Day 0

* If endotoxin free then soak in sodium hydroxide ON
* Make PBS by dissolving 1 tablet/200 mL Dionized water
* Make sure 3% (wt/wt) polyvinyl alcohol (PVA) (in PBS) is made.  PBS tablets are used to create sterile PBS. For every 100 mL PBS, there will be 3 grams of PVA.  Use magnetic stir bar and hot oil bath to quickly dissolve PVA. Otherwise place on hot plate stirrer overnight.

Day 1

1. Weigh out 100mg of polymer in the small scintillation vials (white cap, silicon septa)
2. Add 2 mL of ethyl acetate to polymer and ensure it is fully dissolved (Solvent is variable for each drug)
3. While polymer is dissolving, place 100mL beaker with large magnetic stir bar onto stir plate.  Create 0.3% PVA (in PBS) by adding 18 mL PBS and 2 mL 3% PVA (20 mL total is also variable)
4. At this point make sure your drug of interest is dissolved in PBS or water and has been diluted into 100 uL(volume is variable depending on drug solubility).
5. Grab ice from the ice machine.
6. Set sonicator to Amps 8 with a 30 second process, 1 second on 1 second off.
7. When polymer is fully dissolved, add 100 uL of PBS or water with drug of interest to the polymer solution, this is the primary emulsion.  Vortex briefly.
8. Place scintillation vial in a glass dish with ice and place onto risible platform.  Open up the vial, and place under the sonicator probe. Bring up the platform and place tip right above bottom of tube.  Hit run on the sonicator screen (cover your ears)
9. Add 4 mL of 3% PVA to the already sonicated solution.  This is the secondary emulsion. Vortex briefly. Repeat step 8.
10. Draw up some of the 0.3% PVA from the stirring beaker and pour the contents of the vial into the stirring beaker.  Add the 0.3% PVA into vial to assist in gathering all of the particles.
11. Before using the sonicator again, you must clean it.  First, spray off the probe with water to remove any PVA.  Second spray the probe with acetone. Make sure to wipe the probe dry.
12. Let the solution stir for at least 2 hours to allow for particles to harden.
13. After stirring, add particles to 50 mL conical tubes and centrifuge for 45 minutes at 14,500 RPM at 4 degrees C to pellet (be sure to tare the tube if particle yield is important).
14. Decant and resuspend particles in 10 mL of water.
15. Repeat for 3 spins total (2 wash steps).
16. Resuspend particles in 5 mL of water (make sure completely suspended) and add 20% wt. Glucose in water to ensure cryoprotection and freeze particles.
17. Once completely frozen, place particles on the lyophilizer.  Lyophilizer time is variable. When glass jar or 50 mL tube are no longer cold, particles can be taken off lyophilizer.