Recrystallization of CMBT

Health and Safety:

Wear nitrile gloves, laboratory coat and safety glasses. The process should be done with fume hood ventilation.

Equipment and Materials:

Equipment

2000 mL beaker,

hot plate with magnetic stirring,

3 inch magnetic bar (Fisher, 14-513-68),

Buchner funnel set (diameter size from 60mm to 100mm, e.g., Fisher FB-966-F),

2000 mL filter glass,

filter paper,

2000 Erlenmeyer flask,

Chemicals

5-Chloro-2-mercaptobenzothiazole from Sigma/Aldrich, or other sources; Acetone, HPLC grade; MilliQ water

Procedure

1. Prepare 2000 mL 95% acetone
2. For 100 g CMBT: pour 1800-2000 mL of 95% acetone in a 2000 mL beaker with a magnetic stirring bar and then place the beaker on the hot plate, add 100 g of CMBT in the acetone. A large petri dish that is used to cover the beaker to condense most of the solvent. Start the

stirring and heat up the 95% acetone to 50°C. (the condensing using a petri dish is not efficient, 100mL or 150mL of acetone may need to be added during the heating to compensate the lost solvent). Normally, the dissolution process takes about 2 hours.

3. When the solution from step 2 is totally clear, stop the heating and set the stirring motion at a high speed. Wrap up the beaker with aluminum foil and leave the flask on the hotplate.

4. The fast stirring and slowly cool down process combine to give a slow crystallization of CMBT. During this process, the crystals tend to have a very small size and contain minimum impurity. This process will take a couple hours for the crystals to start to form, and overnight to complete.

5. Filter the crystals of CMBT, rinse with about 200 mL of ice cold 50% acetone and dry in a hood under a dim lighting.

6. Repeat the above procedure one more time with the obtained crystals with adjusted amount of acetone. (10 mL 95% acetone: 1 g CMBT)

7. Store the aliquot of CMBT at -20 to -80°C.

Expected outcome

When at highest purity, CMBT crystals are almost snow white. The color of very fine crystals will slowly change from white to light white.

Reference: please see any basic organic laboratory training book for the recrystallization procedure and mechanism.

Note:

1. Sometimes commercially available matrices have low quality, so recrystallization should be applied at least twice to obtain highest quality crystals.
2. The solution in step 3 is not filtered before crystallization, so, any particles that don't dissolve in acetone are not removed. However, filtration of a large volume of hot volatile solvents under high temperature in practice turns out to be a difficult task and commonly results crystal crushing out and clogging the filter funnel. As a result, the step of filtering the hot saturated solution is removed. The remaining particles in the crystals are the ones that will not dissolve in acetone/ACN, they can be removed by using a syringe filter when the matrix solution is prepared, so, these potential remaining particles won't cause any problems for spraying or analysis.
3. To pursue high purity of the matrix, the yield is sacrificed. Normally, the expected yield of one recrystallization is about 60%. So, after twice recrystallization, the final yield will be around 36%.