Day 0

* If endotoxin free then soak in sodium hydroxide ON
* Make PBS by dissolving 1 tablet/200 mL DI water
* Make sure 3% (wt/wt) poly vinyl 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.  Not the tall ones used for polymer synthesis)
2. Make sure that your drug of interest is weighed out in a small scintillation vial. Create a stock solution of drug such that for every 2mL of solvent, you will have the required amount of drug for 100mg of polymer. (solvent is variable for each drug).
3. Add the 2 mL of ethyl acetate/drug combination to dissolve the polymer.
4. 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)
5. Once fully dissolved, transfer polymer/solvent solution to a 50 mL falcon tube.
6. Add 12 mL of 3% PVA (in PBS) to the 50 mL tube to create the emulsion and homogenize for 30 seconds.
7. Draw up some of the 0.3% PVA from the stirring beaker and pour the contents of the 50 mL tube into the stirring beaker.  Add the 0.3% PVA into the 50 mL tube to assist in gathering all of the particles.
8. Before using the homogenizer again, you must clean it.  Fill a plastic reusable round bottom tube (above Mikes bench) with MilliQ water.  Wash by turning on homogenizer briefly. Dump out the foamy water into the sink and fill again.  Perform this 3 times. Rinse the homogenizer with ethanol or acetone. Wash 3 more times with MilliQ water.
9. Let the solution stir for at least 2 hours to allow for particles to harden.
10. After stirring, add particles to a fresh 50 mL conical tube and centrifuge for 10 minutes at 14,500 RPM at 4 degrees C to pellet (be sure to tare the tube if particle yield is important).
11. Decant and resuspend particles in 10 mL of water.
12. Repeat for 3 spins total (2 wash steps).
13. Resuspend particles in 5 mL of water (make sure completely suspended) and freeze particles.
14. 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.