



out,<sup>1</sup> keeping a high level of crystallinity is important for the mechanical performance of the material. However, PLLA that is too crystalline can yield a brittle product, and so sometimes stereodefects are intentionally incorporated to improve the polymer's mechanical properties. In any case, the degree of stereochemical purity in the polymer product is important for commercial applications, and characterizing the stereochemical properties of PLA is critical.<sup>2</sup>

In this experiment, you will perform the ring-opening polymerization of L-lactide with various amounts of added racemic lactide added. (Racemic lactide is a statistical mixture of 50% L-lactide and 50% D-lactide.) Incorporation of *meso*-lactide and D-lactide will create single and double stereodefects in the polymer that can be quantified by a variety of analytical techniques. At the low fractions of *rac*-lactide you will be using, one would imagine that most of these would be isolated double *R*-defects, and extremely rare single *R*-defects, in an excess of *S*-polymer.

SSSSSSSSSSSS

PLLA  
homopolymer

SSRSSSSSSRS

single stereodefects:  
contaminated with  
*meso*-lactide

SSSSRRSSSSSS

pairs of stereodefects:  
contaminated with  
*D*-lactide

Both types of stereodefects shown above are observed in commercial PLLA polymer. There are also a few complexities to the polymer stereoregularity keep in mind:

1. Transesterification (chain transfer to polymer) processes can randomize the as-synthesized polylactide chains, thus redistributing the stereochemical defects.
2. The “copolymerization” of these two lactide monomers probably isn't an ideal random copolymerization ( $r_1 = r_2 = 1$ ), and therefore the defects may not be distributed randomly.
3. Some polymerization catalysts lead to epimerization of the chiral centers, thus randomizing their absolute configuration.

So, the stereoregularity of the polymer product is not solely determined by the stereochemistry of the monomers used. Nevertheless, in your polymer, the number of defects should be roughly dictated by the amount of racemic lactide impurity added.

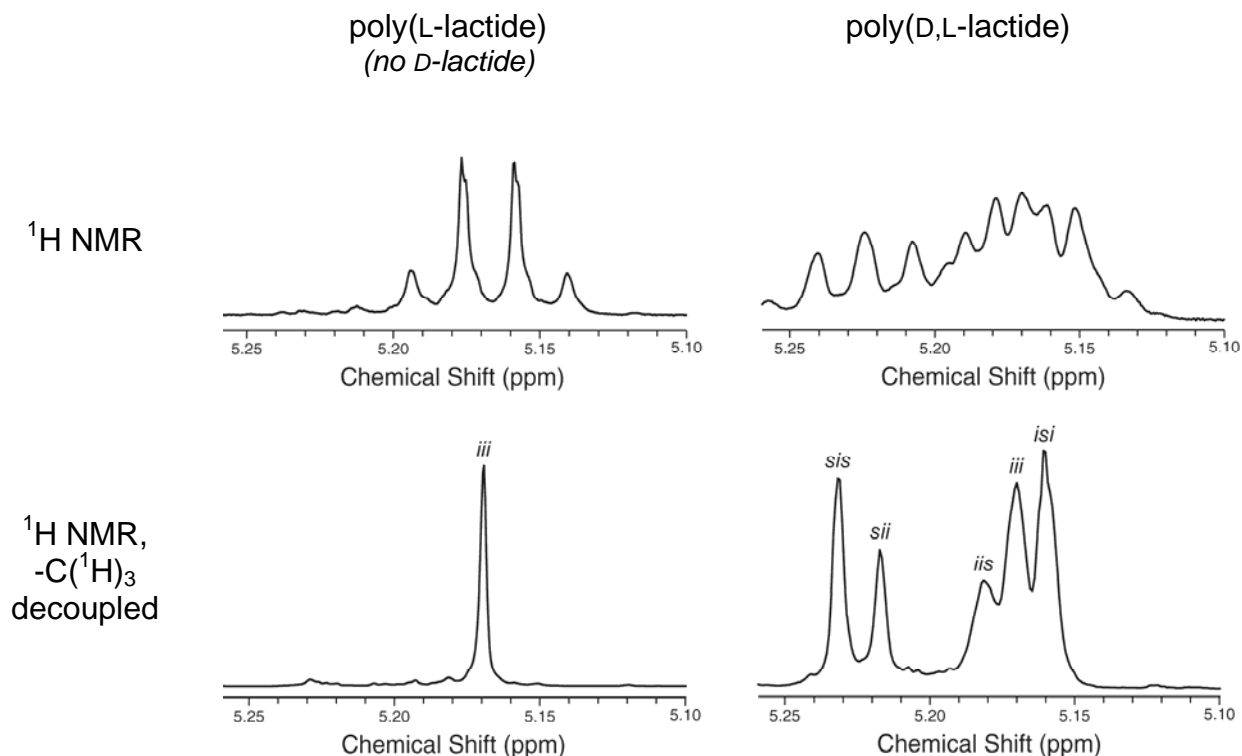
<sup>1</sup>H NMR can be used to quantify the number of *R* defects in a nearly all-*S* polymer, because local variation in tacticity results in measurable variation in chemical shift.<sup>3</sup> The  $\alpha$  (CH) protons of polylactide give rise to <sup>1</sup>H resonances near  $\delta = 5.2$  ppm. In stereopure, poly(L-lactide), all  $\alpha$ -protons are identical, and the <sup>1</sup>H NMR shows a single quartet. By contrast, poly(D,L-lactide) contains multiple  $\alpha$ -protons with different stereochemical neighbors, and each of these protons gives rise to a unique signal in the <sup>1</sup>H NMR. Researchers at Cargill and the U of M determined that the local monomer sequences *SRRS* and *RSSS* showed characteristic resonances at  $\delta = 5.23$  and 5.22 ppm, whereas other sequences, including the stereopure *SSSS* sequence, appear together

<sup>1</sup> Drumright, R. E.; Gruber, P. R.; Henton, D. E. *Adv. Mater.* **2000**, *12*, 1841.

<sup>2</sup> Inkinen, S.; Hakkarainen, M.; Albertsson, A.-C.; Södergård, A. *Biomacromolecules* **2011**, *12*, 523.

<sup>3</sup> Odian, G. *Principles of Polymerization*; Wiley: New York, 1991; chapter 8.

near  $\delta = 5.17$  ppm.<sup>4,5</sup> In tacticity notation, sequences are named based on whether monomer dyads are isotactic (*i*) or syndiotactic (*s*), as shown at right; this means that the signals at  $\delta = 5.23$  and 5.22 ppm correspond to *sii* and *sis* sequences, respectively. These multiple quartets overlap in the <sup>1</sup>H NMR, and that makes it difficult to integrate (and quantify) each signal independently. This can be addressed by <sup>1</sup>H-decoupled NMR, in which the coupling from one type of proton is “turned off” by selectively irradiating that resonance. For polylactide, selectively irradiating the -CH<sub>3</sub> group dramatically simplifies the <sup>1</sup>H NMR spectrum of the  $\alpha$ -protons, and makes it possible to see each stereochemical sequence as a single peak.



<sup>1</sup>H NMR spectra of polylactide polymers containing only L-lactide (left) and containing both L-lactide and D-lactide (right), focusing on the  $\alpha$  (CH) proton.<sup>2</sup>

You will be integrating signals in the <sup>1</sup>H-decoupled NMR to determine the frequency of these different signals, and will use this information to determine the amount of D-lactide and *meso*-lactide contaminant in your polymers.

In this lab, you will also study the effects of stereodefects on the crystallization of PLLA. Pure PLLA is a highly crystalline polymer, but increasing amounts of *R*-stereocenters inhibit the crystallization of the polymer. This effect can be observed by measuring the amount of heat

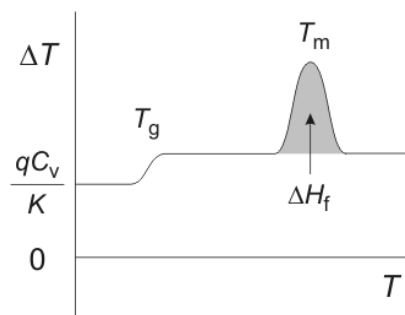
<sup>4</sup> Thakur, K. A. M.; Kean, R. T.; Hall, E. S.; Kolstad, J. J.; Lindgren, T. A.; Doscotch, M. A.; Siepmann, J. I.; Munson, E. J. *Macromolecules* **1997**, *30*, 2422.

<sup>5</sup> Thakur, K. A. M.; Kean, R. T.; Hall, E. S.; Doscotch, M. A.; Munson, E. J. *Anal. Chem.* **1997**, *69*, 4303.

required to melt the crystalline polymer; the more heat that is required, the more crystalline the polymer is. In PLLA, stereodefects have also been shown to depress the crystalline melting temperature ( $T_m$ ). You will be performing this thermal measurement by differential scanning calorimetry (DSC), an instrumental technique that measures heat flow into a material.<sup>6</sup> DSC is an extremely common technique for measuring the temperature and enthalpy of phase transitions in materials. In the case of polymers, DSC can be used to determine glass transition temperatures ( $T_g$ ) and crystallization/melting temperatures ( $T_c$  and  $T_m$ ). At any temperature where no phase change is occurring, the flow of heat into a material (sometimes labeled  $\Delta T$ ) is determined by the material's heat capacity ( $C_p$ ), the heating rate ( $q$ ), and an instrumental calibration factor ( $K$ ):

$$(6.3) \quad \Delta T = \frac{qC_p}{K}.$$

Over most temperature ranges the DSC instrument measures a constant heat flow because heat capacity remains constant. A glass transition is, by definition, a phase change which alters the heat capacity of the material but does not have a transition enthalpy. So, at a material's  $T_g$ , the DSC trace shows a first-order inflection. However, at a material's crystalline melting temperature ( $T_m$ ) the DSC instrument must overcome an enthalpy of fusion ( $\Delta H_f$ ), which results in a second-order peak in the DSC trace. The area underneath the peak corresponds directly to  $\Delta H_f$  for the sample.



Because most crystalline polymers are actually only partially crystalline, they display only a fraction of the theoretical maximum  $\Delta H_f$ . The degree of crystallinity in a polymer sample can be calculated by comparing the  $\Delta H_f$  measured by DSC with the maximum  $\Delta H_f^0$  obtained by other (indirect) methods.

Components of a DSC trace. The trace above would be obtained in the heating direction; in the opposite, cooling direction,  $\Delta T < 0$  and transitions have opposite signs.

$$(6.4) \quad \text{degree of crystallinity} \approx \frac{\Delta H_f}{\Delta H_f^0}$$

For PLLA, a number of values for  $\Delta H_f^0$  have been reported.<sup>5</sup> In this lab, you will use the value  $\Delta H_f^0 = 93 \text{ J/g}$ .

You will also be examining the effects of stereodeflect on the crystallization of PLLA using polarized optical microscopy.<sup>7</sup> In polarized optical microscopy, a polymer sample is imaged

<sup>6</sup> For a brief introduction to DSC, see Macrogalleria's DSC webpage, (<http://www.pslc.ws/macrog/dsc.htm>). For a longer one, see Menczel, J. D.; Judovits, L.; Prime, R. B.; Bair, H. E.; Reading, M.; Swier, S. "Differential Scanning Calorimetry", in *Thermal Analysis of Polymers: Fundamentals and Applications*; Menczel, J. D., Prime, R. B., Eds.; John Wiley & Sons: New York, 2009; chap. 2.

<sup>7</sup> For more information on polarized light microscopy, see MicroscopyU's interactive webpage (<http://www.microscopyu.com/articles/polarized/polarizedintro.html>). You may also want to check out the other pages at the MicroscopyU site to learn about materials microscopy.

between two polarizing films. When the polarization axes of the two films are oriented at a 90° angle to one another, the illuminating light cannot ordinarily pass through to the eyepieces or camera. However, if the material between the crossed polarizers exhibits some order, birefringence in the sample will cause the light to pass. As a result, growing spherulites of ordered, crystalline material can be distinguished from the surrounding disordered melt by characteristic bright and dark patterns against a dark background.

The growth of these crystalline spherulites can be imaged by time-lapse digital photography. The rate of growth ( $G$ ) of a spherulite is defined as  $dr/dt$ , where  $r$  is the radius of the spherulite. A plot of  $r$  vs.  $t$  usually gives a linear plot with slope  $G$ ,<sup>8</sup> until the measured spherulites grow into one another. There are two conflicting influences on how  $G$  varies with temperature: viscosity and undercooling. The viscosity of the sample determines how quickly molecules can organize themselves at the crystalline front, and because viscosity increases with decreasing temperature, low temperatures slow crystallization. However, the difference between the sample temperature and the crystallization temperature ( $T_c - T$ ), or “undercooling”, thermodynamically drives the growth of crystals. In this case, temperatures that are too high ( $T_c \approx T$ ) also suppress crystallization. As a result of these two competing effects, the rate at which polymer crystals grow is usually maximized tens of degrees below  $T_c$ . Stereodefects will also impact the rate at which PLA crystallizes from the melt. You will image the effect of stereodefects on crystallization kinetics for your PLLA in this lab, by imaging the growth rate of spherulites for different materials on a temperature-controlled microscope stage.

## Experimental

### Polymerization of pure and impure L-lactide (Apr 2/4)

*The goal of this experiment is to synthesize polylactide by ring-opening polymerization.*

- Check out two small stirbars from the stockroom, and pick up two vials with black phenolic caps from the TA's.
- In addition to polymerizing a sample of pure L-lactide, you will have been assigned a ratio of L-lactide to *rac*-lactide monomer to copolymerize. For each of these samples, weigh out 3 g *total* lactide and place it into a vial. Label each vial appropriately.
- Use a microsyringe to add 0.1 mL of a 0.2 M solution of tin 2-ethylhexanoate initiator in methylene chloride to each vial.
- Place the vials in the oven, set at 170 °C, *without their caps* for 3 minutes. By this time the methylene chloride should have evaporated and the lactide mixture should melt. If not, you may put the vials back in for 2 more minutes.
- Using tongs or gloves, remove the vials from the oven. Tightly cap the vials and then shake them vigorously for 30 seconds. (This helps disperse the tin.) Place the capped vials back into the oven at 170 °C and leave them for 1 h. The polymerization is sensitive to

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<sup>8</sup> “Nucleation and Growth Kinetics: Measurements”, in Schultz, J. M. *Polymer Crystallization*; Oxford University Press: New York, 2001; chapter 7.

temperature, so try to follow TA's instructions on keeping the oven door closed and on placement of your vial in the oven. Ideally, everyone should place their polymerizations in the oven and remove them at the same time.

- Remove the vials from the oven and let them cool at room temperature for 5 minutes. Open the vials and add 5 mL chloroform and a small magnetic stir bar to each. VERY loosely recap the vials and place them on a magnetic stirrer/hot plate. VERY gently warm the mixture to dissolve the polylactide. You will need to rapidly stir the mixture until the entire polymer sample has dissolved. This can take up to 30 minutes.
- Let the solution cool back to room temperature and precipitate the polymer by pouring it into a beaker containing 30 mL methanol. If you see solid material remaining in your vial, repeat the previous step with 10 mL more chloroform. Recover the polymer using a Büchner funnel vacuum filtration apparatus. Dry the material as best you can on the filter, and measure the yield of your product.
- Put 0.2 g of each polymer into a separate aluminum pan and melt the samples using a hot plate. Be careful not to touch the polymers with your fingers. Once the polymer samples have melted, check to make sure that the normal oven has been set to 100 °C, and put your pans into the oven for 30 minutes to anneal. After annealing, save these samples separately from your bulk material for next week's DSC experiments.
- Dry the remainder of your polymers in labeled aluminum pans in the vacuum oven. You will pick these samples up next week.

### **<sup>1</sup>H-NMR spectroscopy of polylactide: Determining tacticity (Apr 9/11)**

*The TA's will collect ordinary and <sup>1</sup>H-decoupled NMR spectra for each of your two polymer samples. One consequence of the <sup>1</sup>H-decoupled NMR experiment is that all useful information in the irradiated,  $\delta = 1.6$  ppm region is lost. If you see anything at  $\delta = 1.6$  ppm in the <sup>1</sup>H-decoupled NMR spectrum, ignore it.*

- Check out two NMR tubes from the stockroom.
- Prepare <sup>1</sup>H-NMR samples by dissolving 30 mg of PLA in 1 mL of CDCl<sub>3</sub>. It is important to have a low concentration to control the broadening of the peaks in the NMR spectrum due to increased solution viscosity.
- A TA will collect traditional and decoupled <sup>1</sup>H NMR spectra for of each of your PLA samples, and the data will be available for FTP transfer and processing as in Lab 2.

### **Differential scanning calorimetry (Apr 9/11 or 16/18; see schedule)**

**Caution:** Never touch the PLA samples or crucibles with your hands. Oils from your fingers will alter the results of the DSC analysis. Use tweezers and a razor blade to hold and cut your sample.

- Remove one pan and one lid for each of your three polymer samples from the container labeled "hermetically sealed". Record the mass of the pan and lid before adding any polymer. Record the masses in your laboratory notebook. Carefully load the pan with about 5 mg of your polymer sample. Try to make sure that no polymer is on the lip of the pan.
- Carefully place the lid on the pan, and crimp the pan using the press next to the balance. Press the pan first right side up, and then upside down. The TAs will assist you with this process. It is important that the pan is well sealed, so that the polymer does not melt and leak out into the heating/cooling chamber.
- Place your sample pans into the auto-sampler, noting its position number. On the computer that controls the DSC, insert all pertinent information for your three samples. Make you're the output filenames include your group number and the polymer identity.
- The DSC will expose your sample to three temperature ramps: from room temperature to 200 °C, back to room temperature, and then up to 200 °C again. The TA will program the instrument with the following temperature ramp instructions:
  - Step 1:* Equilibrate at 40 °C.
  - Step 2:* (Fill cooling unit if level is below 25%.)
  - Step 3:* Equilibrate at 25 °C.
  - Step 4:* (Set sampling interval to 1 sec/point.)
  - Step 5:* Keep isothermal for 1 min.
  - Step 6:* Ramp from 25 °C to 200 °C at a rate of 10 °C/min.
  - Step 7:* Keep isothermal for 5 min.
  - Step 8:* (Mark end of cycle 1.)
  - Step 9:* Keep isothermal for 1 min.
  - Step 10:* Ramp from 200 °C to 25 °C at a rate of 20 °C/min.
  - Step 11:* (Mark end of cycle 2.)
  - Step 12:* Keep isothermal for 1 min.
  - Step 13:* Ramp from 25 °C to 200 °C at a rate of 10 °C/min.
  - End.*
- The data that is generated will be posted to the course Data website, as individual files. You will analyze your files using TA Instruments' Advantage software package; instructions for using this software are in Appendix A of these instructions.

### **Polarized optical microscopy (Apr 9/11 or 16/18; see schedule)**

*You will be performing this experiment in groups of four. The group should collect a complete set of variable-temperature images on one PLLA sample, and then collect as much data as possible on both optically impure samples.*

- The TA will help you familiarize yourself with the polarizing microscope. Make sure you can identify the polarizer, analyzer, condenser, and objective. In addition, make sure you

understand how to control the temperature of the hot stage, and how to insert a sample into the viewing area.

- You will be using a program called TSView to save images. Load a sample of crystalline imidazole onto the stage. Then, on the computer, make sure that the program is open, and shows an image. Focus the microscope while watching the computer screen. (Sometimes the focal plane of the camera is slightly different from the focal plane of the eyepieces.) *Notice that the area imaged by the camera is smaller than the view in the eyepieces; as a result, sometimes it helps to use the eyepieces for rough positioning of features into the camera's view.* Rotate the sample stage to see the effect of polarizer and analyzer angle on the features of the crystal.
- In TSView, press the **Snap** button to take a picture. In the dialog box that pops up, type in the name you want to give the image file.
- With the heat stage temperature set near room temperature, place the stage micrometer in the stage, in the viewing column. Remove the analyzer so that you can see the micrometer. (It isn't polarizing, so you can't see it if you don't.) The micrometer slide has a set of ruled lines that you will use to calibrate distances in your other images. Focus on these lines in the eyepieces, and then center and straighten them (horizontal/vertical) in the camera view. Take a picture.
- Sprinkle a small amount of your filtered PLLA onto the last third of a 3" x 1" microscope slide, and then cover this with another slide. Be careful to keep the slides as free of dust as possible; you can help this by wearing gloves while you prepare your samples, and by keeping the box of unused slides closed.
- Set the hot stage to 120 °C. Wait until the stage reaches the set temperature before continuing.
- Heat your sample slide on a hot plate, pre-set to ~190 °C, in order to melt the polymer solid. A minute or so after you see your material melt, press down on the hot cover slip with something other than your finger to spread the liquid material.
- Immediately slip your slide sandwich into the hot stage on the microscope. As your material cools, move your sample around and try to find spherulitic nuclei in your sample using the eyepieces. As soon as you center a few spherulites nucleating in the TSView window, focus as best you can, and immediately take an image. Continue to take images in TSView every 20 seconds or so, and record the run time of each image. If you don't ever observe spherulites, make another sample and try again. If you still don't observe spherulites at this point, cool the stage to 90 °C (instead of 120 °C), make another sample, and try again. If you don't observe spherulites at this point, it may be because your polymer does not crystallize.
- Continue to collect images until the spherulites have grown into one another. Even if one side of a spherulite has stopped growing, you can always use a different direction to determine the radius, so keep imaging until nothing is growing.
- Repeat these instructions for all of the group's samples. (You may want to use different filename prefixes for your different runs.)
- Once you are finished, save your files to a flash drive.



### *Image analysis:*

- In order to obtain radii  $r$  for your growing spherulites, you will have to open your image files in an image analysis program that allows you to make diagonal distance measurements. The image viewer that comes with Microsoft Windows won't do this, but the NIH-supported freeware program ImageJ will. There are a number of ways you can use ImageJ. The program is available in the Microcomputer Lab in Smith 101D; you can download it to your computer <http://rsb.info.nih.gov/ij> as a resident executable; or you can use it within your web browser, either as a signed applet (<http://rsbweb.nih.gov/ij/signed-applet/>) that runs in a separate window, or embedded within the browser (<http://imagej.sourceforge.net/applet.html>). If you choose to run ImageJ on your own computer, it will need to run the latest version of Java as well (<http://java.com/getjava>). You can also do measurements in image files with commercial image editing programs such as Adobe Photoshop. The instructions below are written for ImageJ.
- First, open the image of your stage micrometer in ImageJ. In the ImageJ Toolbar, select the Straight Line tool. Click-and-drag a line between two of the micrometer lines. Information about your line, including angle and length in pixels, are located underneath the Toolbar, or you can select *Analyze* → *Measure* to get the information in a separate window.
- Calculate a conversion factor to turn micrometer image pixels into real  $\mu\text{m}$ .
- Using the same Straight Line tool, you should now be able to measure spherulite radii  $r$  in  $\mu\text{m}$  for each of the images you collected. Try to pick the center point of each spherulite as consistently as you can, and you may want to make multiple measurements for different spherulites in each image for accuracy.

### **Lab Writeup (due *In Lecture*, April 29)**

For this report, you will be responsible for providing both a structural and a logical framework for your experiments—meaning your report should not only have all the appropriate sections and data, but it should also say something about stereodefects in polylactide and what effects those defects have on the spectroscopic, optical and thermal properties of PLA. You will probably find some conclusions on these subjects in the references given in these instructions—does your data agree? What characteristics of crystalline polymers are your results consistent with?

Be particularly critical of your Experimental section—would someone who did not have my instruction sheet be able to reproduce your experiments based on your description alone? However, do not simply copy the Experimental, as there are many details that aren't described there.

In your report, make sure to include the following:

- NMR data, both coupled and decoupled. What does the decoupled spectrum tell you about the tacticity of your polymer?
- DSC data. Show traces, and label those traces with  $T_g$  and  $T_m$  values, if you observed them. Make sure your labels are readable.

- Polarized microscope data. Show at least three images from one of your time series to demonstrate what the growing crystallites looked like; make sure these images include scale bars. Do different domains in the same experiment grow at the same or at different rates? In addition, graph the growth data for domain growth at different temperatures, if you did that.
- How did stereodeflect impact the crystallization kinetics in your material compared to your pure PLLA? Does this match results from the literature?

I am available for comments on this report, and you are welcome to bring drafts to me anytime up until the due date to ask how I feel about what you've written; I promise to help you improve your manuscript. (You may also want to speak with the writing tutors at the Center for Writing.) Good luck!

## Chem/MatS/ChEn 4223W

### Lab 6 Appendix A

#### Processing DSC Data with TA Instruments Advantage (Thermal)


*It may help to read Assignment 18 before following these instructions.*

In this lab, you will be analyzing your DSC data using Advantage (Thermal), a software package that accompanies the TA Instruments Q1000 DSC. This software has already been installed on computers in the Microcomputer Lab (Smith 101D), but you can also install it on your own computer, as long as it runs a Windows operating system.

*To install the software on your own computer:*

- Go to the TA Instruments Advantage software download page (<http://tinyurl.com/taadv>), and click on “Advantage Software v5.4.0” to download the installation .exe file.
- Run the downloaded file to install the software. You can do this automatically by clicking a check box during the download (“Run this file after download”), or by finding the file on your computer after it is downloaded. During installation, as you follow the prompts, the wizard will give you the choice of two “setup types”: *Complete* or *Custom*. I recommend you choose *Custom*, and then choose only to install “Universal Analysis” in the next window.
- The program will finish by placing “TA Universal Analysis” shortcuts on the desktop and Start menu.

*To use the software to analyze your DSC data:*

- Download your data files from the course website to your local computer. The files are text files, although they may not have “.txt” for a suffix.
- Open the Advantage software. Open one of your data files by choosing *File* → *Open* or the  icon. The program will display information about your sample; if you see anything that needs to be corrected (*e.g.*, the mass of your sample), do it in this window.
- Your DSC thermograms will be displayed in the main window, with the “endotherm up”—that is, with the vertical axis inverted so that negative heat flow values (where heat is put into the system) are up and positive values are down. If your run involves multiple passes, it will show all of them. You can toggle which runs are visible in *Edit* → *Cycle List*. If you are analyzing a peak or feature from a specific pass, it may help to toggle the others off.
- To zoom in on a particular region of the spectrum, select *Rescale* → *Manual...*, and enter the axis boundaries you would like to focus on. To return to the full spectrum, select *Rescale* → *Zoom Out*.
- To analyze a glass transition,
  - Select *Analyze* → *Glass transition*.

- The program will create two red cross cursors in the window that you can click-and-drag, and instructions at the bottom of the window that tell you to “Enter step transition limits”. Place the two cursors on the DSC trace, one far to the left of the transition and one far to the right.
- When you have put the cursors where you want them, right-click in the window, and select *Accept limits*.
- The program will calculate lower and upper bounds for the temperature range of the transition, and then determine  $T_m$  as the inflection point (marked “(I)”).
- If you don’t like the analysis—maybe you set the boundaries in the wrong places, or it obscures other features in the spectrum—select *Edit* → *Delete Results* → *Last*.
- To analyze a melting transition,
  - Choose *Analyze* → *Integrate Peak*. The submenu has four choices related to how the baseline in your graph looks; choose one based on the recommendations in Menczel and Prime’s chapter on DSC (p. 100).
  - The software will give you red cross cursors, and ask you to “Enter baseline limits.” Move the cursors onto the curve, before and after the peak. Once you’ve set them, right-click on the graph and select “Accept limits”.
  - The program will give you  $T_{m,peak}$  (in °C) and  $\Delta H$  (in J/g).
- To transfer your graph to a word processing program, select *Edit* → *Copy Plot*, and then *Paste* the graph directly into your report. Alternately, under *File* → *Export Plot*, you can create a bitmap or metafile file that you can later *Insert* into a document.