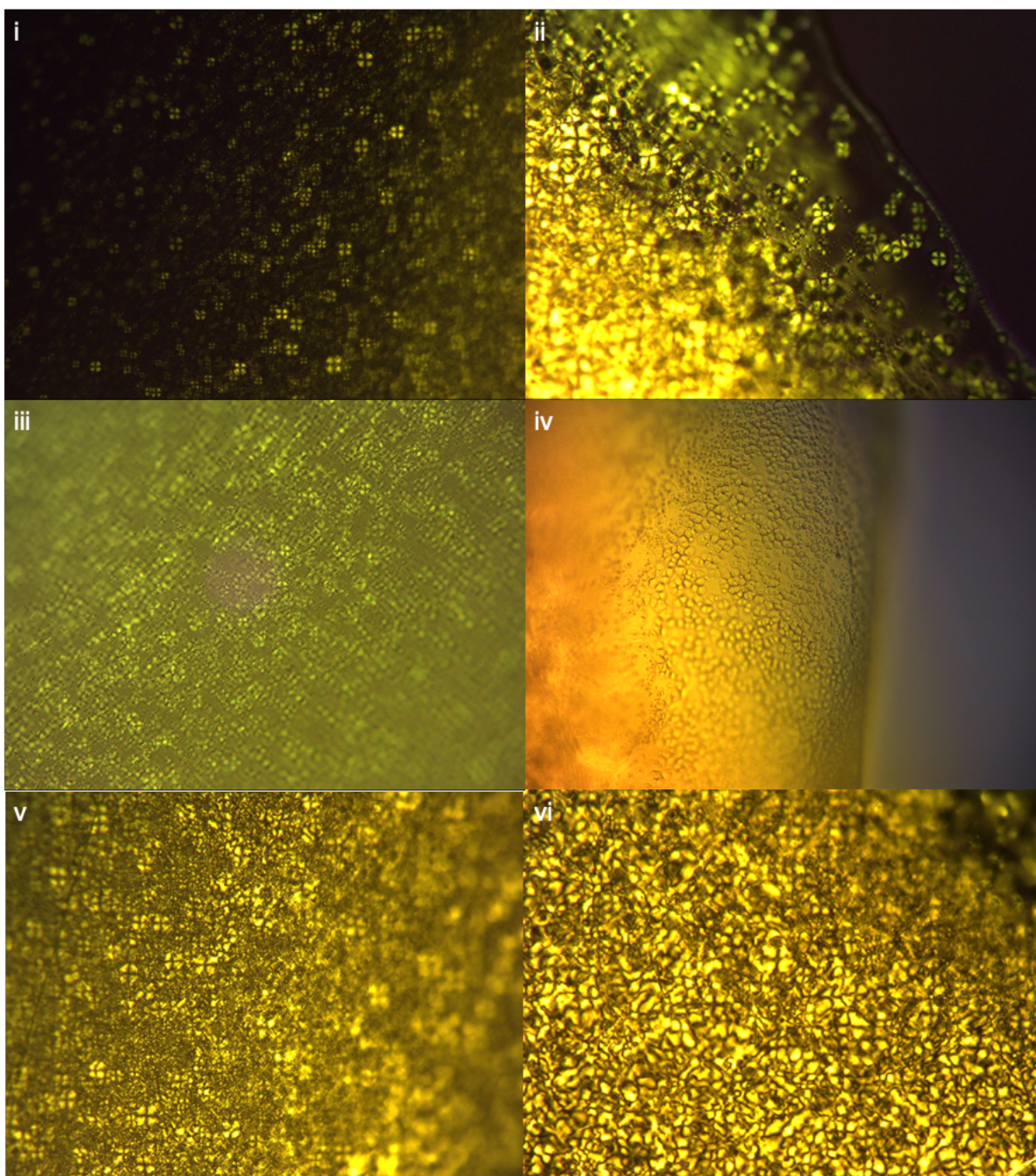
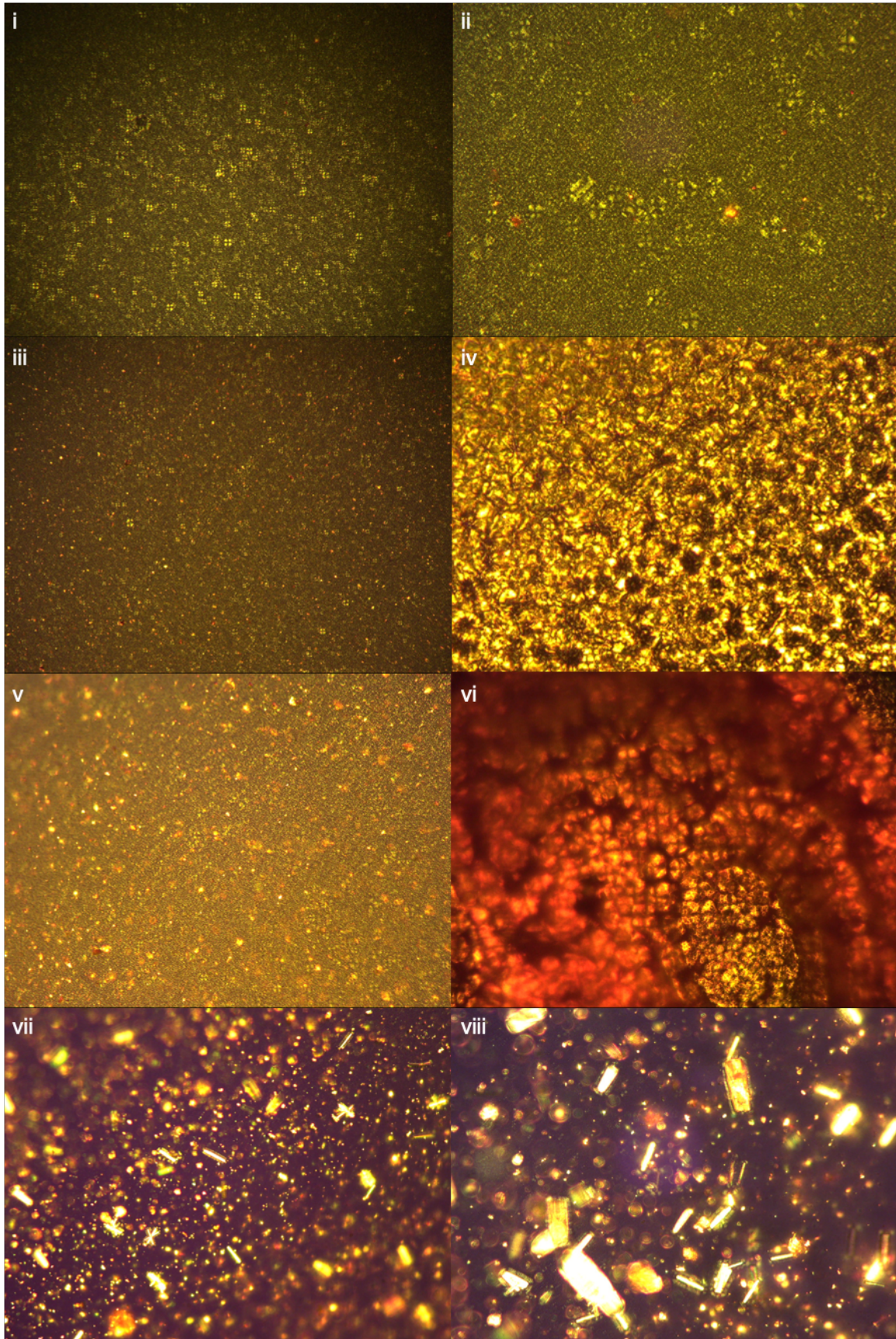
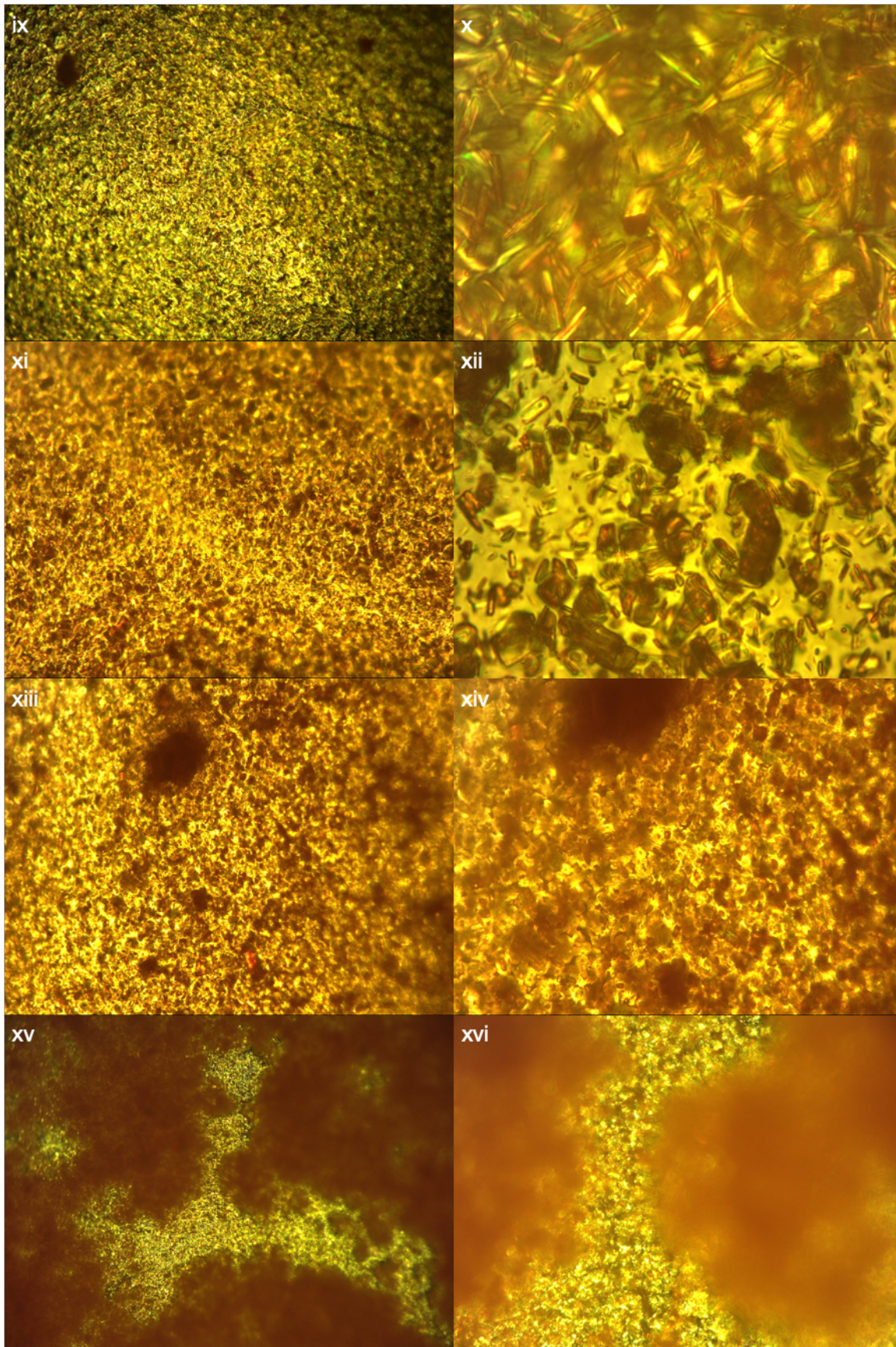


Figure 2. Different magnifications of PLM captures for 1%Cur_PLA film cast. 5X (iv), 10X (i, ii), 20X (iii, v), and 40X (vi).





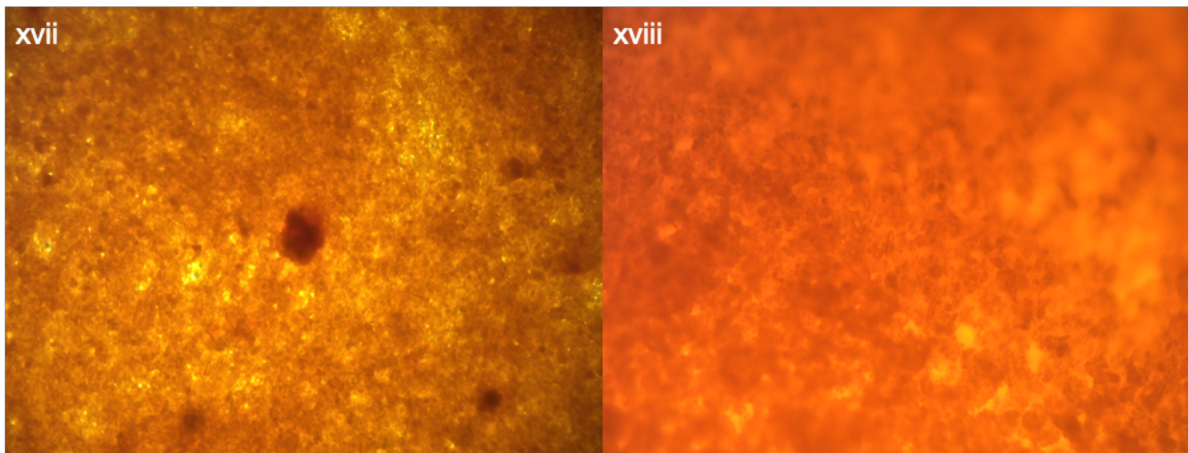


Figure 3. Different magnifications of PLM captures for film casts with curcumin concentration of 2%, 3%, 4%, 5%, 7.5%, 10%, 15%, 30%, and 50% in PLA. i: 2%Cur_PLA 10X, ii: 2%Cur_PLA 20X, iii: 3%Cur_PLA 10X, iv: 3% Cur_PLA 40X, v: 4%Cur_PLA 10X, vi: 4%Cur_PLA 40X, vii: 5%Cur_PLA 20X, viii: 5%Cur_PLA 100X, ix: 7.5%Cur_PLA 4X, x: 7.5%Cur_PLA 100X, xi: 10%Cur_PLA 10X, xii: 10%Cur_PLA 100X, xiii: 15%Cur_PLA 20X, xiv: 15%Cur_PLA 40X, xv: 30%Cur_PLA 10X, xvi: 30%Cur_PLA 40X, xvii: 50%Cur_PLA 10X, xviii: 50%Cur_PLA 20X.

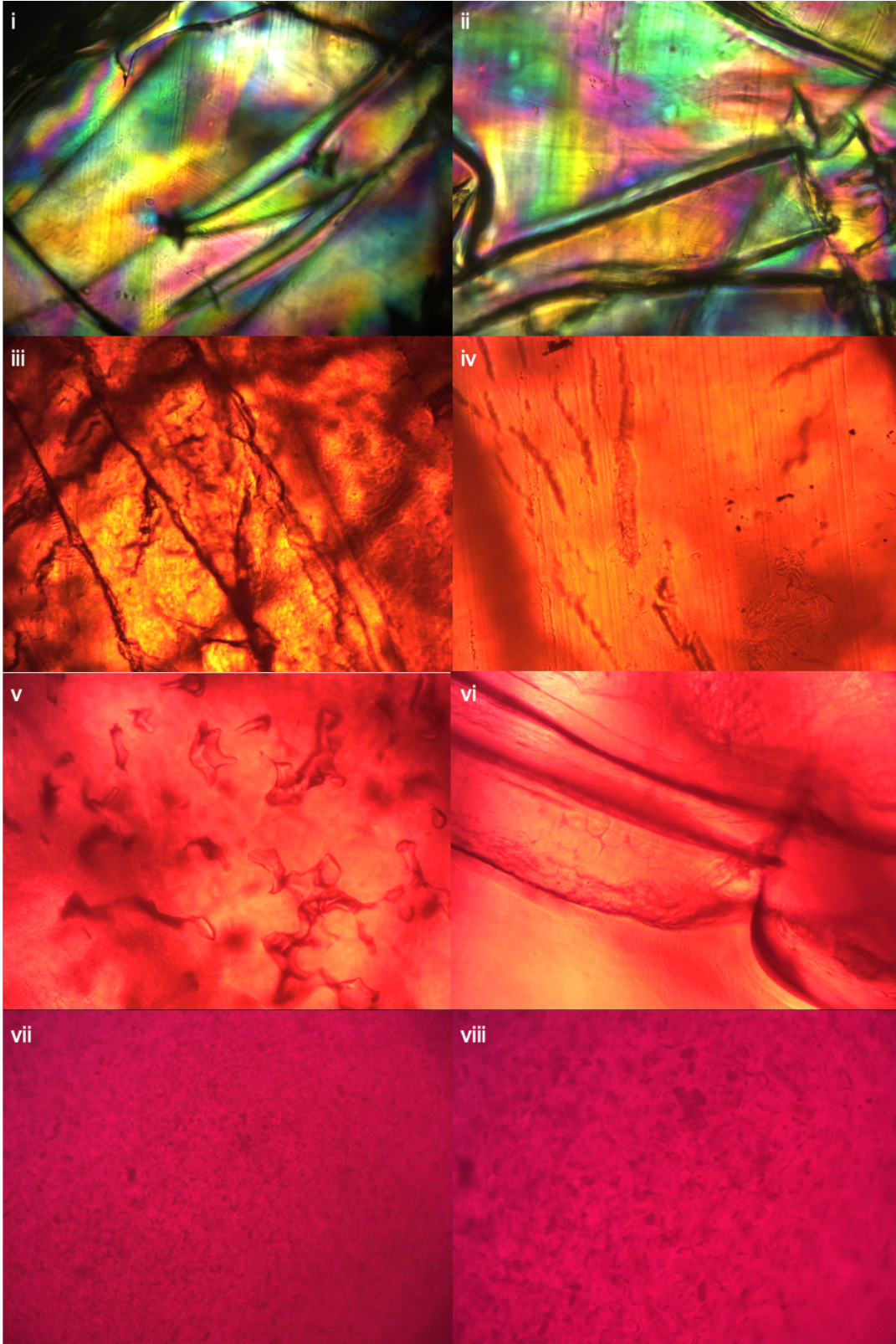


Figure 4. Different magnifications of PLM captures for first presses of 100%PLA (i, ii: 20X), 1%Cur_PLA (iii, iv: 20X), 5%Cur_PLA (v, vi: 20X), 7.5%Cur_PLA (vii: 10X, viii: 20X).

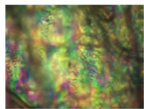
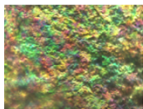
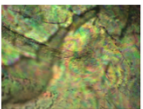
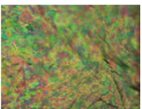
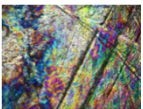
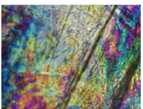
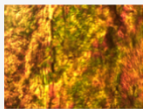
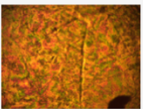
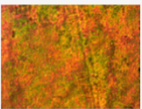
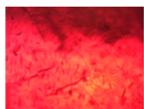
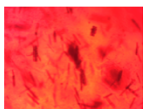
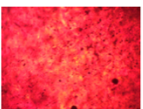
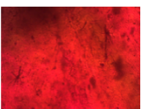
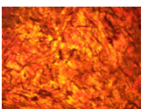
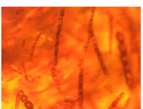
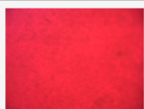
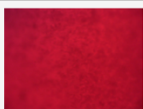
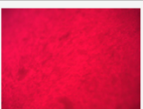
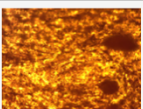
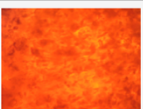
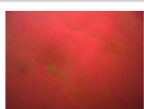
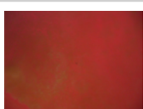
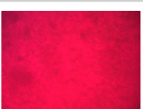
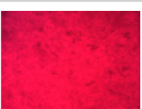
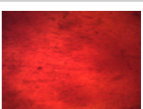
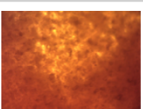
	64 degrees Celsius		85 degrees Celsius		125 degrees Celsius	
100%PLA						
	10X	20X	10X	20X	10X	20X
1%Cur_PLA						
	10X	20X	10X	20X	10X	20X
5%Cur_PLA						
	10X	20X	10X	40X	10X	40X
10%Cur_PLA						
	10X	20X	10X	40X	20X	40X
15%Cur_PLA						
	10X	20X	10X	20X	4X	20X

Table 2. PLM captures for heat pressed samples of 1%Cur_PLA, 5%Cur_PLA, 10%Cur_PLA and 15%Cur_PLA at 64, 85 and 125 degrees Celsius.

XRD

X-ray diffraction technique was used to determine how much crystallinity our Cur + PLA samples have. Figure 5 are XRD scanning that were transferred from txt files to Excel XY scatter plots of film cast samples of 100%PLA, 1%Cur_PLA, 2%Cur_PLA, 3%Cur_PLA, 4%Cur_PLA, 5%Cur_PLA, 7.5%Cur_PLA, 10%Cur_PLA, 15%Cur_PLA, 30%Cur_PLA, and 50%Cur_PLA.

The XRD of 100%PLA showed a narrow peak on the top with a prominent broadening on the bottom. This represents the existence of semi-crystalline state with some order. Based on Cameron et. al and combining with Figure 6, it is suggested that the structure of 100%PLA_FC was nematic mesophase. From 1%Cur_PLA_FC to 4%Cur_PLA_FC, both Figure 5 and Figure 6 indicate the gradual fading of PLA crystallinity, and the films became more and more amorphous. This phenomenon might be due to secondary interaction between curcumin and PLA. It is worth noticing that the occurrence of peaks at around 10 (2 theta) might be due to the amorphous glass slide used for XRD measurements (see supplement Figure S1). Because the glass slide also possesses another peak at around 16-17 (2 theta), though not high, it would still have affected the morphology of those films. Figure 5 5%Cur_PLA_FC was a transition point where peaks of PLA were contributing again to the XRD plot. Figure 6 5%Cur_PLA_FC started to reveal some orange halo core in the middle right of the 2D capture, as well as a clear orange ring on the right and an inconspicuous orange ring to the left. Both results represented the possible emergence of liquid crystals (with minimum order) and some condic crystals (with medium order). When the concentration of curcumin increased to 7.5% and beyond, the peaks of PLA and curcumin diverged from each other. The two tall and sharp peaks near 15-20 (2 theta) represented the typical PLA peaks, whereas other minor ones represented the curcumin peaks. Figure 6 7.5%Cur_PLA_FC, 10%Cur_PLA_FC and 15%Cur_PLA_FC showed 2 bright curves that represent the partial crystallinity of PLA, and all other dim curves that represented partial crystallinity of curcumin. This phenomenon might be due to the phase separation of curcumin from PLA. On Figure 6 30%Cur_PLA_FC and 50%Cur_PLA_FC, the typical PLA curves faded, and curcumin curves became more dominant. This was because the concentration of curcumin became so high that it hindered the PLA crystals from x-ray diffraction. Another possible

explanation could be that curcumin became too concentrated to allow PLA to crystallize freely. Figure 7 is a group comparison between film casts of 1%Cur_PLA, 5%Cur_PLA, 7.5%Cur_PLA, 10%Cur_PLA, and 15%Cur_PLA and samples with same concentrations that were pressed under 5000 lbs. for 5 minutes at room temperature. 1% and 5% FC samples transitioned from low and broad peaks to much more intense and narrow peaks. We suggest that when curcumin concentration is lower than the threshold concentration, PLA has enough space to form considerable sized crystals under 5000 lbs. for 5 minutes at room temperature. Not much mesophase had formed in 1% and 5%Cur_PLA by first press. At 7.5%cur, the peaks became much more intense after the first press. This means that not much mesophase has formed. 10%Cur_PLA and 15%Cur_PLA showed different results in the first press which the bottom of the peaks became widened, indicating that mesophase started to appear.

Figure 8 was the 2D-XRD captures for first pressed samples of 100%PLA, 1%Cur_PLA, 5%Cur_PLA, 10%Cur_PLA, and 15%Cur_PLA. Compared to Figure 6, 100%PLA_FP induced a certain amount of mesophase due to the appearance of a slightly brighter orange region. The broadening of PLA peaks of 10%Cur_PLA and 15%Cur_PLA curves indicate the induction of mesophase of the polymer, meaning that curcumin with higher concentration tends to phase separate completely instead of interacting with PLA and preventing PLA from recrystallize.

Figure 9 is an overall picture of 2D-XRD captures of heat pressed samples at 64, 85 and 125 degrees Celsius under 20000 lbs. of pressure for 5 minutes (except 100%PLA_125C cool downed for 2 hours to room temperature due to machine malfunction) for a general visualization. I have integrated each capture to the corresponding XRD plots for a case-by-case analysis. Figure 10 is XRD plot and 2D_XRD captures of 100%PLA heat pressed samples at 64, 85 and 125 degrees Celsius. At 64 degrees Celsius, comparing with its XRD plots and that of the cold

press on Figure 7, 100%PLA_64C became relatively more amorphous with only nuanced orientational order due to the decrease of peak intensity and increase of peak broadness, representing the disappearing of nematic mesophase from the cold press (Cameron et al.). The focus intensity of the Debye-Scherrer ring around the equator on the 2D-XRD capture did indicate some orientational order, but completely lacking positional order and conformational order. This phenomenon was coherent to Cameron et al.'s observation. The increase of focused intensity of the Debye-Scherrer ring around the equator of PLA peaks on the 2D of 100%PLA_85C_20K_5min indicated the induction of condic crystals (Cameron et al.), exhibiting crystals with both the orientational and positional order. The 2D rings gave a hint that heat press at 125 degrees Celsius combined with slow cooling had induced strong positional, orientational and conformational order for 100%PLA (Cameron et al.). This processing condition induced highly crystalline morphology was because when the temperature was close to the melting point, PLA bonds became mobile enough to recrystallize, while slow cooling allowed the PLA chains to have extended time to form longer and more ordered crystals. Figure 11 illustrates the XRD and 2D captures of heat pressed 1%Cur_PLA. While press at 64 degrees Celsius generated almost completely amorphous structure on 1%Cur_PLA, even more amorphous than film cast and first press at room temperature, at 85 degrees Celsius, the broadened rings on the 2D and the raise of peaks on the XRD plot both indicated the occurrence of mesophase. In this case, possible more condic-crystal mesophase had formed compared to liquid crystals. 125 degrees Celsius on the other hand, induced the most condic crystals because of the rapid elevation of peak intensity on the XRD plot and the broadening of Debye-Scherrer rings. It is also suggested that 125 degrees Celsius created more conformationally disordered mesophase than 85 degrees Celsius due to less sharpness of the rings on the 2D. Figure 12

illustrates the XRD and 2D captures of 5%Cur_PLA tablets that were pressed at 64, 85 and 125 degrees Celsius. Based on the 2Ds, pressing at 64 degrees Celsius induced condic crystals comparing to Figure 8, where crystals were more ordered positionally and conformationally. Both 64 and 85 degrees Celsius created smaller condic crystals compared to 125 degrees Celsius press, whereas at 85 degrees Celsius there was a much higher peak of PLA meaning that the crystals formed were in a greater size than at 64 degrees Celsius. 125 degrees Celsius created almost perfect crystalline state on 5%Cur_PLA. The reason why heat press of 5%cur output the most structurally ordered phase might be that 5% is the threshold concentration at which curcumin and PLA prefer to phase separate more completely on their own instead of interacting during the thermal processing, giving each other more room to recrystallize into their lowest energy state more easily, also observed in the first press at room temperature (Figure 7). Figure 13 depicted the XRD and 2D captures of 10%Cur_PLA tablets that were pressed at 64, 85 and 125 degrees Celsius. All 3 thermal processing induced a great amount of mesophase from 10%Cur_PLA_FP tablets based on the widened and elevated peaks, while at 64 degrees Celsius the induced mesophase could be mostly liquid crystals with only orientational order, at 85 degrees Celsius the induced mesophase could be an even mix of liquid crystals and condic crystals, and at 125 degrees Celsius could be mostly condic crystals (Cameron et al.). Figure 14 indicated the XRD and 2D captures of 15%Cur_PLA heat pressed at 64, 85 and 125 degrees Celsius. 64 degrees Celsius lowered the intensity of PLA peaks from 15%Cur_PLA_FP, indicating the disappearing of nematic-like morphology. This seemed coherent to Cameron's observation that by applying external heat (near glass transition temperature) and pressure the plastic-like morphology of PLA decreased into nematic-like orientation due to the more limited degrees of freedom of the polymer chains with rotational and translational motion (Cameron

et.al). However, any concentration above 1%cur led to a much smaller drop of XRD PLA peak intensity at 64 degrees Celsius heat press compared to their first pressed counterparts. Though not proved by DSC analysis, I still suggest that curcumin acted like a thermal stabilizer which retained the morphology of PLA at its rubbery state. At 85 and 125 degrees Celsius, 15%Cur_PLA XRD plots showed increase intensity of PLA peaks without much broadening, indicating the induction of condis-crystal morphology. The merging rings on the 2D-XRD captures also verified the fact. It is worth noticing that as the pressing temperature increased, the peaks of curcumin were hindered more thoroughly. The reason behind this might be that PLA under much higher pressing temperature (closer to its melting temperature) tended to gain more degrees of freedom, allowing it to crystalize into orientationally and positionally much more ordered crystals whereas curcumin did not have such ability though being phase separated from PLA.

Because I found that first press at room temperature under 5000 lbs. for 5 minutes had the ability to induce decent condis-crystal mesophase in 1%Cur_PLA (different from the cause of broadening of XRD peak of 5%Cur_PLA_FP due to the PLM observation), and heating to the melting temperature induced even more condis-crystal, I wonder if first press with different pressure and duration would induce better nematic-like mesophase for individual study of such mesophase without the need of secondary press at 64 degrees Celsius. Figure 15 to 20 depicted the XRD and 2D captures of 2 random sites on the 100%PLA_FP under the corresponding first press conditions as the legends indicated. I used them as the reference for the same first press conditions of 1%Cur_PLA. I found that except 5k_10min and 15k_10min conditions, 10k_5min, 10k_10min, 15k_5min and 15k_15min all could induce a decent number of liquid crystal morphology. 100%PLA_5k_10min was mostly amorphous, whereas 15k_10min induced peaks

that were sharper than nematic-like morphology. Figure 21 depicted the XRD and 2D captures of 2 random sites for 1%Cur_PLA first press under 5000, 10000 and 15000 lbs. of pressure for 5, 10, and 15. minutes. Only 10k_5min and 10k_10min induced the nematic-like morphology that we wanted. The peak of my controlled first press at 5k_5min was too sharp. I can't yet explain why 5k_10min had less intensity, but I suppose because the 1%Cur_PLA film cast had a certain degree of plasticity, and sudden application of pressure for a short period would induce a good amount of condis crystals that were endowed with a certain orientational and positional order, but after an extended time of similar pressure that was insufficient to lower the degree of freedom, the polymeric chains began to absorb energy from the environment and relocate themselves into greater orientation. 15k lbs. of pressure in another way may be too much for the induction of liquid crystals because it dramatically decreased the degrees of freedom for PLA chains, forcing the chains to become ordered in a certain orientation and position.

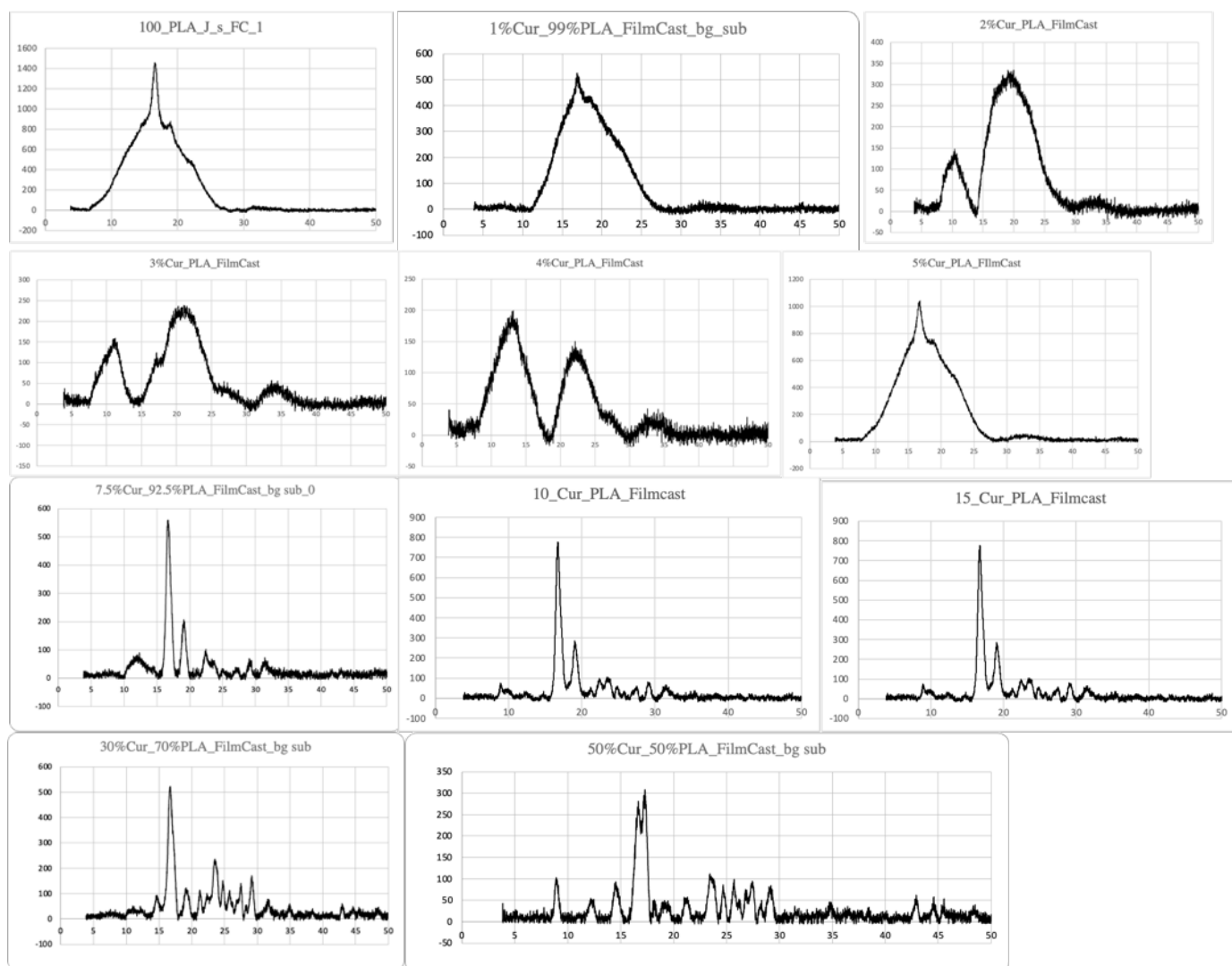


Figure 5. XRDs for film cast samples from 1%Cur_PLA to 50%Cur_PLA.

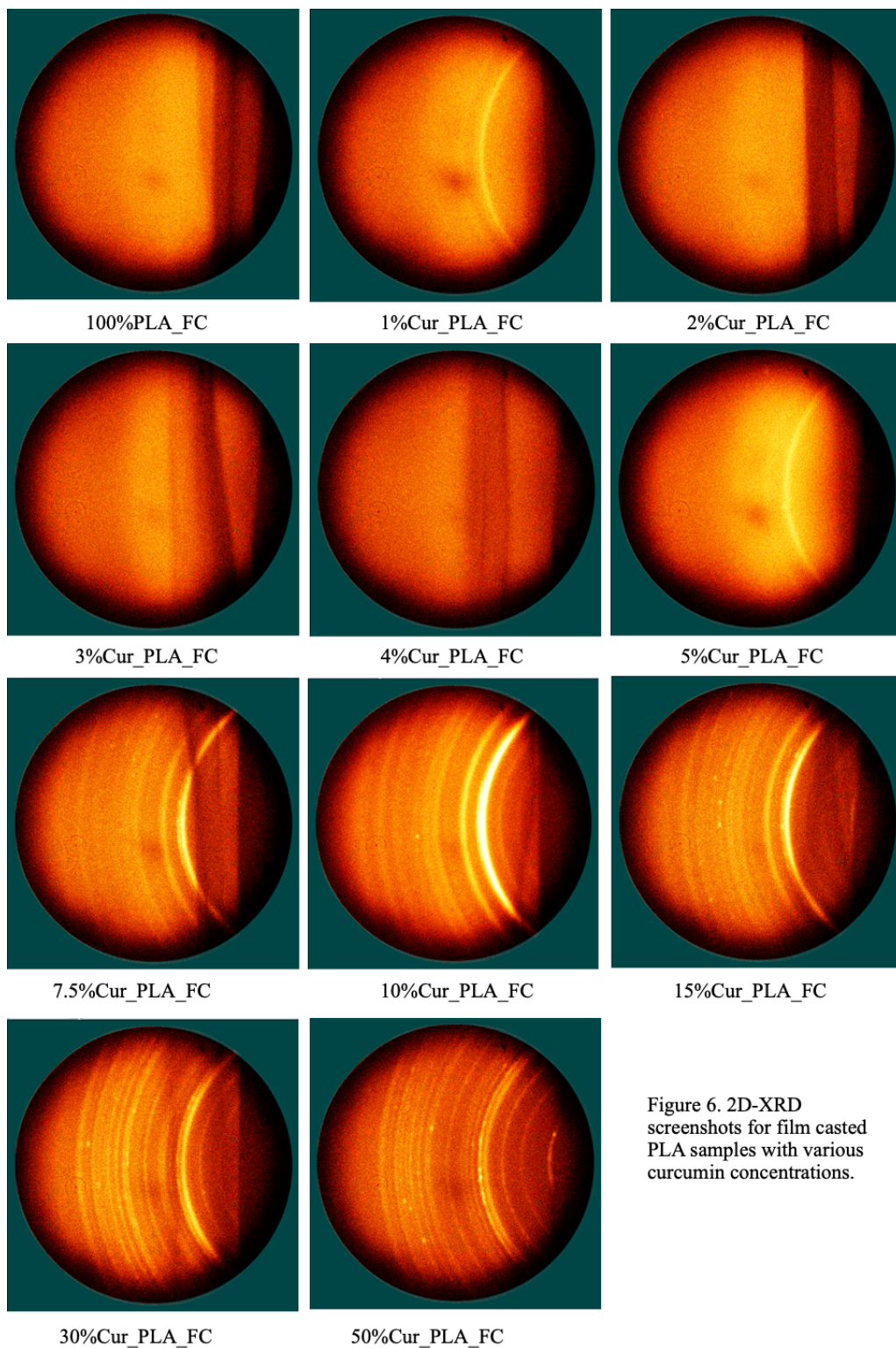


Figure 6. 2D-XRD screenshots for film casted PLA samples with various curcumin concentrations.

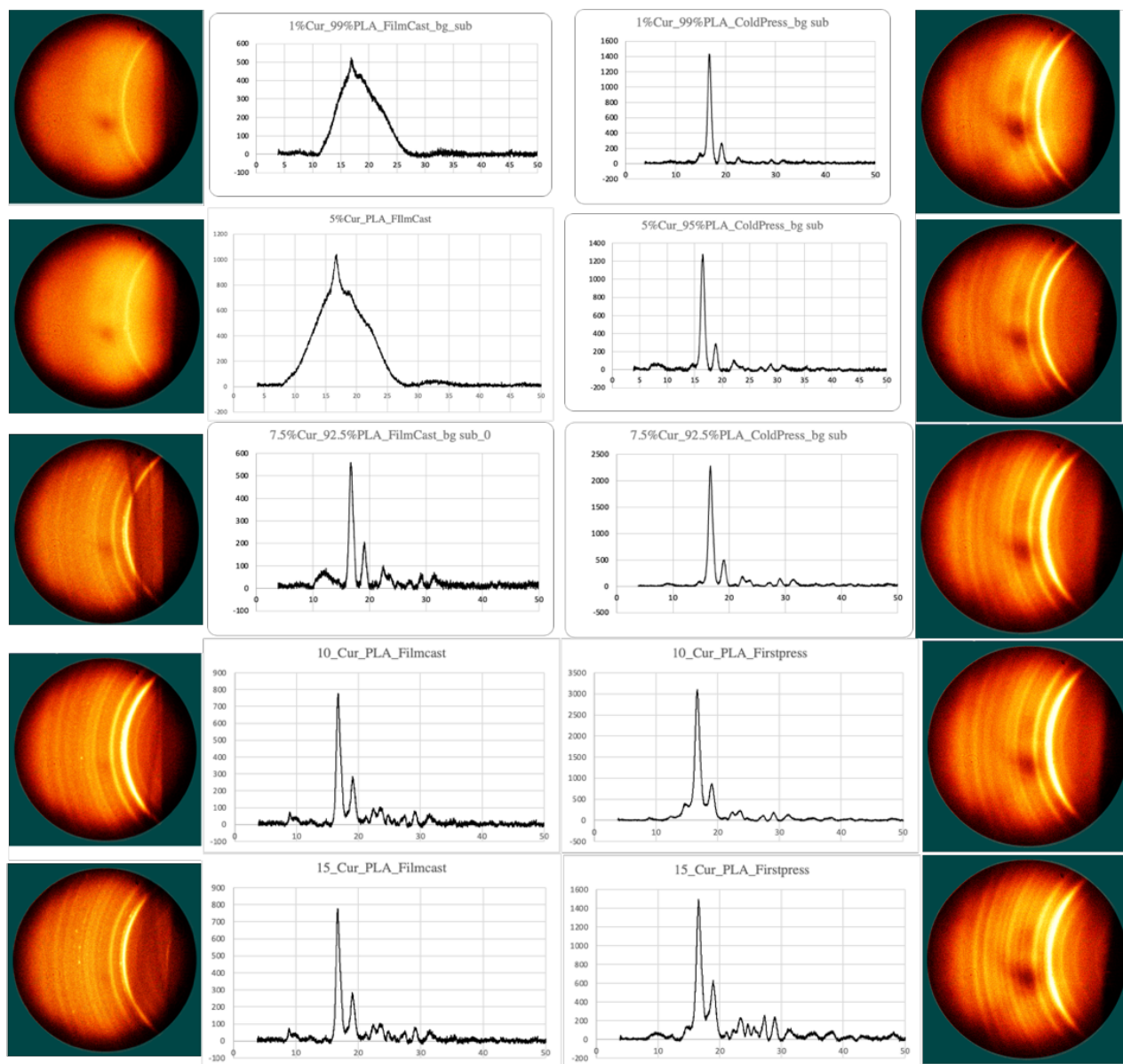


Figure 7. XRD and 2D comparisons between film cast and cold press samples for 1%Cur_PLA, 5%Cur_PLA, 7.5%Cur_PLA, 10%Cur_PLA, and 15% Cur_PLA.

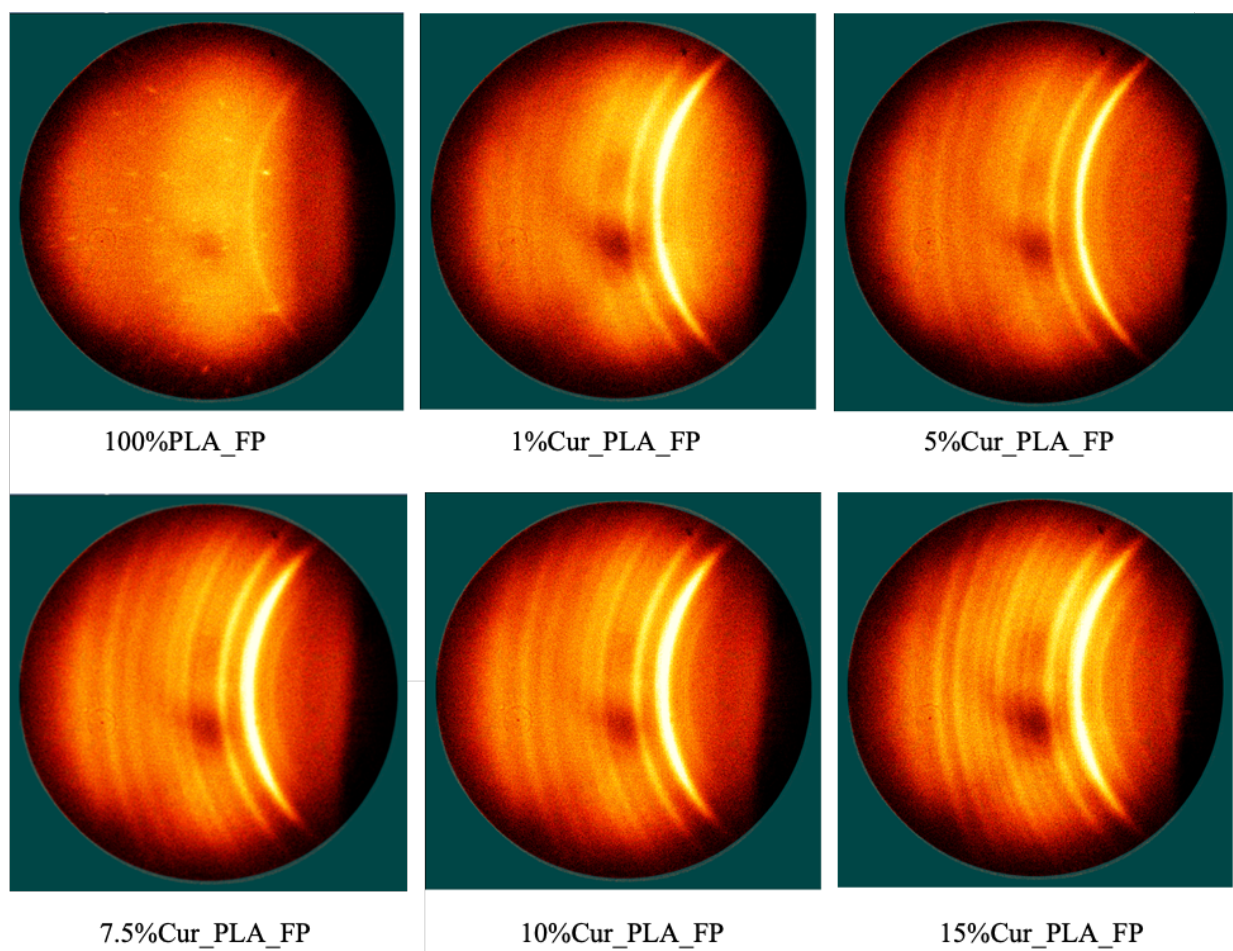


Figure 8. 2D-XRD screenshots for first pressed PLA samples with various curcumin concentrations.

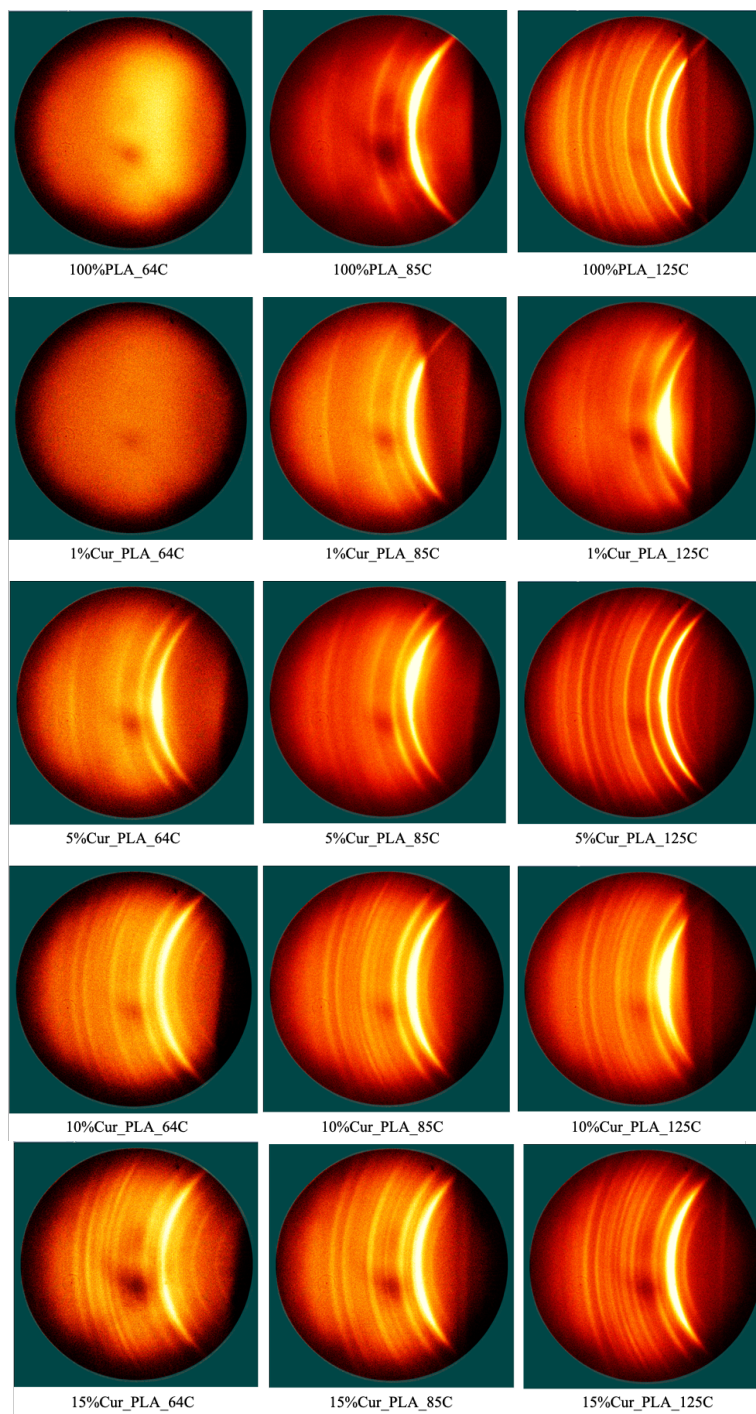


Figure 9. 2D-XRD screenshots for heat pressed samples at 64, 85 and 125 degrees Celsius for 100%PLA, 1%Cur_PLA, 5%Cur_PLA, 10%Cur_PLA and 15%Cur_PLA.

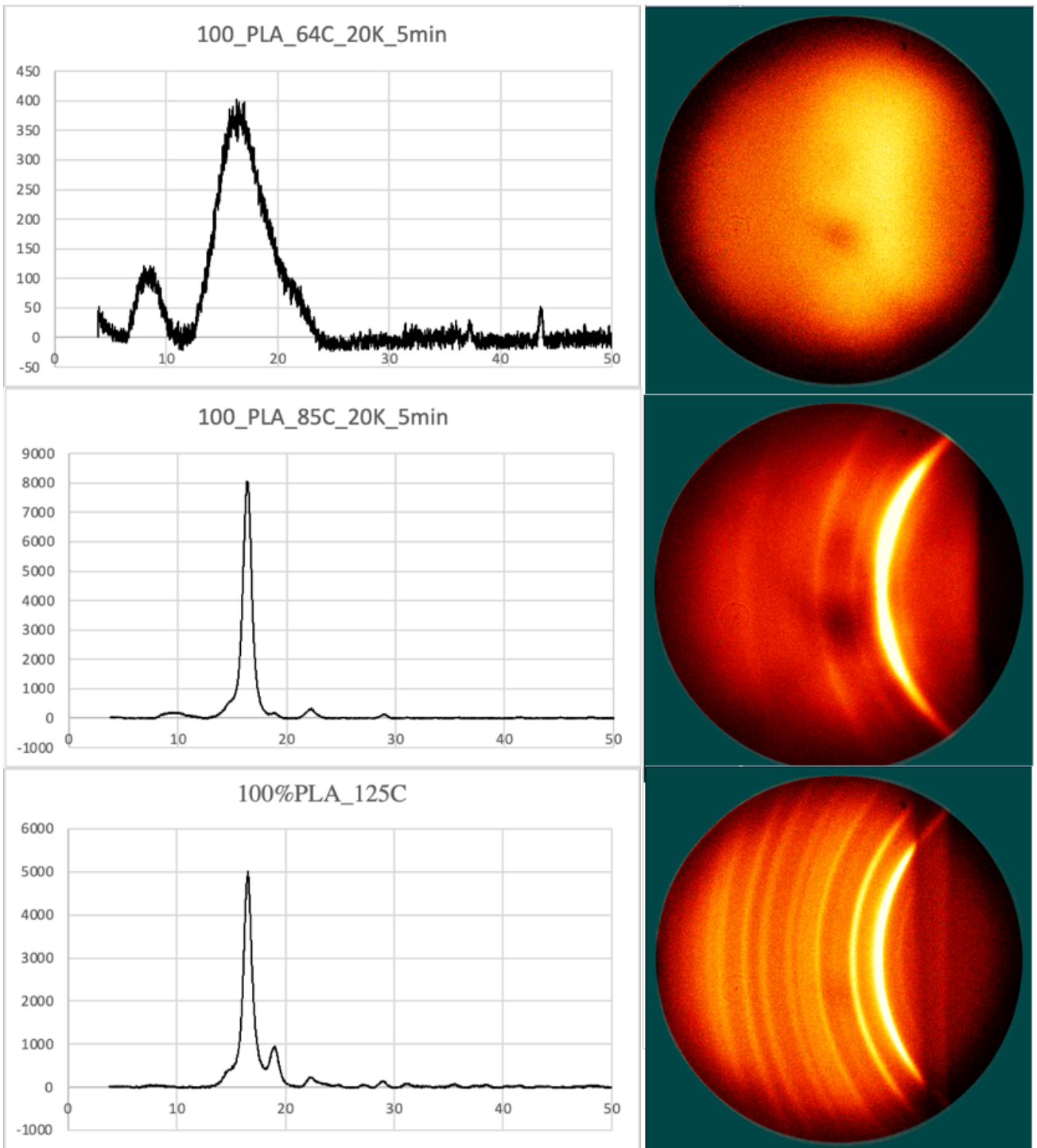


Figure 10. XRD for heat pressed 100%PLA samples at 64, 85 and 125 degrees Celsius.

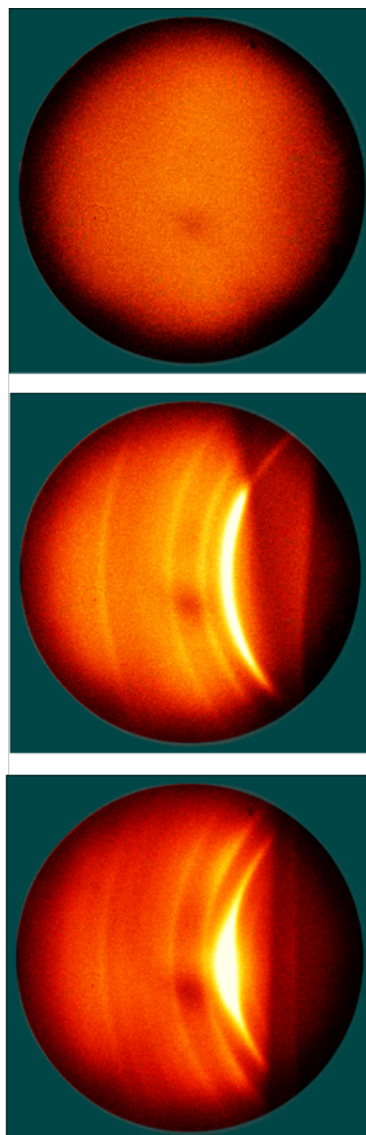
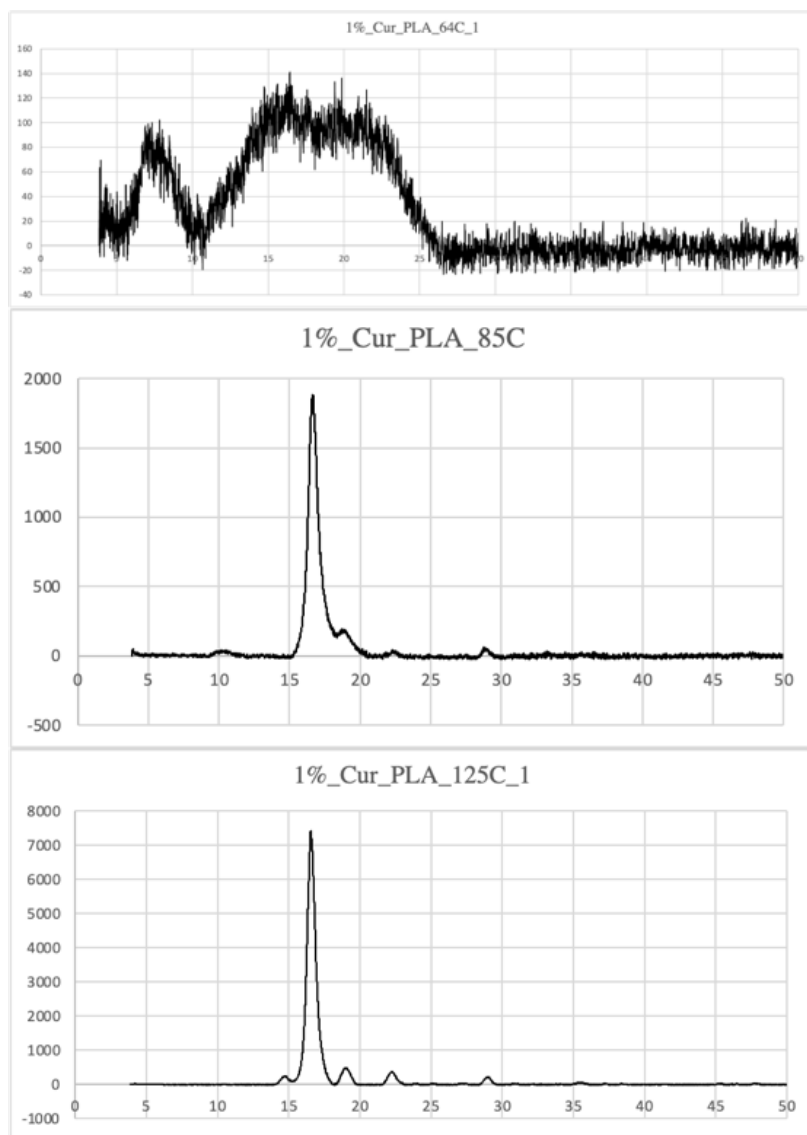


Figure 11. XRD for heat pressed 1%Cur_PLA samples at 64, 85 and 125 degrees Celsius.

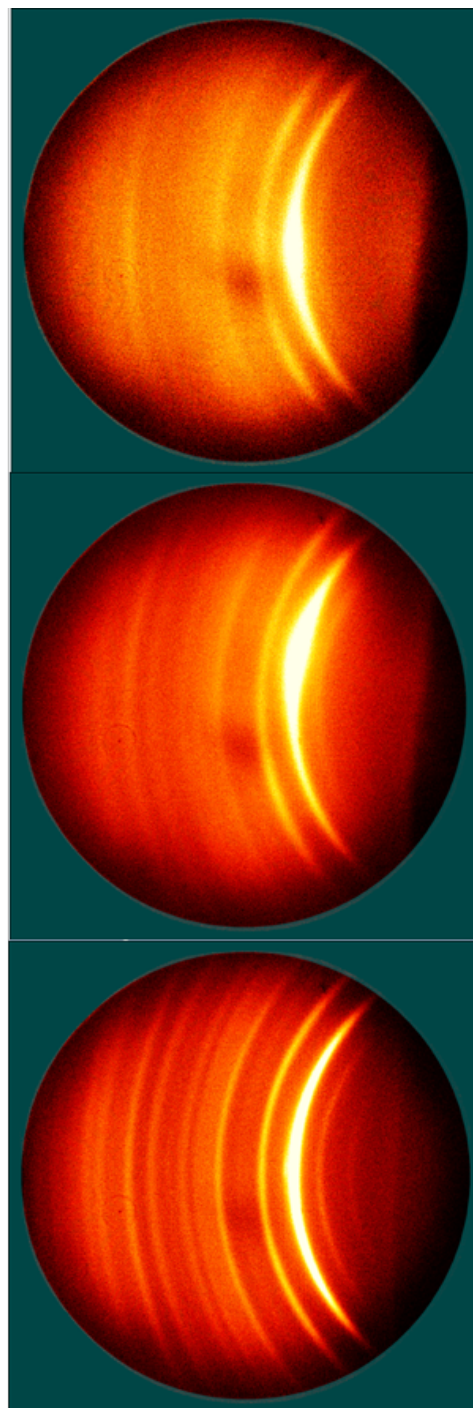
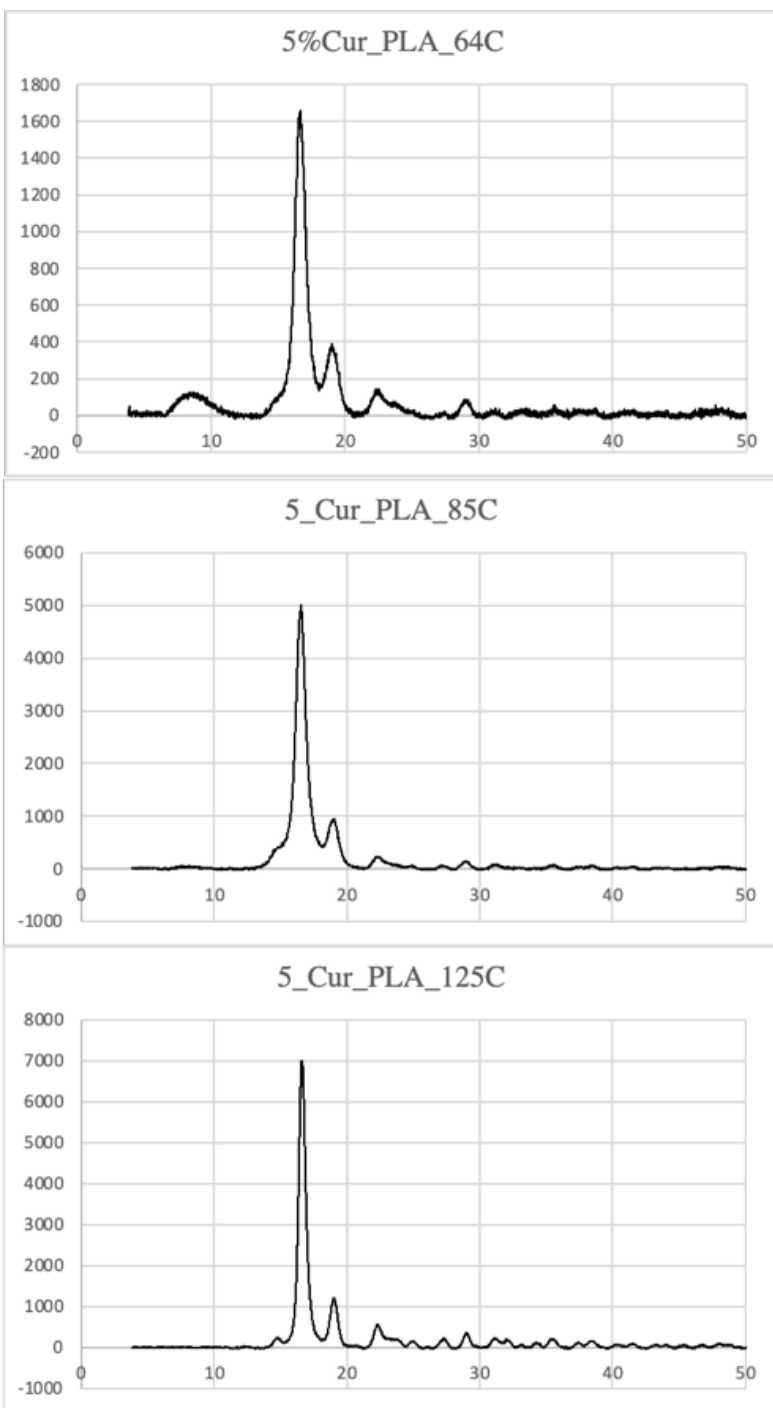


Figure 12. XRD for heat pressed 5%Cur_PLA samples at 64, 85 and 125 degrees Celsius.

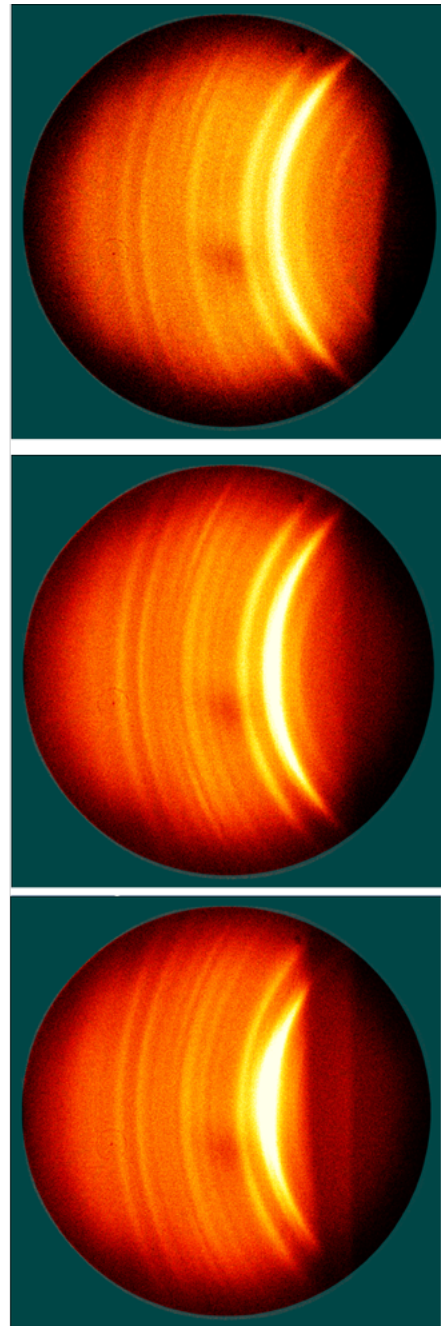
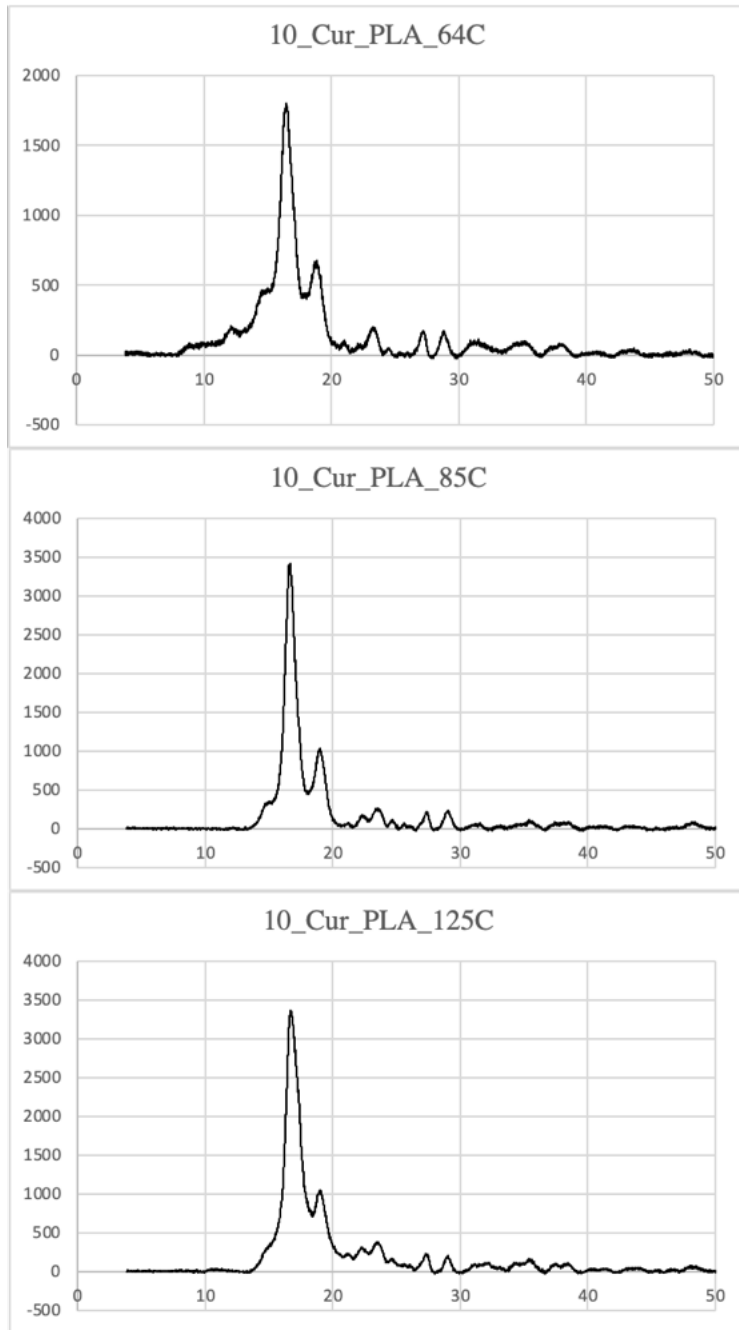


Figure 13. XRD for heat pressed 10%Cur_PLA samples at 64, 85 and 125 degrees Celsius.

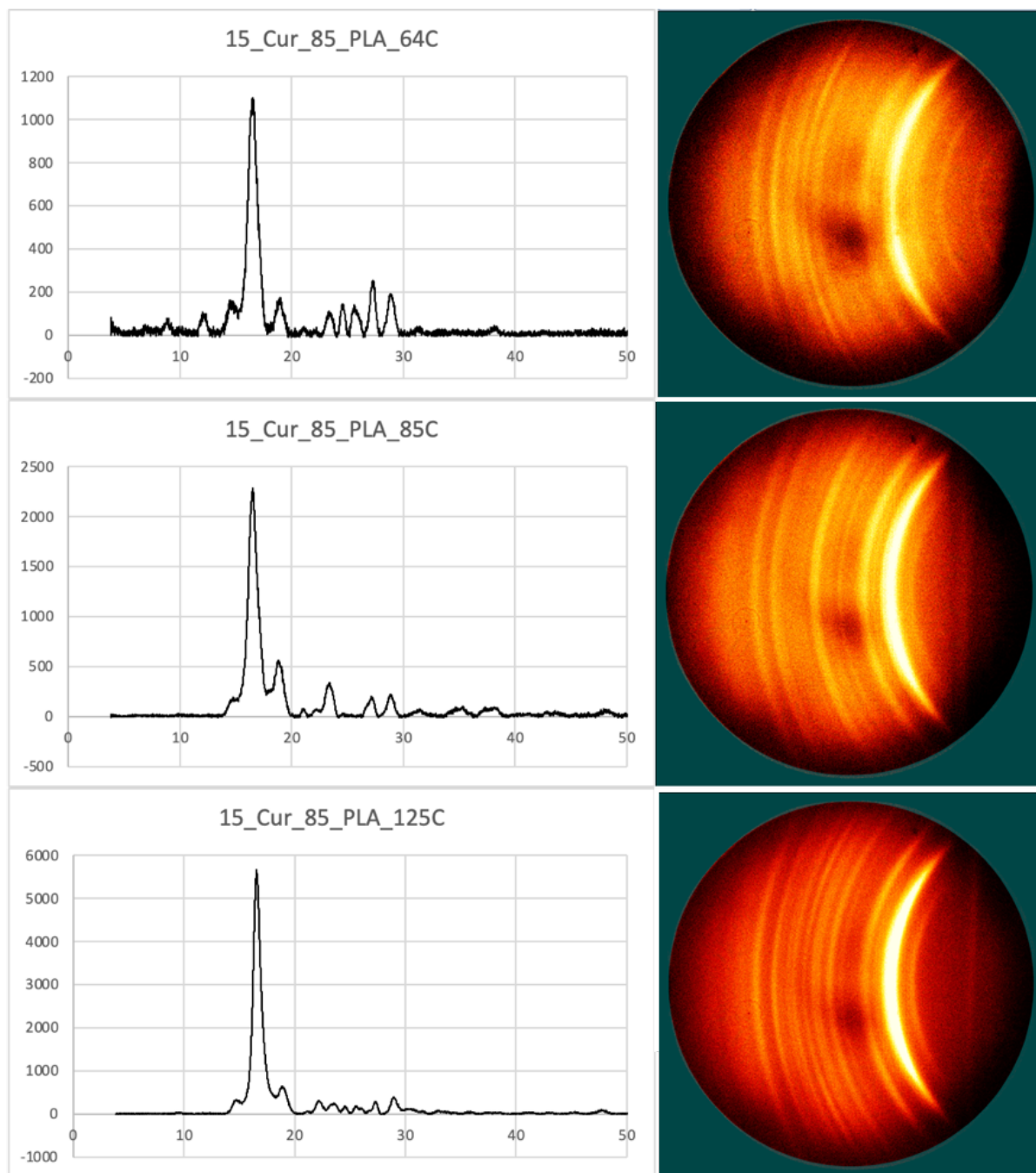


Figure 14. XRD for heat pressed 15%Cur_PLA samples at 64, 85 and 125 degrees Celsius.

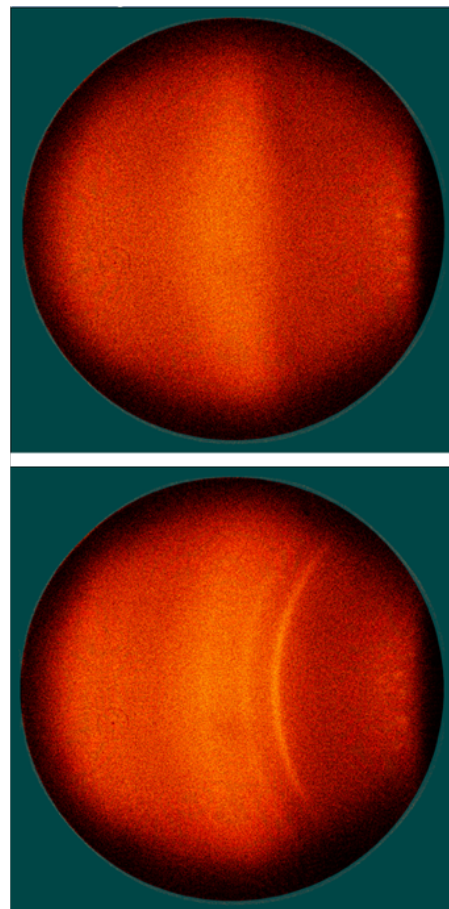
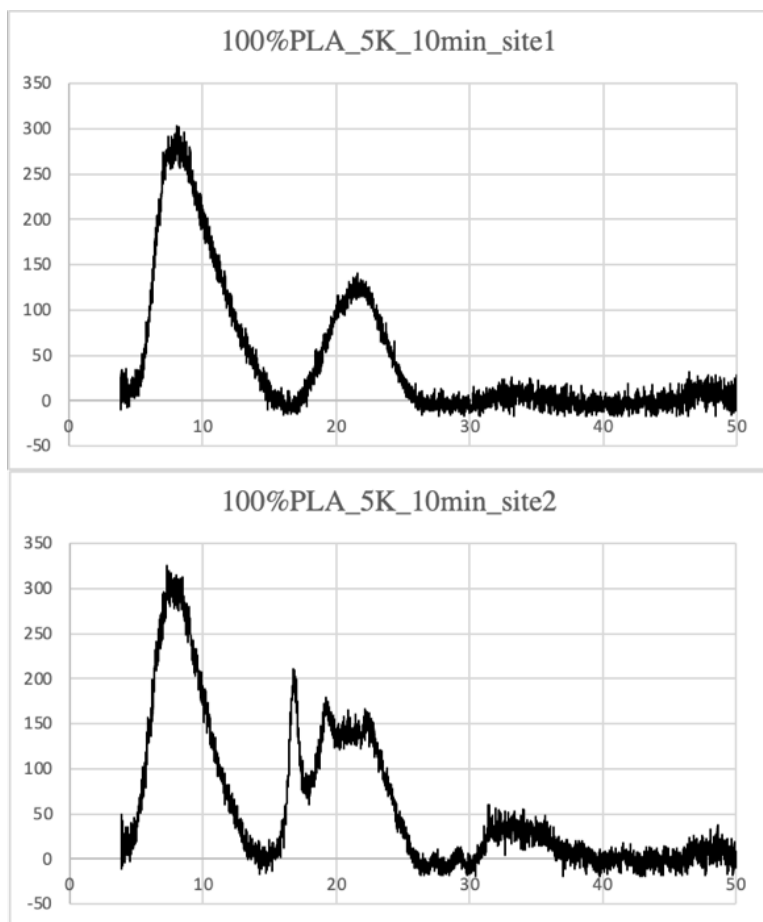


Figure 15. XRD of 2 random sites for 100%PLA first press under 5000 lbs. of pressure for 10 minutes.

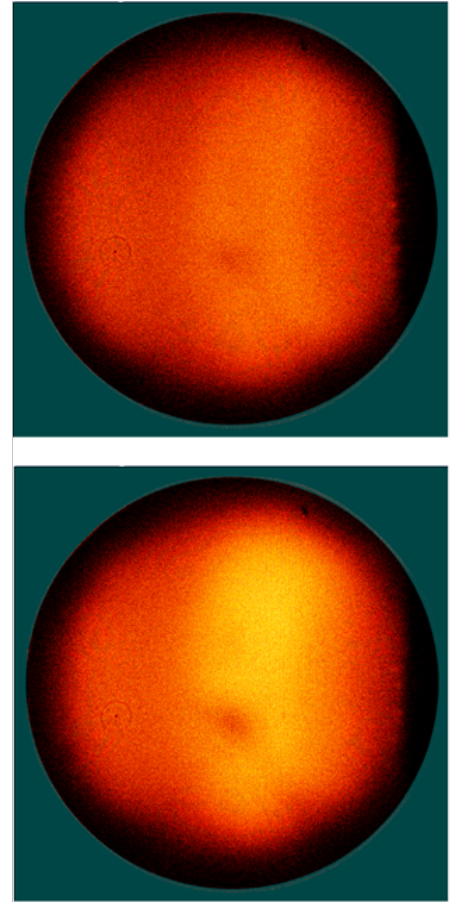
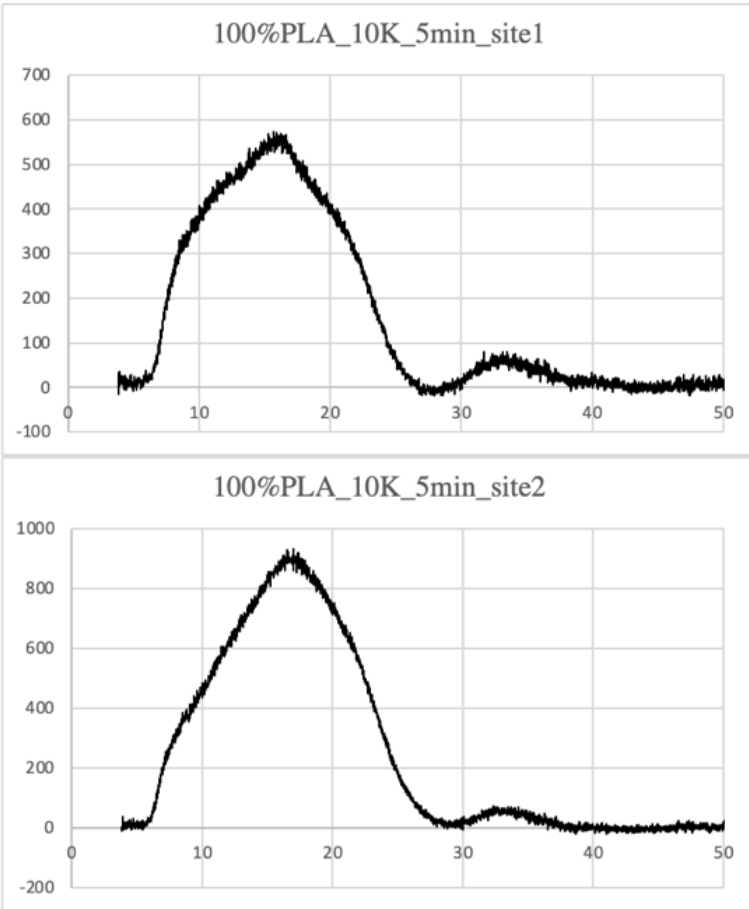


Figure 16. XRD of 2 random sites for 100%PLA first press under 10000 lbs. of pressure for 5 minutes.

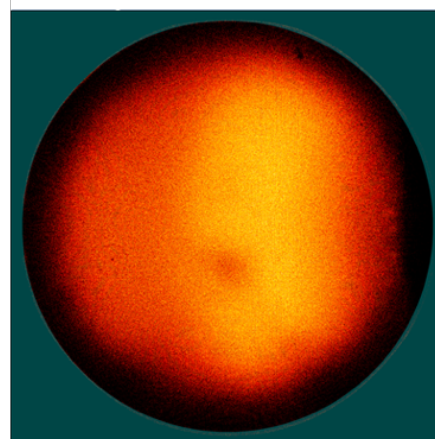
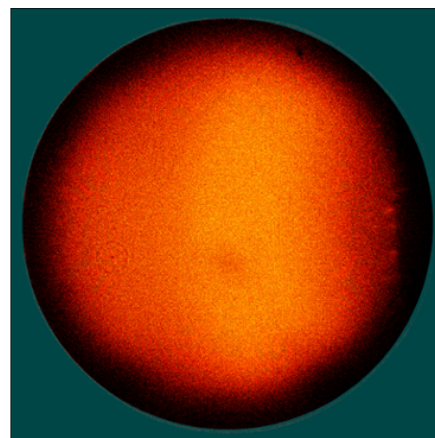
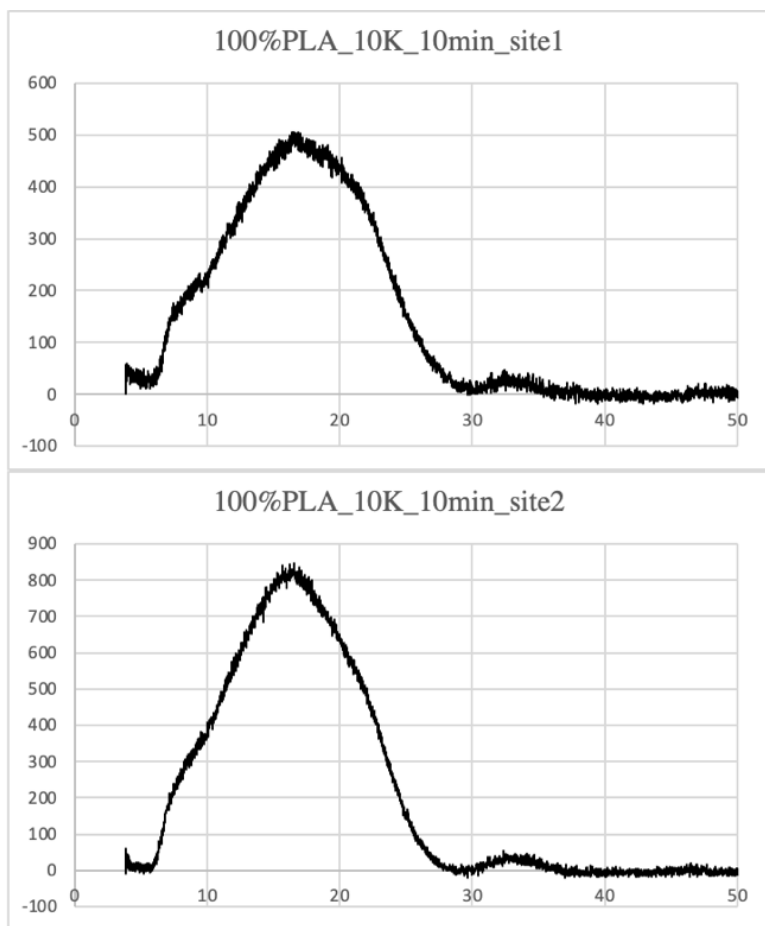


Figure 17. XRD of 2 random sites for 100%PLA first press under 10000 lbs. of pressure for 10 minutes.

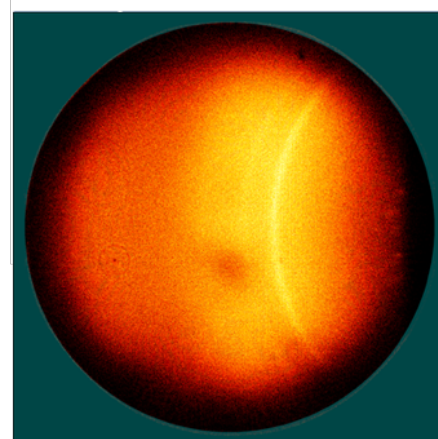
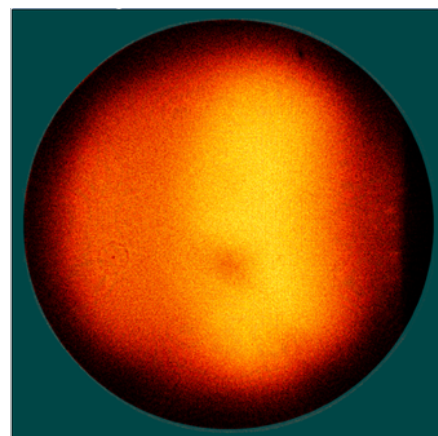
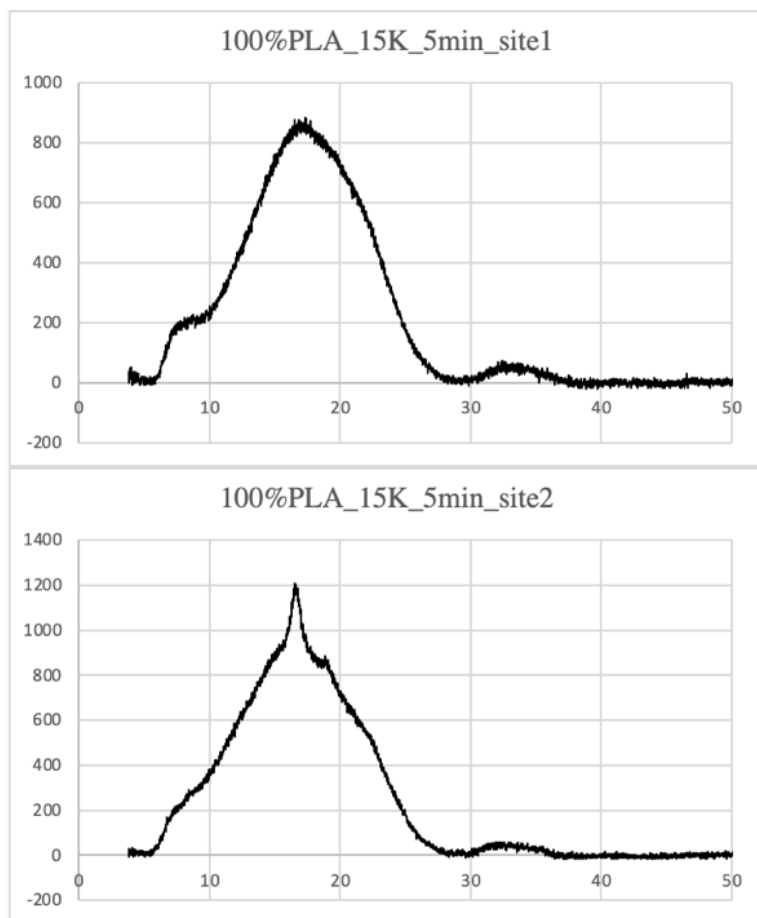


Figure 18. XRD of 2 random sites for 100%PLA first press under 15000 lbs. of pressure for 5 minutes.

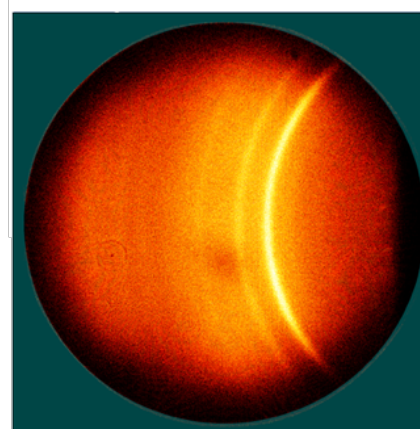
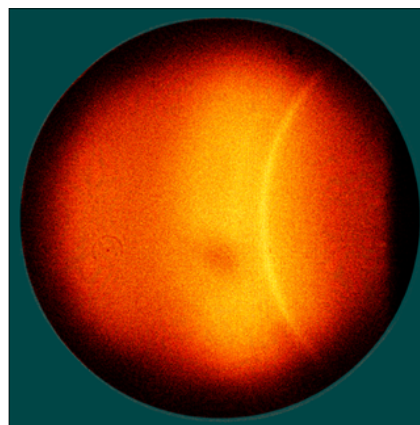
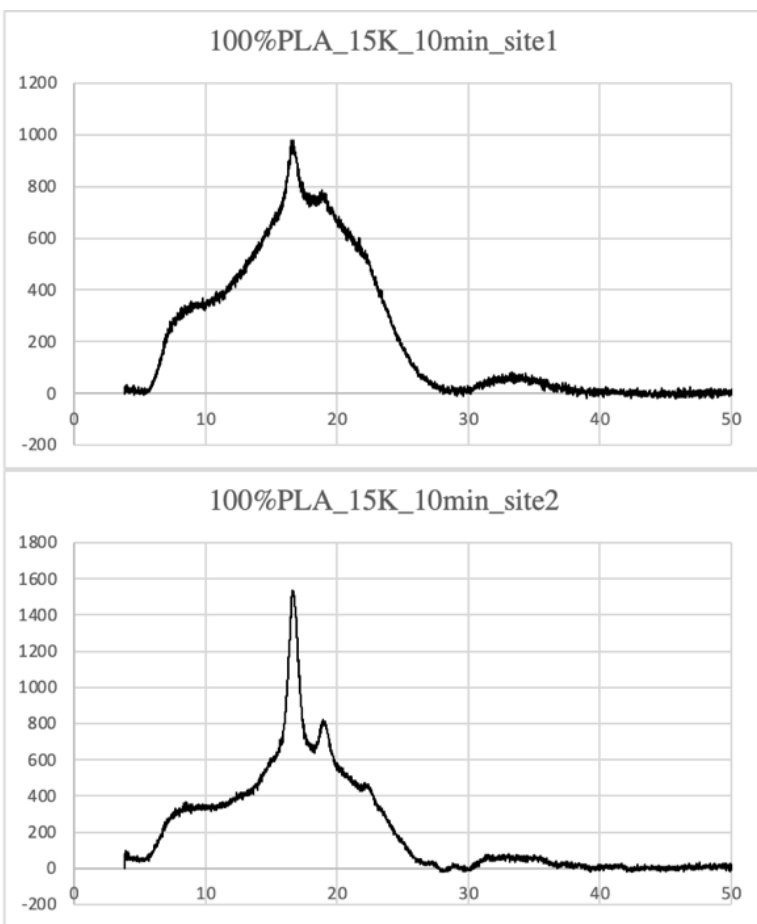


Figure 19. XRD of 2 random sites for 100%PLA first press under 15000 lbs. of pressure for 10 minutes.

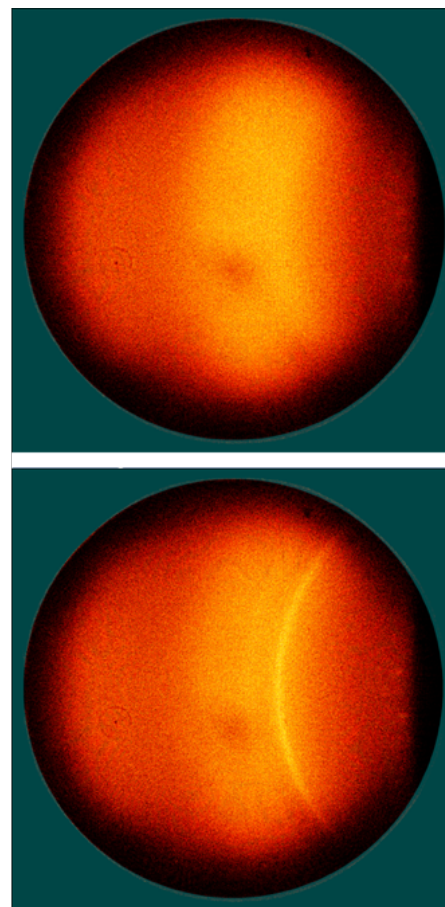
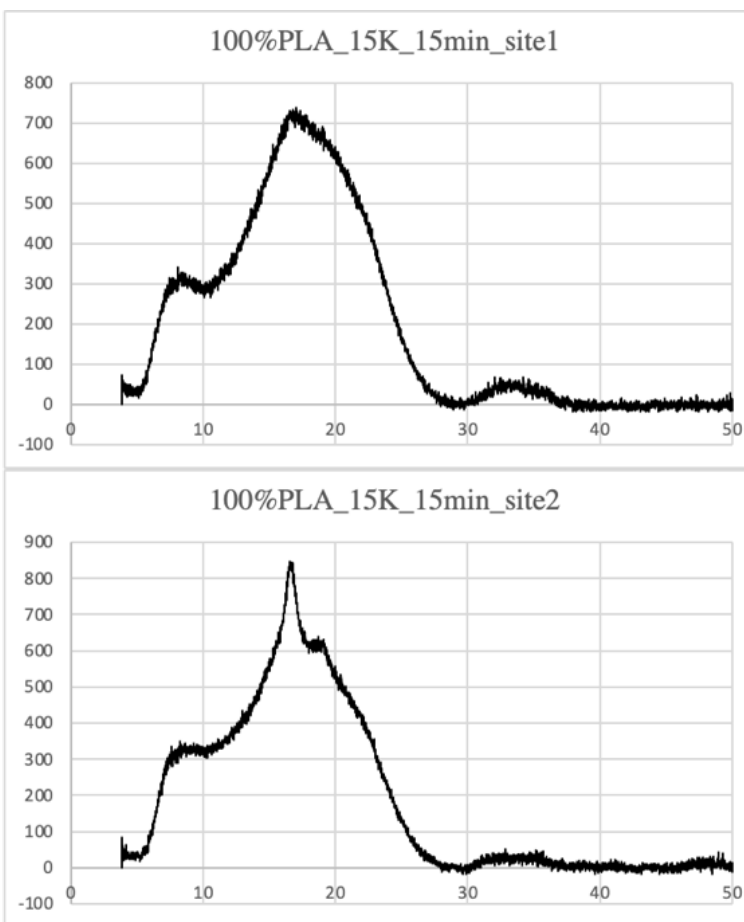


Figure 20. XRD of 2 random sites for 100%PLA first press under 15000 lbs. of pressure for 15 minutes.

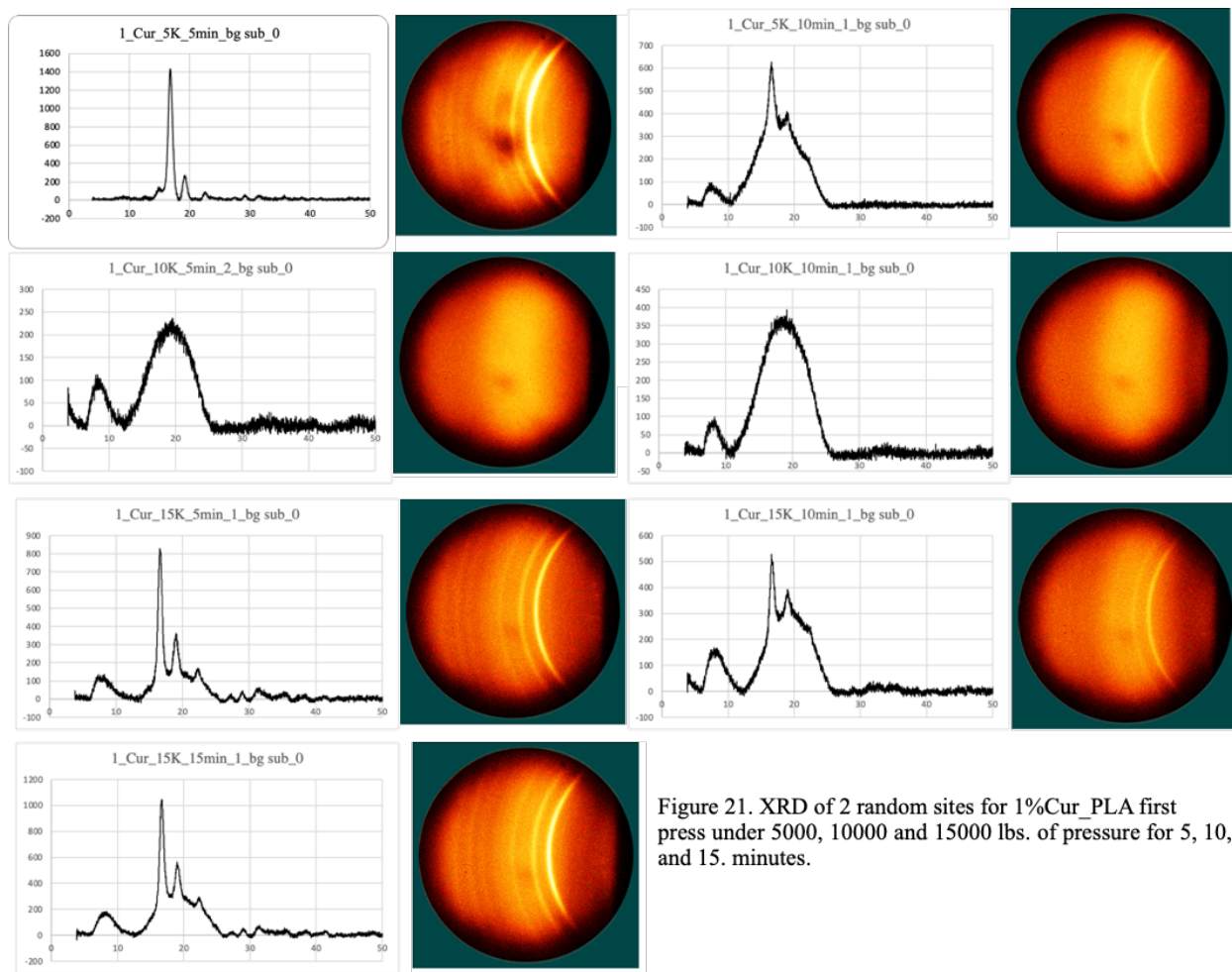


Figure 21. XRD of 2 random sites for 1%Cur_PLA first press under 5000, 10000 and 15000 lbs. of pressure for 5, 10, and 15. minutes.

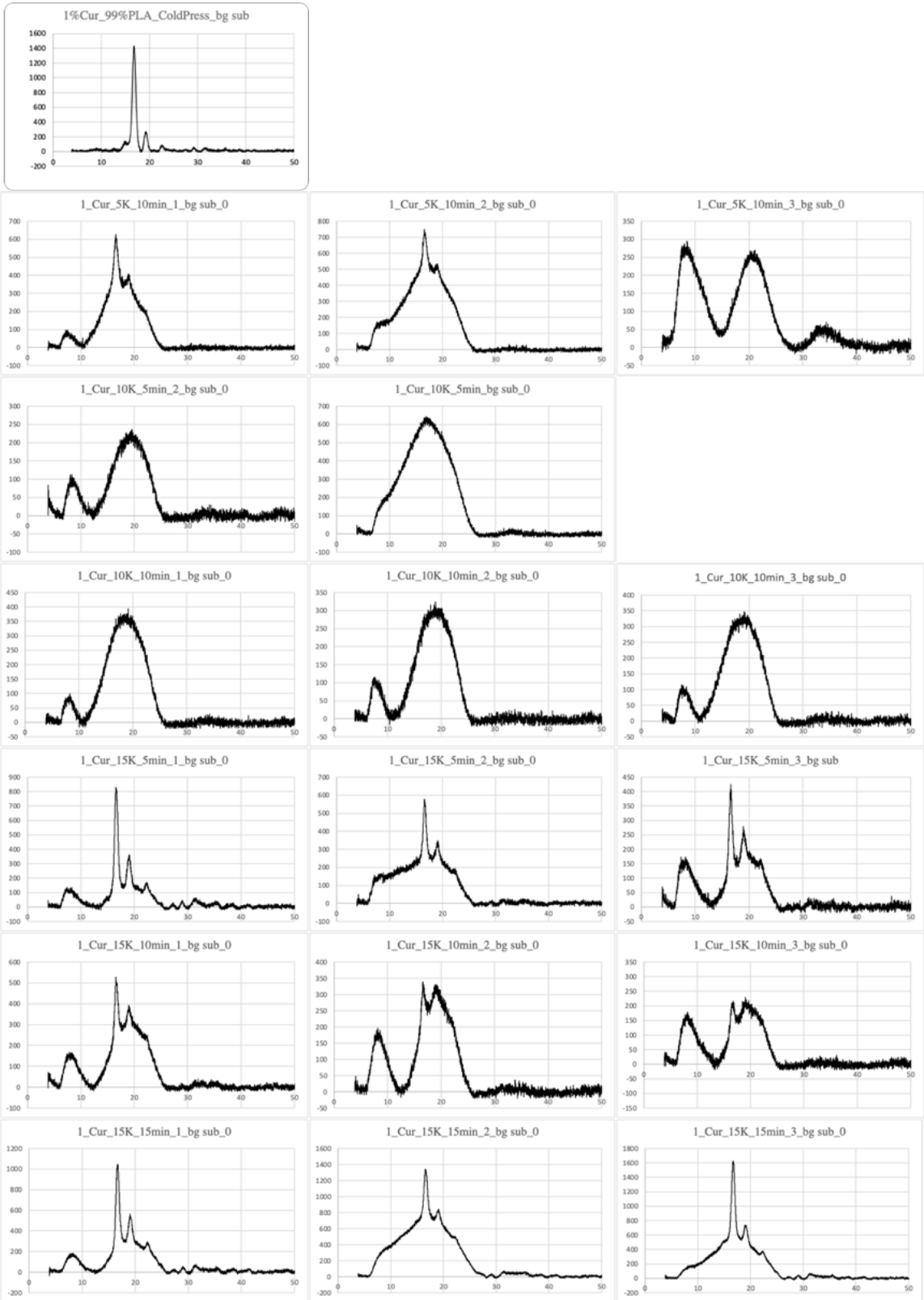


Figure 22. XRDs of samples that were first-pressed at different conditions. Three (except for the 1%Cur_10K_5min condition) random spots on the samples were taken to visually and computationally evaluate the variations.

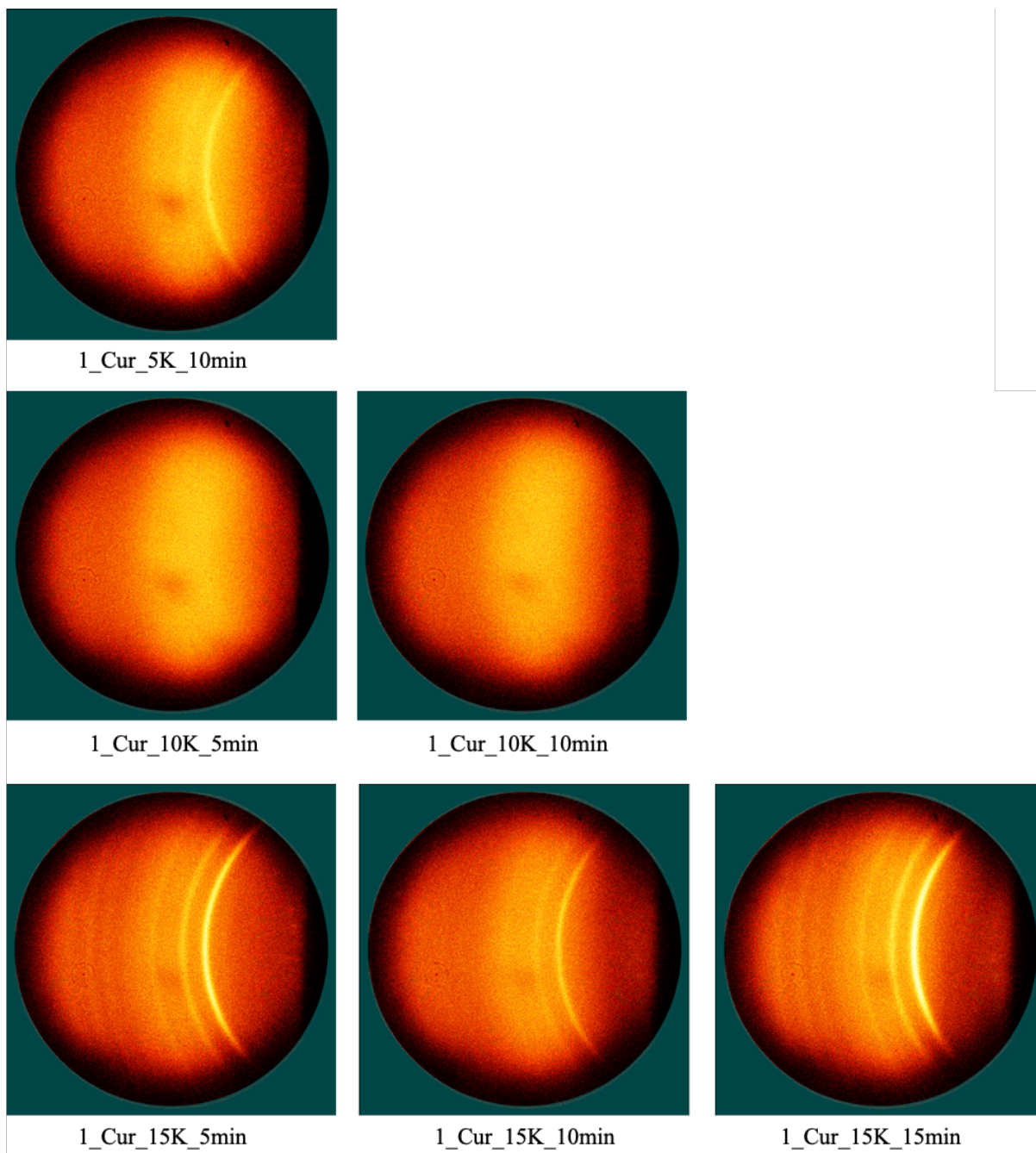


Figure 23. 2D-XRD screenshots for first pressed 1%Cur_PLA samples under different processing conditions.

ATR

ATR was used for pig cadaver skin release project. The first group of pig cadaver skin release study was composed of release of 100%PLA_FC, 10%Cur_PLA_FC, 15%Cur_PLA_FC and 50%Cur_PLA on day 0 as reference, 5%Cur_PLA_FC and 5%Cur_PLA_FP on day 0, 5 and 8, and 30%Cur_PLA_FC on day 0, 1, 3 and 4 (Figure 24 to Figure 37). The absorbance peaks from 1550 to 1650 wavenumber represented the existence of curcumin, as it was not observed with the 100%PLA_FC, but observed with all other films casted with curcumin. As the concentration of curcumin increased, the peak absorption height of curcumin visually increased after being normalized by the highest PLA absorption peak. Unfortunately, I was unable to say if any of my film cast or first press samples truly released curcumin because the curcumin absorption peak was strongly interfered by the occurrence of absorption at 2800-3000 wavenumber. We supposed that such gained absorption peaks came from the shredded skin, protein, fat, mineral and other unknown skin composition. Figure 38 to 44 depicted the release of curcumin from 3 different sites on 1%Cur_PLA_64C, 5%Cur_PLA_85C, 5%Cur_PLA_125C, 5%Cur_PLA_FC, 10%Cur_PLA_FC, 15%Cur_PLA_FC, and 30%Cur_PLA_FC at hour 1, 2, and 3. Both 5%Cur_PLA_85C showed solid signs of continuous release of curcumin, as the curcumin absorption peak decreased with increasing duration on the skin. 5%Cur_PLA_125C had a release in the first hour but stopped at hr2 and hr3. 1%Cur_PLA_64C experienced an increase of curcumin absorption peak at hr1 but dropped back to original state at hr2 and 3. This could mean that pig cadaver skin had hydrophobically extracted some curcumin that was trapped in the mid-layers of the tablet to its surface layer since the tablet was in a completely amorphous state (Figure 9), and polymer at such state may have a greater tendency to release drug compared

to the more crystalline state (Susan). 30%Cur_PLA_FC also exhibited evidence of drug release, as the absorption peak of curcumin decreased as duration of the contact with the pig cadaver skin increased. 30%Cur_PLA_FC had both intense PLA peaks and curcumin peaks on the XRD (Figure 5), meaning that drug and polymer might have phase separated to some extent that the surface drug could be readily extracted hydrophobically by the skin. 5%Cur_PLA_FC, 10%Cur_PLA_FC and 15%Cur_PLA_FC did not show such results might because the drug concentration was not sufficient to induce such a phase separation that drug could be readily extracted.

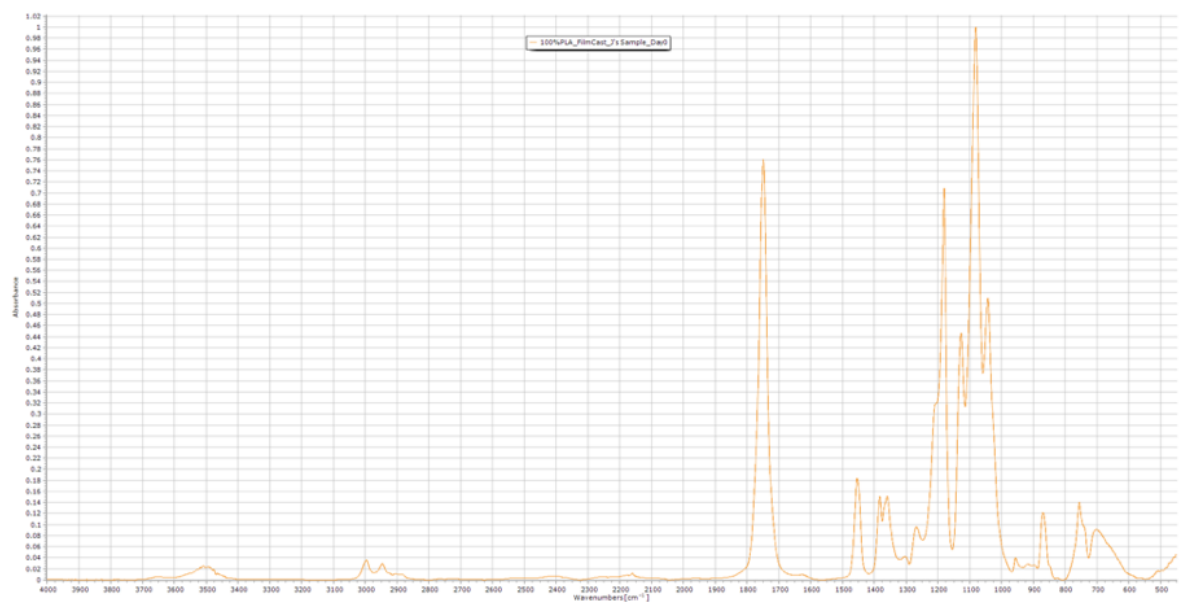


Figure 24. ATR of film cast sample of 100%PLA on day 0.

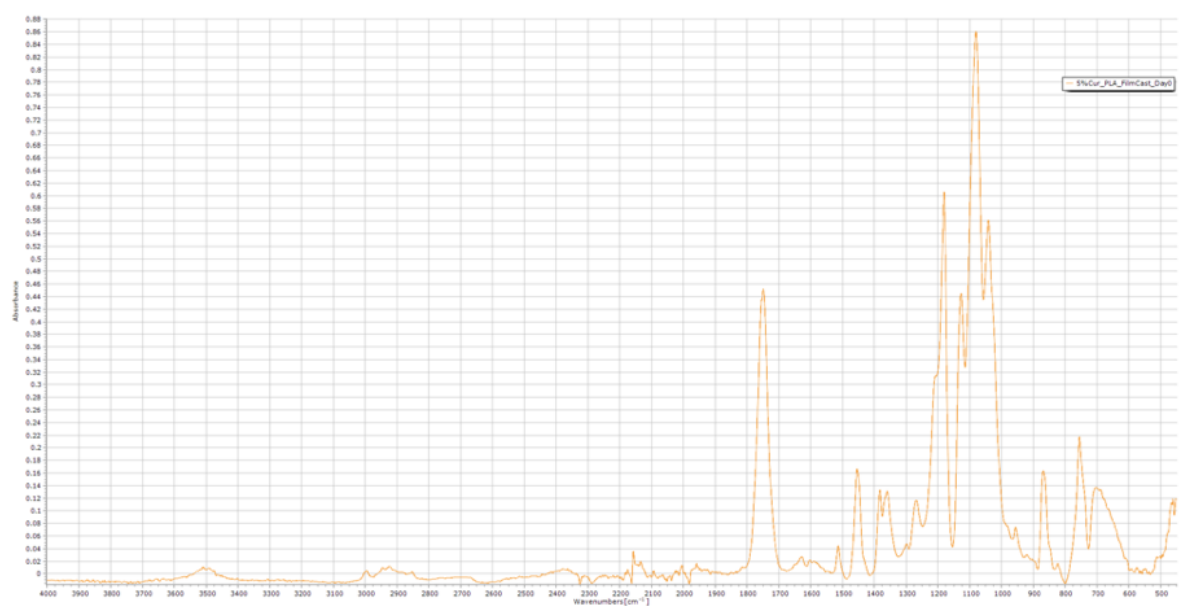


Figure 25. ATR of film cast sample of 5%Cur_PLA on day 0.

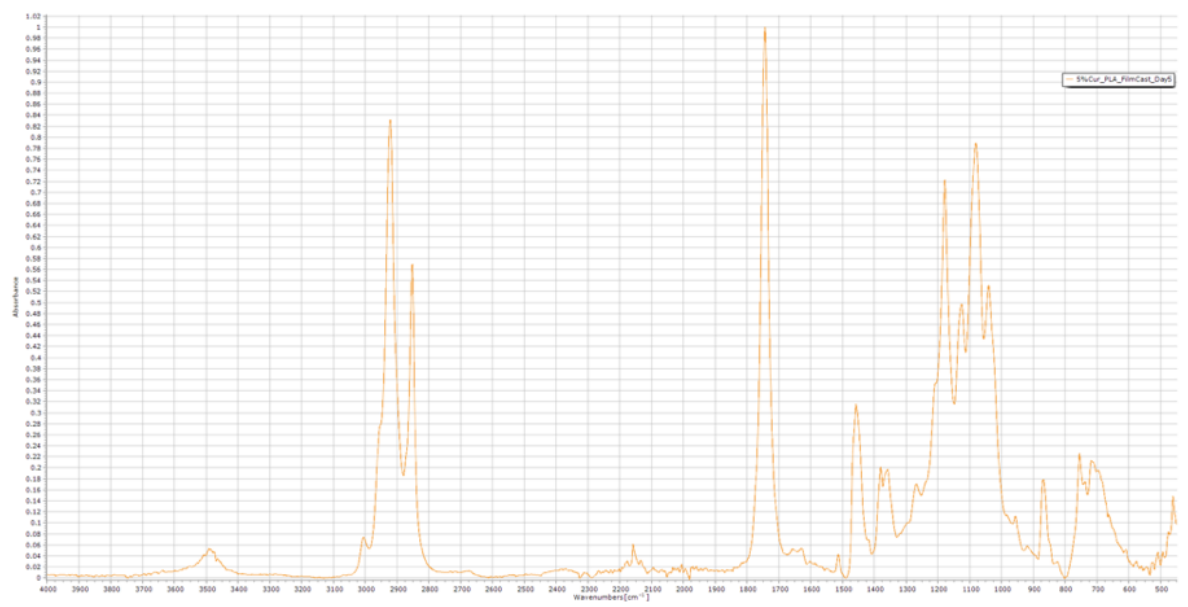


Figure 26. ATR of film cast sample of 5%Cur_PLA on day 5.

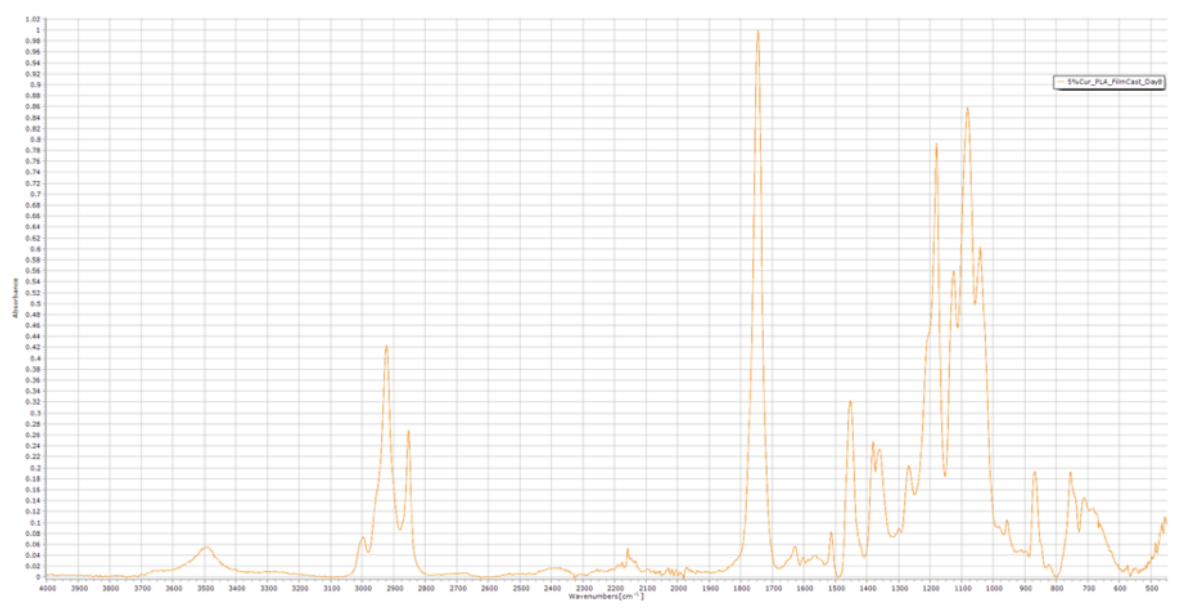


Figure 27. ATR of film cast sample of 5%Cur_PLA on day 8.

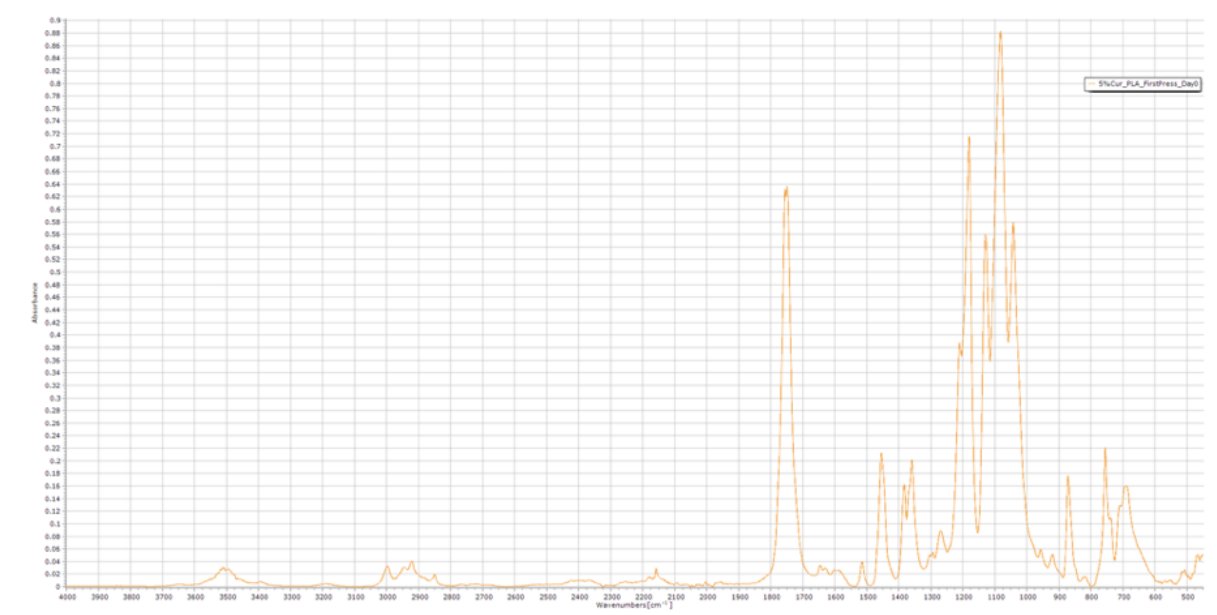


Figure 28. ATR of first press sample of 5%Cur_PLA on day 0.

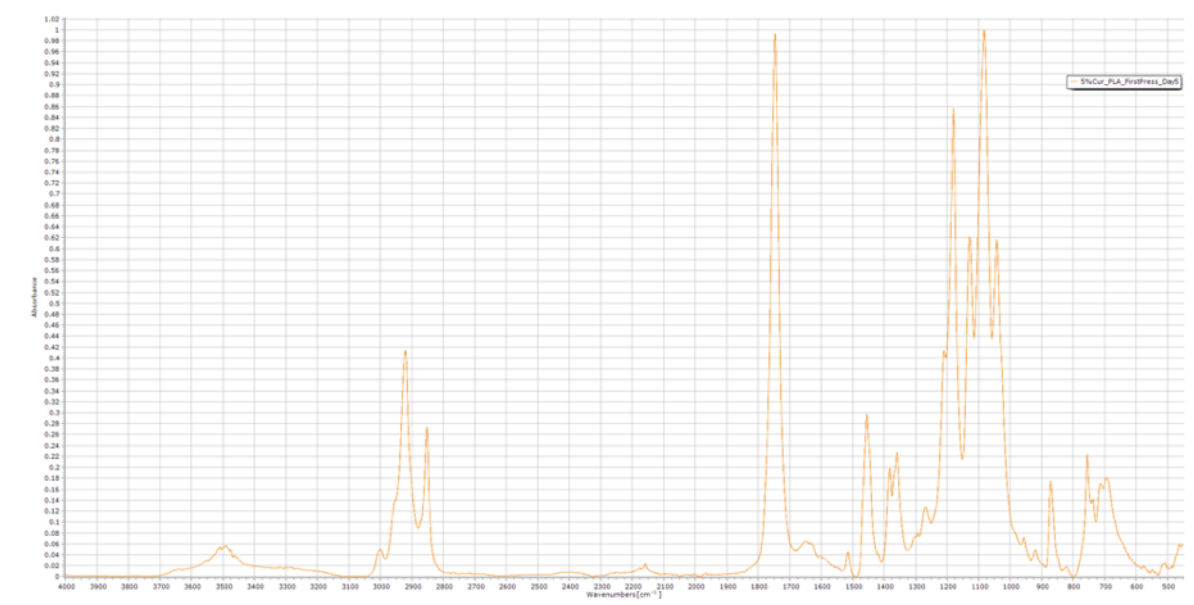


Figure 29. ATR of first press sample of 5%Cur_PLA on day 5.

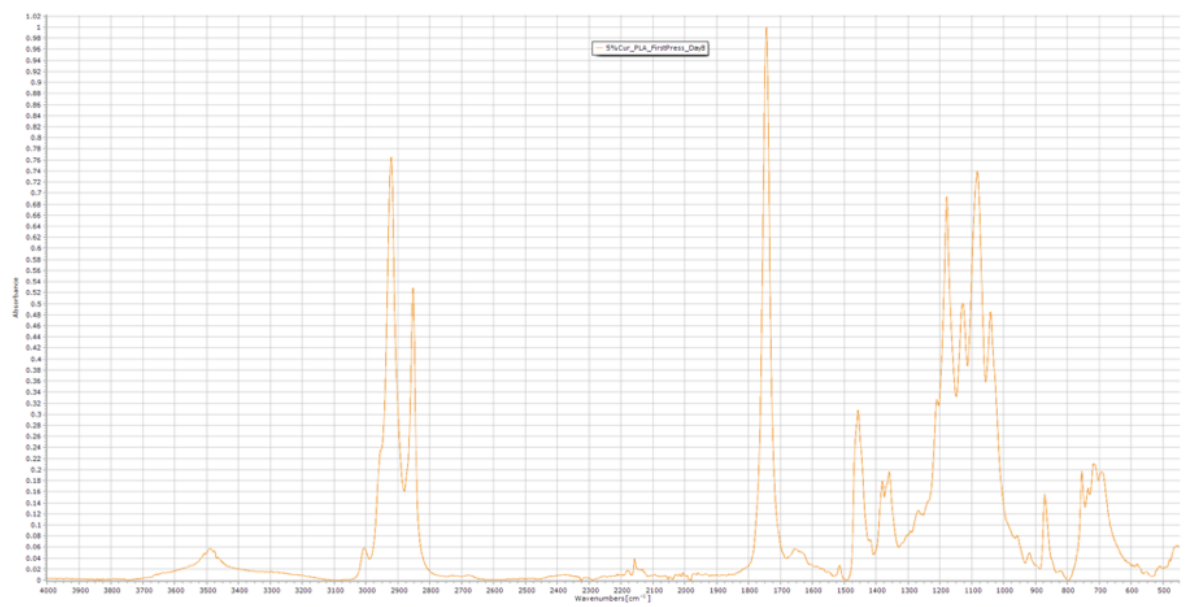


Figure 30. ATR of first press sample of 5%Cur_PLA on day 8.

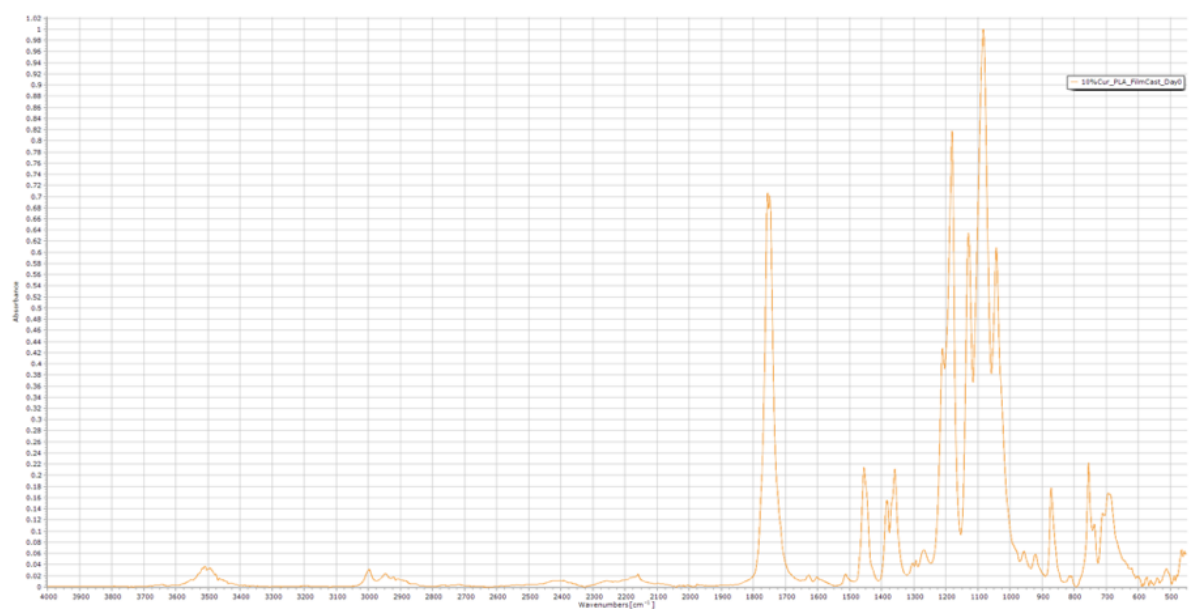


Figure 31. ATR of film cast sample of 10%Cur_PLA on day 0.

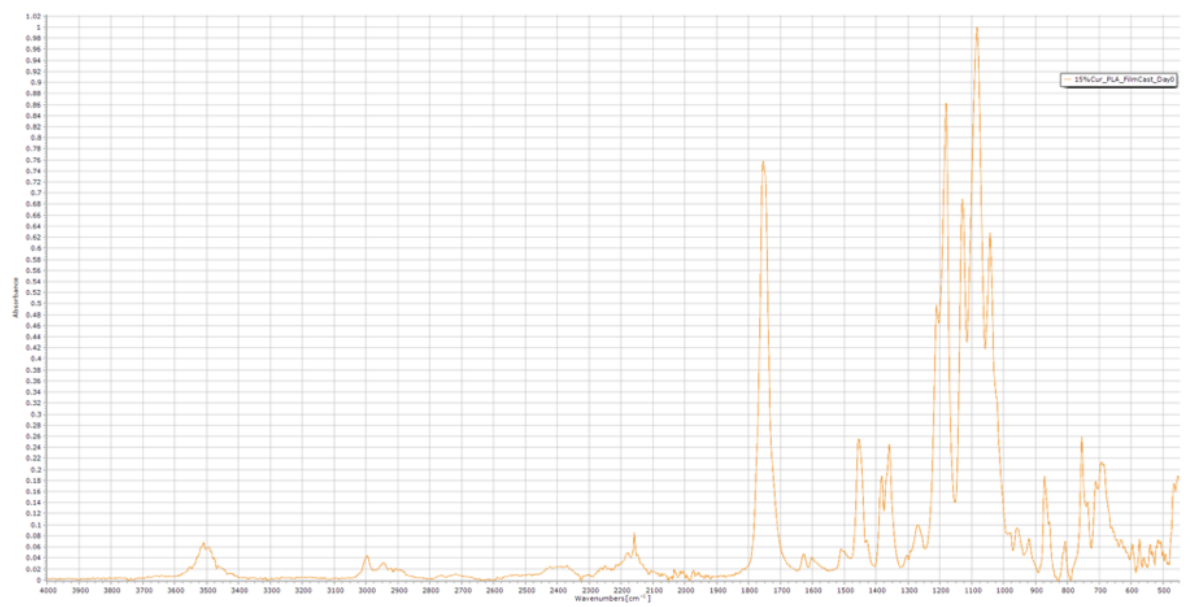


Figure 32. ATR of film cast sample of 15%Cur_PLA on day 0.

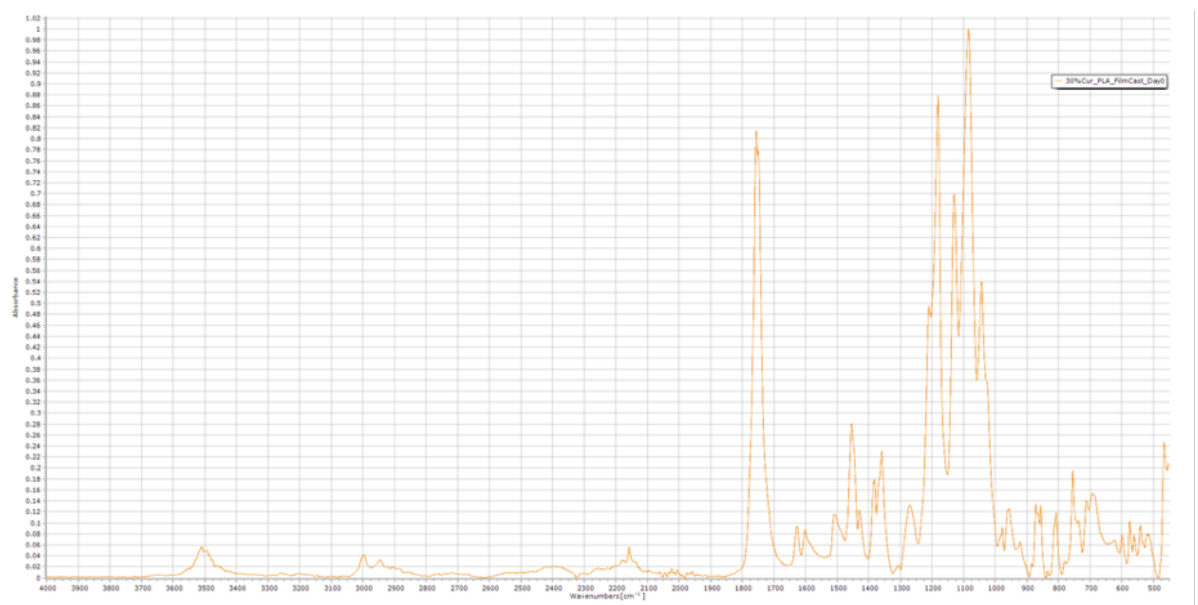


Figure 33. ATR of film cast sample of 30%Cur_PLA on day 0.

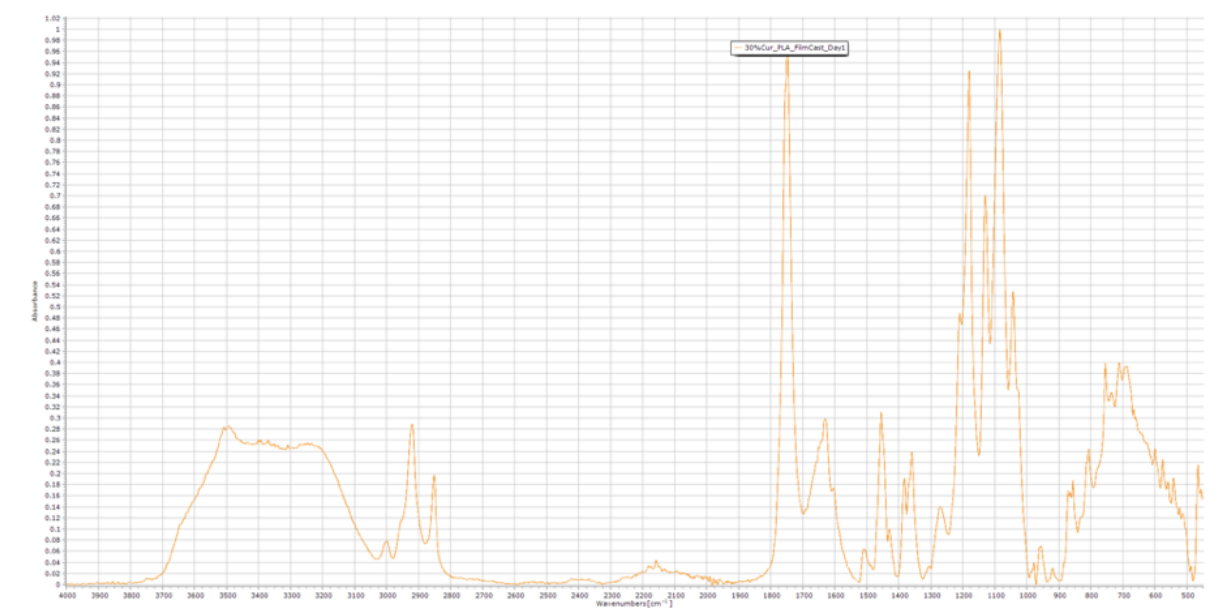


Figure 34. ATR of film cast sample of 30%Cur_PLA on day 1.

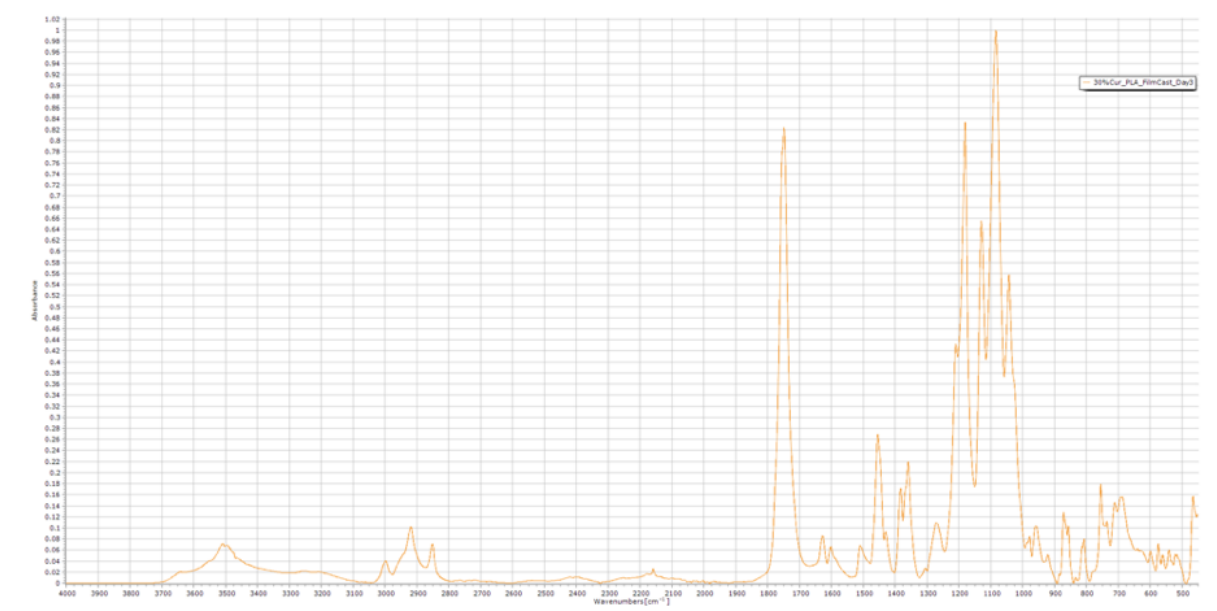


Figure 35. ATR of film cast sample of 30%Cur_PLA on day 3.

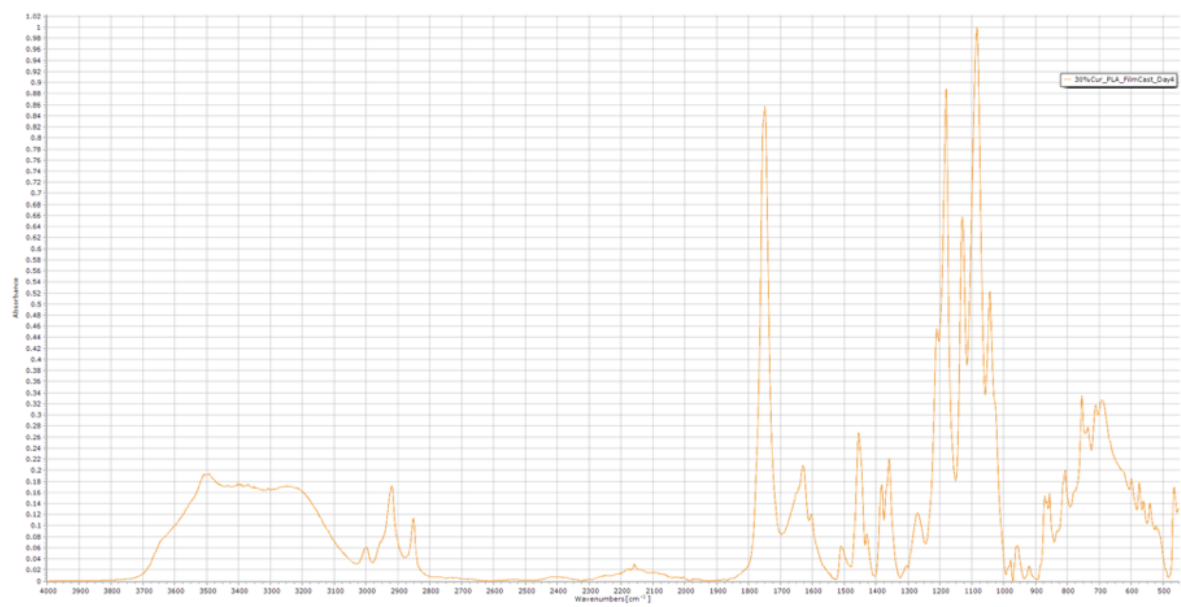


Figure 36. ATR of film cast sample of 30%Cur_PLA on day 4.

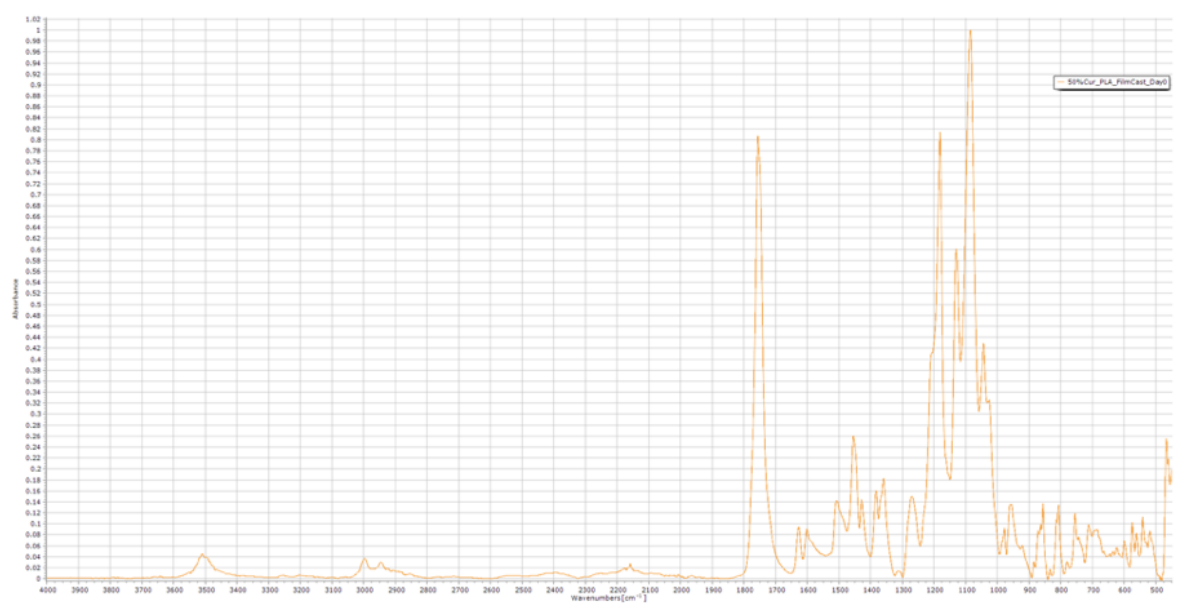


Figure 37. ATR of film cast sample of 50%Cur_PLA on day 0.

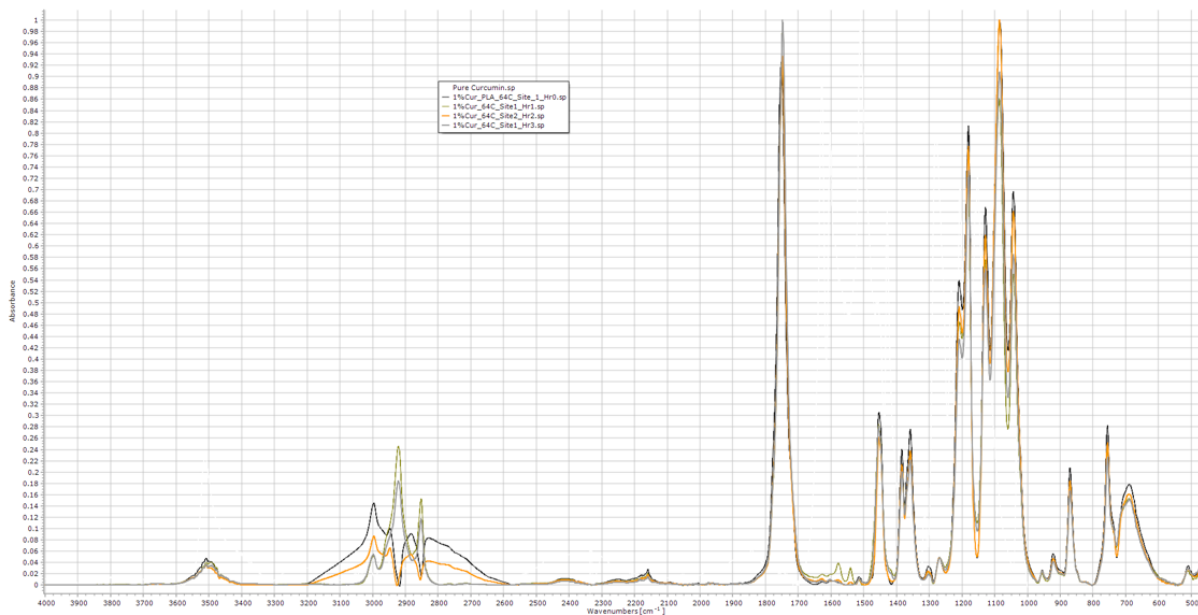


Figure 38. Averaged ATR of the release at 37 degree Celsius for 1, 2 and 3 hours of heat pressed sample for 1%Cur_PLA at 64 degrees Celsius at 3 different sites with pure curcumin as a background reference.

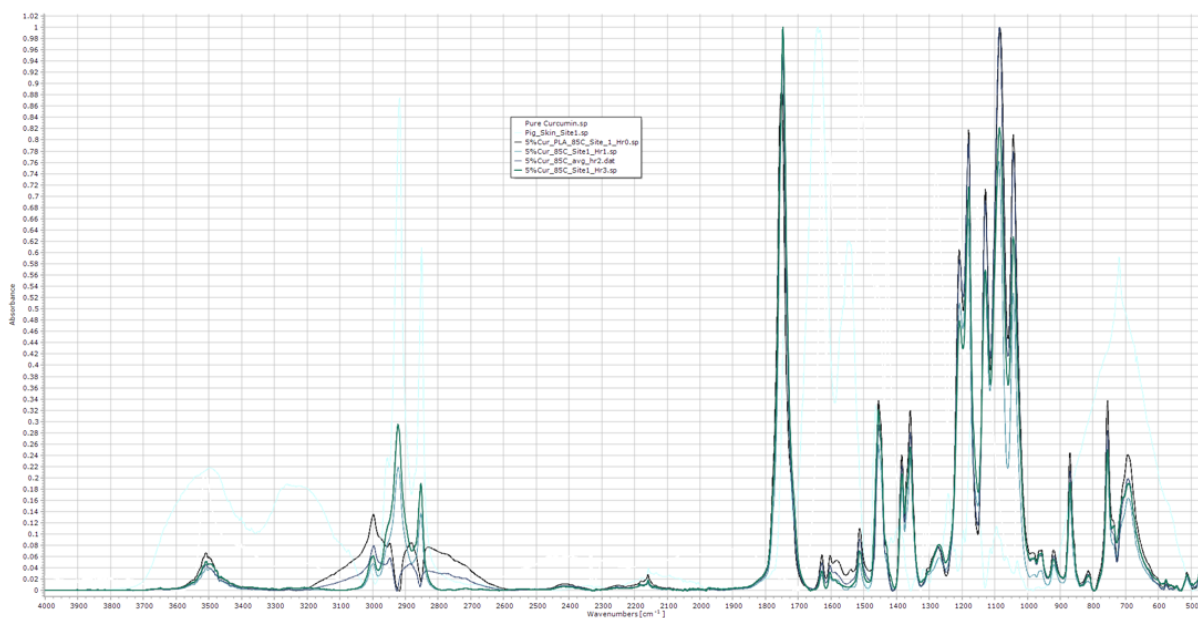


Figure 39. Averaged ATR of the release at 37 degree Celsius for 1, 2 and 3 hours of heat pressed sample for 5%Cur_PLA at 85 degrees Celsius at 3 different sites with pure curcumin and pig skin as a background reference.

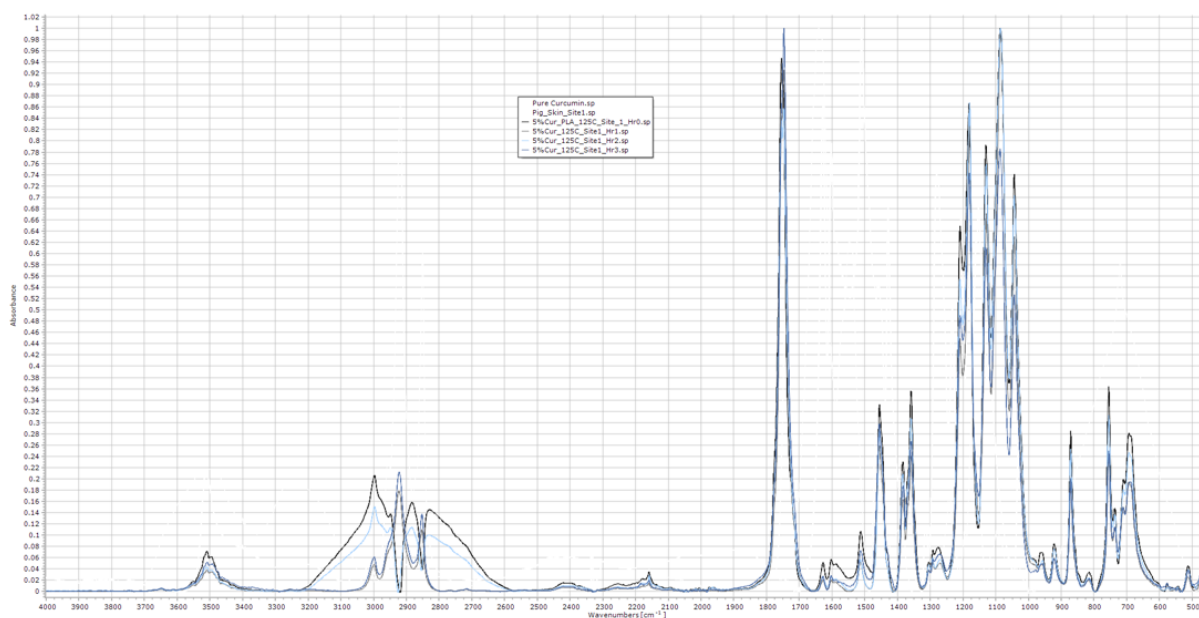


Figure 40. Averaged ATR of the release at 37 degree Celsius for 1, 2 and 3 hours of heat pressed sample for 5%Cur_PLA at 125 degrees Celsius at 3 different sites with pure curcumin and pig skin as a background reference.

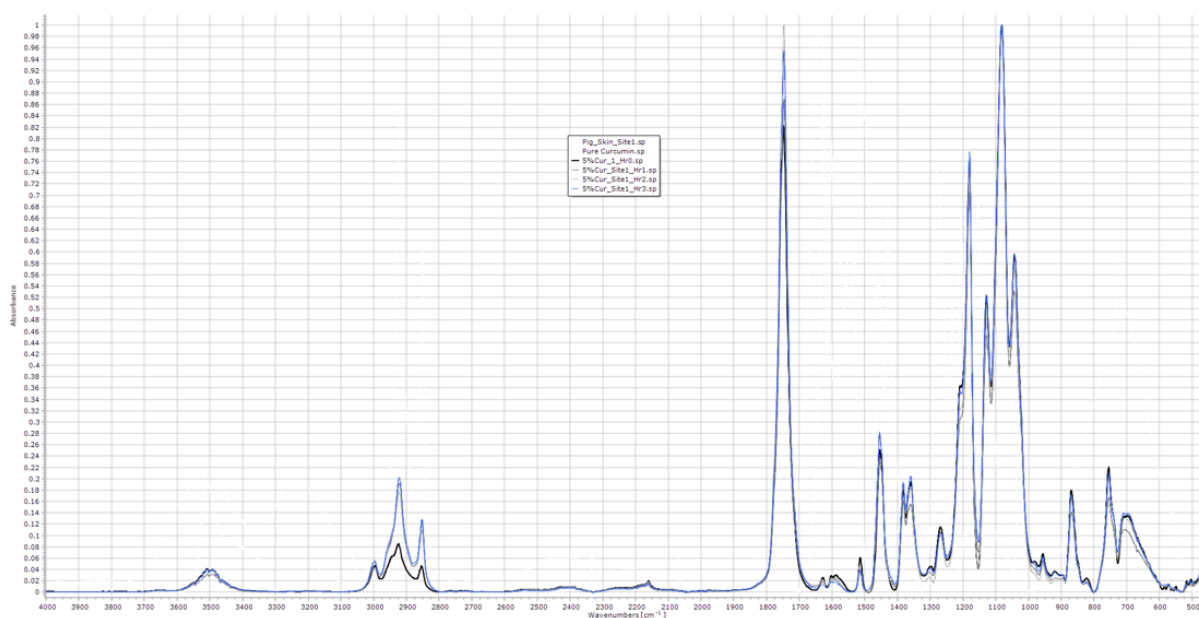


Figure 41. Averaged ATR of the release at 37 degree Celsius for 1, 2 and 3 hours of film cast sample for 5%Cur_PLA at 3 different sites with pure curcumin and pig skin as a background reference.

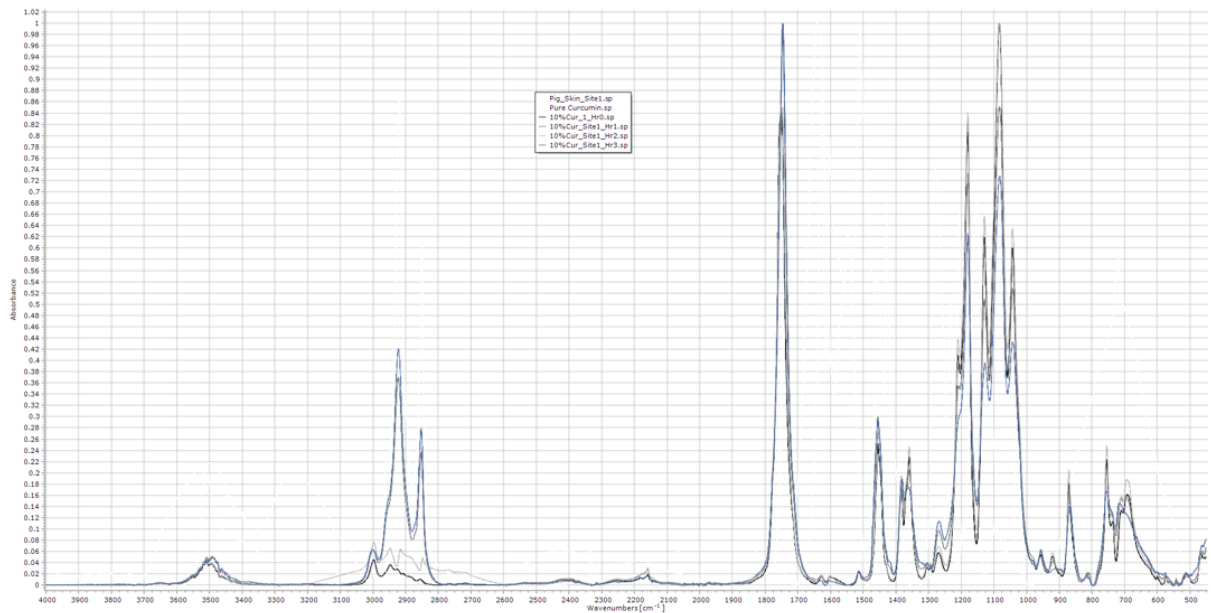


Figure 42. Averaged ATR of the release at 37 degree Celsius for 1, 2 and 3 hours of film cast sample for 10%Cur_PLA at 3 different sites with pure curcumin and pig skin as a background reference.

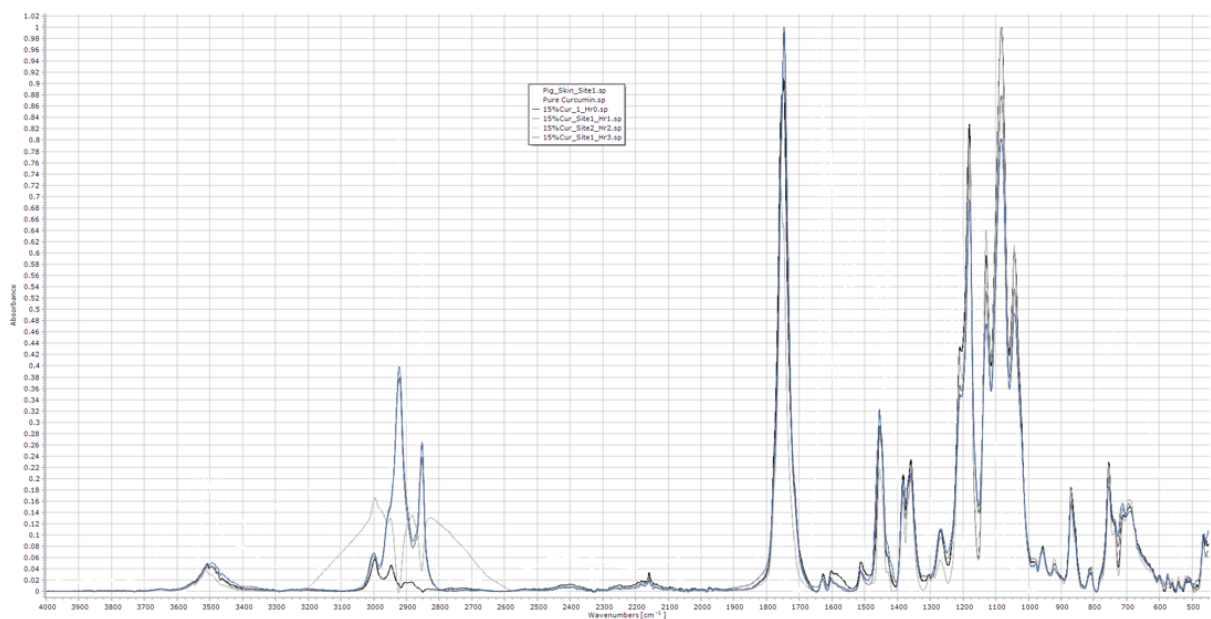


Figure 43. Averaged ATR of the release at 37 degree Celsius for 1, 2 and 3 hours of film cast sample for 15%Cur_PLA at 3 different sites with pure curcumin and pig skin as a background reference.

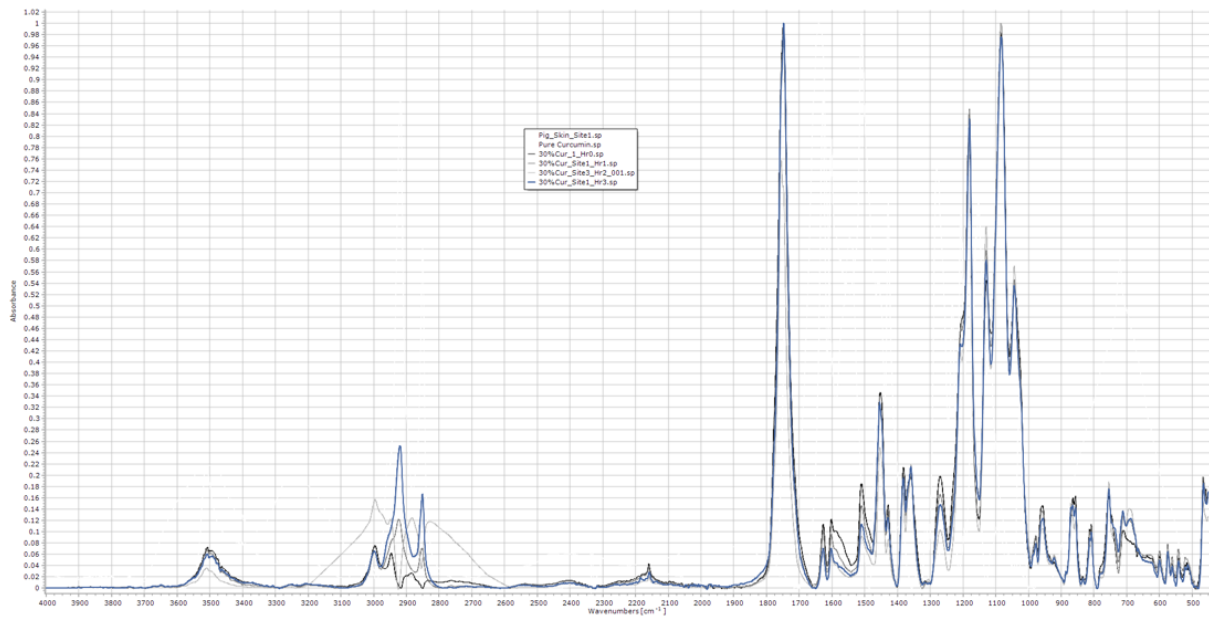


Figure 44. Averaged ATR of the release at 37 degree Celsius for 1, 2 and 3 hours of film cast sample for 30%Cur_PLA at 3 different sites with pure curcumin and pig skin as a background reference.

Discussion and Conclusion

I did not do analysis on the percentage of mesophase formed on each sample yet. The main purpose of the PLM, ATR and XRD was to give a general look on the change of states at various conditions for the specific combination of curcumin and PLA.

I found that curcumin at lower concentrations (1%-4%) interfered the formation of the clover-shaped spherulites in film casted samples that naturally 100%PLA_FC would form. I consider this as the secondary bond interaction between curcumin molecules and PLA polymeric chains, an interaction that prevents PLA from creating homogenous nucleation locally and crystallize. At the same time, concentration of 5% curcumin and above contributed to the crystallization of PLA to a rod-shaped structure, and curcumin tended to phase separate out from the PLA crystals. This phenomenon became more pronounced from 2% to 4% curcumin loading due to the occurrence of phase separated curcumin crystals in red-orange color, though rod-shaped PLA crystals remained unobservable. Heat pressed samples revealed the possibility that curcumin might played a role as thermal stabilizer since lower concentrations of curcumin yielded less crystalline in both PLM and XRD compared to higher concentrations of curcumin at ascending temperature.

In the pig cadaver skin release study, 5%Cur_PLA_85C, 5%Cur_PLA_125C, 5%Cur_PLA_FC, 30%Cur_PLA_FC showed a decrease of ATR intensity after time 0 from wavenumber 1550 to 1700, reflecting the potential release of curcumin on the surface region.

The PLM captures of 1% and 2%Cur_PLA_filmcast samples looked distinct from those of the rest of others, seeing the fact that the existence of the clover-shaped spherulites that were visible under the polarized light. At the same time, Figure 6i and 7i both showed a

distinguishable void of darkness, reflecting the fact that a great portion of the films may compose of amorphous state. However, after a 5000lbs-5min press at room temperature, 1%Cur_PLA_FC became a tablet and XRD on Figure 6 (1%Cur_99%PLA_ColdPress_bg sub) showed a much higher peak, representing the transition of morphology from largely amorphous to semi-crystalline. During the transition, mesophase may have occurred. The main difference of film casts between 1%Cur and others was that orange clusters could be observed among all samples except the 1%Cur. On Figure 3, every magnification of any percentage of curcumin would show a trace of small orange dots among the crowd of spherulites. This phenomenon might indicate that curcumin could not be completely dissolved with concentration higher than 2% in PLA using dichloromethane. Or it could also mean that curcumin had a greater chance of phase separation from PLA above 2% in concentration during the air drying/film casting process. From both XRDs and PLMs of 100%PLA_Filmcast, 1%, 2%, 3%, and 4%Cur_PLA_Filmcast, we can observe a trend: 100%PLA had the highest and broadest x-ray intensity and the most numbered clover-shaped spherulites; the x-ray intensity and the number of clover-shaped spherulites decreased with the increase of curcumin concentration within PLA films. Hypothetically, curcumin with less than 5% concentration within PLA film casts plays a role of prohibiting the nucleation of PLA spherulites, while the higher the percentage (closer to 5%), the more phase separation of curcumin and PLA by themselves would occur.

Roy et al. has claimed that the strength and thermal stability of PLA may be increased by mixing with increasing amount of curcumin (Roy). This might explain a fact that when curcumin concentration increased to higher than 30%, the film became tougher when processing (like punching, tearing, and packaging) than the 10% and 15%. However, 1%-5% film casted samples showed a completely different texture and higher toughness/flexibility than others (visually and

sensually). Threshold concentration of curcumin in PLA can be the ultimate explanation for why such trend would occur. I believe that 5% curcumin is such concentration that determines the hindrance or the arise of increasing strength when mixing with PLA. Hopefully, experiments can be done in the future to measure the exact tensile strength of each PLA film mixed with a certain concentration of curcumin.

When we move on to the first pressed (at room temperature, 5000lbs./5min) samples, the first thing worth noticing is that 100%PLA was no longer highly crystallized based on Figure 9 and Figure 4i. However, 1%, 5%, 7.5%, 10%, and 15%Cur_PLA_FP all showed a rapid increase in XRD intensities. This phenomenon highly correlates to the fact that curcumin increases the structural stability when exerted with a great amount of pressure.

Heat press

Figure 11 and 13 are the horizontal comparisons between the XRD trends of 1%Cur_PLA and that of 10%Cur_PLA heat pressed samples at same temperature and pressure conditions (64, 85 and 125 degrees Celsius, 20klbs., 5 minutes). 1%Cur_PLA heat pressed samples revealed a significant increase in XRD intensity (dominantly PLA peaks) from 120 at 64 degrees Celsius to 1800 at 85 degrees Celsius to 7300 at 125 degrees Celsius. However, though 10%Cur_PLA (one with 10 folds higher curcumin loading than 1%Cur_PLA) increased from 1750 at 64 degrees Celsius to 3400 at 85 degrees Celsius, it stayed 3400 at 125 degrees Celsius. This observation also supports the idea that curcumin helps to stabilize PLA from completely melting and recrystallize when the concentration becomes higher. I am not yet sure about the threshold concentration of curcumin that can induce such thermal stabilization effect. In the future, DCS analysis for more precise readings needs to be conducted.

By comparing Table 1, the PLM captures of the heat pressed samples of the multiple curcumin concentrations at 64, 85 and 125 degrees Celsius, there was some trend worth noticing. Firstly, the transition of coloration and texture from 64 to 125 degrees Celsius of 1%Cur_PLA appeared relatively comparable with those of 100%PLA. The greenish color that occurred with all 100%PLA and 1%Cur_PLA samples from 64 to 125 degrees Celsius heat press disappeared with 5%Cur_PLA, 10%Cur_PLA, and 15%Cur_PLA under any thermal conditions. Since 100%PLA showed such greenish color, I suppose that this represented the induction of PLA's semi-crystalline states. The reason why it disappeared with 5%Cur_PLA, 10%Cur_PLA, and 15%Cur_PLA might be that lower concentration of PLA with higher concentration of curcumin had a longer move-free state during the cooling stage, making it recrystallized less than 1%Cur_PLA and 100%PLA. As a result, the new semi-crystalline states could not form, leaving themselves with rod-shaped crystals. This phenomenon can also be explained by the induction of different semi-crystalline states due to the difference between spherulites formed with 1%Cur_PLA_FC and other curcumin concentrations.

Pig cadaver skin release

I have conducted two separate pig cadaver skin release studies. The reason I called the first one a pre-experimental study is because we did not understand anything about the release characteristics of any curcumin/polymer mixture, either relatively fast due to chemical and biological similarities between the skin oil and amino acids and curcumin's hydrophobic groups, or relatively slow due to the low biodegradability of PLA, or simply no release due to the way that curcumin interact with / got trapped in the polymer. As a result, we tested the possibility of

release by employing Cur/PLA film casted and heat pressed samples that were random in size and shape. From Figure 34 to 37, 100%PLA_FC, 5%Cur_PLA_FC, 5%Cur_PLA_FP, 10%Cur_PLA_FC, 15%Cur_PLA_FC, 30%Cur_PLA_FC, and 50%Cur_PLA_FC samples were analyzed with ATR, some just for reference (100%PLA, 10%Cur_PLA_FC, 15%Cur_PLA_FC, and 50%Cur_PLA_FC), some for horizontal comparisons (5%Cur_PLA_FC and 5%Cur_PLA_FP). The 100%PLA ATR curve was used as a reference for the peaks of curcumin existed in other samples. The peaks occurred from 1550 to 1700 wavenumber represented trace of curcumin, and that was why 100%PLA did not reveal such peak. But in the second part of the pig cadaver skin release study, I realized that FTIR for pure curcumin should also be included as a more detailed background reference, and that was why I added both 100%PLA and pure curcumin IR spectrums for the analysis. Based on the data from Day 0 to Day 8 study of 5%Cur_PLA_FP and from Day 0 to Day 4 study of 30%Cur_PLA_FC, no obvious decreased in the ratio between curcumin peak and the highest (PLA) peak after normalization.

One main reason we did not see any solid sign of release was that the temperature at which the release study took place was -20 degrees Celsius. We did not test the release at body temperature in the first place because at that point we had not planned out how to set up a platform that would allow us to keep the skin at the optimum temperature steadily without drying up (due to lacking an incubator that did not require a good level of sterilization). Also, we only hoped to see just a faint amount of release at -20 degrees Celsius because if so, there would be very likely a prominent release at body temperature due to greater polymer chain mobility at higher temperature (Suryanegara).

Since we did not observe any reliable sign of release at -20 degrees Celsius, the completely new release study was then designed to further explore the possibilities of release at 37 degrees

Celsius. Figure 38 to 44 illustrate the surface composition of 1%Cur_PLA_64C, 5%Cur_PLA_85C, 5%Cur_PLA_125C, 5%Cur_PLA_FC, 10%Cur_PLA_FC, 15%Cur_PLA_FC, and 30%Cur_PLA_FC before and after 1, 2, and 3 hours of release at 37 degrees Celsius on the pig cadaver skin, with ATR of clean pig cadaver skin (before contact with drug/polymer), 100%PLA, and FTIR for pure curcumin/KBr as background reference. This time, I measured 3 different and random sites on each sample for every hour of release and calculated them to an averaged fit using Spectragryph. 5%Cur_PLA_85C, 5%Cur_PLA_125C, 5%Cur_PLA_FC, 30%Cur_PLA_FC all showed a decrease after time 0 from wavenumber 1550 to 1700, reflecting the potential release of surface curcumin. The reason why 1%Cur_PLA_64C showed a completely different trend at the curcumin region on ATR curves could be that most of the curcumin was trapped inside the pressed tablet, and it was till the second hour the substrate on the pig cadaver skin extracted the drug out of the inner layer to the surface. But it could also just be an averaged measurement for a specific region that contained higher curcumin concentration on the tablet. The better illustration of a constant release is the 5%Cur_PLA_85C, which the intensity kept decreasing with the increase of time of contact with the pig cadaver skin. Though the curves were all normalized to 1, it is still recommended to calculate the peak-to-peak ratio between curcumin and PLA ATRs in the future for a more precise mathematical analysis on the release.

In the future studies, I highly recommend that the antibiotic effect of PLA + Curcumin can be tested, since drug release was observed on the pig cadaver skin at body temperature. Also, the bio-adhesion property of PLA with curcumin shall be studied at the same time. Both projects would give an idea if PLA can be used as a carrier polymer for antibiotic/wound healing transdermal patch.

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