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Protocol for 3,5-DTBC oxidation trials monitored by UV-visible spectrophotometry

Recommendations

- Before starting, make sure each of the volumetric flasks that will be used are washed with acetone and completely dry.
- Prepare the solution in under 2 minutes rather than having the solution prepared and sitting on the bench in order to avoid autoxidation and to get a true zero mark. This 2 minute time frame can be measured from as soon as the solvent touches the solid 3,5-DTBC until the cuvette is placed in the UV-Vis instrument.
- Prepare each sample containing 3,5-DTBC one at a time and only when it will be going straight into the cuvette to use in the UV-Vis instrument to minimize autoxidation.
- For larger concentrations of 3,5-DTBC (20 mM and higher) the solution should be mixed using a vortex mixer for at least 20 s to fully dissolve the catechol and make a homogeneous solution.

Procedure

This procedure includes the details for a reaction run in methanol with 0.01 mM catalyst and base, but can be adapted to other solvents and catalyst/co-catalyst concentrations.

A. Before making the sample

- The UV-Vis instrument should be turned on and allowed to warm up.
- To control the temperature, use a Peltier accessory and set it to the desired temperature. **Make sure to add water to the aquarium pump before plugging it in, or else it'll burn out. Order should be water in pump, plug in, turn on Peltier, and set temperature.*
- Set up the UV-Vis to the appropriate parameters for the length intended of the run. For example, to conduct a zero mark check followed by an hour long run with scans every 5 minutes, use the program Scanning Kinetics and setup based on the following information and table.

  600.00nm - 300.00nm, scan rate 4800.00nm/min, baseline correction on, first order, ave time 0.0125, advanced collect, y min 0, y max 2.00

Stage	Cycle (min)	Stop (min)
1	0.1	0.1
2	5.0	60.0

- Zero and baseline the UV-Vis machine, making sure that for the baseline run, the cuvette has the same solvent that will be used to prepare your samples.
- Once at the step for naming the sample on the computer, preparation of the solution can be done.
- Prepare a stock solution of the catalyst that will be used.
  - To prepare a stock solution of 0.10 mM **$PyAm_2Cu^{II}(MeCN)$**, mass out 0.0015 g of complex into a 25 mL volumetric flask and fill to the line with methanol.
- Prepare a stock solution of the base co-catalyst, KOH.
  - To prepare a 1.0 mM KOH stock solution, mass out 0.0014 g of KOH and dissolve it in methanol in a 25 mL volumetric flask.
  - To prepare a 0.10 mM KOH stock solution, transfer 1.0 mL of the 1.0 mM KOH solution into a 10 mL volumetric flask and fill to the mark with methanol.

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B) Making the solution

- Mass out the required amount of 3,5-DTBC for your desired concentration directly into a 10 mL volumetric flask (the starting material should be a colorless solid).
- If using catalyst, transfer 1.0 mL of the 0.1 mM catalyst stock solution to the volumetric flask. This will result in a 0.01 mM catalyst concentration.
- If using base, transfer 1.0 mL of the 0.1 mM base stock solution to the volumetric flask after the 3,5-DTBC and catalyst stock solution. This will result in a 0.01 mM catalyst concentration.
- Fill to the line with methanol.
- Mix with a vortex mixer for 20 s.
- Pipette the prepared solution into a clean cuvette and run the sample on the UV-Vis.
- Once the UV-Vis is running, look for a peak at 400 nm on the absorbance spectra indicative of the formation of 3,5-DTBQ.