

Double emulsion particles using a homogenizer

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Day 0

- If endotoxin free then soak in sodium hydroxide ON
- Make PBS by dissolving 1 tablet/200 mL deionized 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 glass scintillation vials (white cap, silicon septa)
2. Add 2 mL of solvent to polymer and ensure it is fully dissolved (Solvent is variable for each drug and polymer)
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. Once fully dissolved, transfer polymer/solvent solution to a 50 mL falcon tube.
6. Add 100 uL of drug in PBS or water into the polymer solution. You will notice droplets as these two solutions are immiscible. This is the primary emulsion.
7. Put tube onto homogenizer set to 18,000 RPM and rock back and forth to allow the small volume to be homogenized. Perform this for 30 seconds. Do not turn on homogenizer unless the blade is submerged in the solution.
8. Add 12 mL of 3% PVA (in PBS) to create the second emulsion and homogenize again for 30 seconds.
9. 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.
10. 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.
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 10 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 basic water.
14. Repeat for 3 spins total (2 wash steps).
15. Resuspend particles in 5 mL of basic water (make sure completely suspended) 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.