

Single emulsion sonicated particles

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Day 0

- If endotoxin free then soak glassware in sodium hydroxide
- 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. 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 1mL of solvent, you will have the required amount of drug for 100mg of polymer. (solvent is variable for each drug).
3. Add 1 mL of the 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. 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 2 mL of 3% PVA to the vial to create the 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)
 - a. Once the sonication is complete the energy used should be between 1000-2000 J.
9. 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.
10. 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.
11. Let the solution stir for at least **2 hours** to allow for particles to harden.
12. 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).
13. Decant and resuspend particles in 10 mL of water.
14. Repeat for **3 spins total** (2 wash steps).
15. Resuspend particles in 5 mL of water (make sure completely suspended) and add 20% wt. Glucose in water to ensure cryoprotection and freeze particles.
16. 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.