Homogenization of Drug Particles

(April 2020)

Introduction

This protocol describes the homogenization and zeta sizer protocols for most formulations in the Nano medicine lab.

Definitions

Reagents and Materials

- 20 mL Glass vial
  Manufacture: Fisher Scientific

- Polymer (see Formulation QC Form)

- Buffer (see Formulation QC Form)

- Drug (see Formulation QC Form)

- Histology grade Isopropanol
  Lot#: _____
  Manufacture: Fisher Scientific

- HPLC grade Methanol
  Lot#: _____
  Manufacture: Fisher Scientific

- 0.5 M Sodium Hydroxide (NaOH)
  Manufacture: In House

- HPLC grade Water
  Manufacture: In House

- .2% P 407 polymer solution
  Manufacture: In House

- Low volume disposable sizing cuvettes
  Lot#: _____
  Manufacture: Malvern

- Malvern Disposable folded capillary cells
  Manufacture: Malvern

- Pipetmen
  P 1000
  P 20
  Manufacturer: Fisher Scientific

- Pipetmen Tips
  P 1000
  P 20
  Manufacturer: Fisher Scientific

Instrumentation

Mettler Toledo Excellence Plus 205 Balance;
SN __B151560880_____
Equipment ID __00784397_____
Manufacture: Mettler Toledo

Magnetic stir plate
Manufacture: Fisher Scientific

High-Pressure Avestin EmulsiFlex-C3 Homogenizer
Manufacture: Avestin

Malvern ZetaSizer Nano series, Nano-ZS
Manufacture: Malvern

Thermo Fisher Haake A-40 chiller
Manufacture: Fisher Scientific

Protocol

1. For all formulations, weigh polymer (as per Formulation QC Form) into a glass vial
2. Disperse the polymer into 15 mL of buffer, as per Formulation QC Form
3. Mix at medium speed using a magnetic stirrer at room temperature until polymer is completely dissolved
4. Weigh drug (as per Formulation QC Form) onto weigh paper
5. Carefully add weighed drug to polymer solution
6. Mix overnight using a magnetic stirrer to obtain complete suspension
7. Turn on homogenizer, compressed air, and pressure gauge
8. Connect tubing to homogenizer and homogenizer coil
9. Wash homogenizer thoroughly
    a. Rinse homogenization vessel with 500 ml isopropanol
    b. Rinse homogenization vessel with 500 ml methanol

   c. Rinse homogenization vessel with 500 ml water
   d. Fill homogenization vessel approximately half full with 0.5 M NaOH; apply pressure up to 20,000 psi, alternate pressure on/off
   e. Rinse homogenization vessel with 500 ml water
   f. Fill homogenization vessel with .2% P 407 solution, apply pressure up to 20,000 psi, alternate pressure on/off
   g. Rinse homogenization vessel with 500ml water
   h. Fill homogenization vessel approximately half full with water; apply pressure up to 20,000 psi, alternate pressure on/off
   i. Use compressed air to blow out any water in homogenizer/tubing
10. Turn chiller and set to -2 °C.
11. Remove an aliquot (10 mcL) of suspension and add to 990 mcL fresh HPLC grade Water for pre-homogenization particle size, PDI, and zeta potential determined by dynamic light scattering (DLS); mix well
12. Add diluted suspension to low volume disposable sizing cuvette
13. Place cuvette in ZetaSizer with arrow pointing forward, close lid
14. Read size and PDI; select size measurement, name sample, change equilibration time to 30 seconds, change cuvette type to low volume disposable sizing cuvette, change number of runs to 3, start measurement
15. Once done measuring size, remove sizing cuvette and carefully transfer suspension to disposable folded capillary cell (zeta cell)
16. Place zeta cell in ZetaSizer with Malvern logo pointing forward, close lid
17. Read zeta potential; select zeta measurement, name sample, change equilibration time to 30 seconds, change number of runs to 3, start measurement
18. Once done measuring zeta, highlight the 6 runs (3 size, 3 zeta) and record the averaged size, PDI, and zeta
19. Remove and clean zeta cell with HPLC grade Methanol and then HPLC grade Water
20. Transfer the suspension to the homogenization vessel, route the end of the tubing back into the homogenization vessel to create a continuous loop
21. Increase the pressure gradually to 20,000 $\pm$ 1,000 psi and homogenize at -4 °C, note starting time
22. Regularly (i.e. every hour, every 30 minutes, etc.) monitor the size, PDI, and zeta by taking a 10 mcL aliquot from the homogenization vessel and adding to 990 mcL fresh HPLC grade Water; measure size, PDI, and zeta as before (11-19)
23. Continue to homogenize at 4 °C until the target particle size and PDI are achieved
24. Reduce pressure down to 0 and carefully remove nanosuspension from the homogenizing vessel into a clean glass vial; note finishing time
25. Keep the nanosuspension stirring using a magnetic stirrer to avoid aggregation
26. Clean homogenizer thoroughly, as before (9a-i)