**Citrate-coated Prussian blue nanoparticle (Cit-PBNP) synthesis**

**Materials:**

* Iron (III) chloride hexahydrate, FeCl3.6H2O (MW 270.3; source Sigma)
* Potassium hexacyanoferrate (II) trihydrate, K4[Fe(CN)6].3H2O (MW 422.39; source Sigma)
* Citric acid (MW 192.12; source Sigma)
* Acetone
* NaCl

**Protocol:**

1. Prepare 20mL of 10.0 mM aqueous FeCl3 solution under stirring at 60 °C (set hot plate to 60C and that is good enough). Add 5 mmol of citric acid powder to a stirring FeCl3 solution after complete dissolution**. Original protocol scaled** up 10x.
2. Prepare 20mL of 10.0 mM aqueous K4[Fe(CN)6] solution containing the same amount (5 mmol i.e. 961mg) of citric acid under stirring at 60 °C.
   1. Mix step 2 into step 1 quickly by pouring whole beaker
3. After stirring at 60 ºC for 10 min, allow the solution to cool to room temperature with the stirring continued for another 5 min at room temperature.
4. Divide the solution formed in two 50mL falcon tubes (20mL each)
5. Add an equal volume of acetone to the dispersion in each tube
6. Add 5mL of 5.0 M NaCl to each tube (facilitated the pellet formation)
7. Centrifuge at 10,000 rpm for ~15 min to form a pellet of Prussian Blue nanoparticles
8. Re-disperse the pellet in ~20 mL distilled water by sonication (amp 40%; 30 sec) and separate again by adding equal volume of acetone, mix well and add 5 mL of 5.0 M NaCl
9. Centrifuge at 10,000 rpm for 15min
10. Repeat this purification process (steps 9 & 10) one more time
    1. If there is discoloration in the supernatant, do one more wash
11. Use the air nozzle to dry the pellet
12. Allow pellet to sit overnight in oven for further drying.
13. Use balance to weigh out desired amount of Prussian Blue for use and then resuspend in DI water.
    1. Always sonicate particles after resuspension in DI water.
14. Measure DLS, Zeta potential, UV-Vis (characteristic peek at 680-700nm), Absorbance to measure the concentration from standard curve (or) dry the sample to powder and weigh to get mg/mL concentration of the prepared batch of PBNPs.