(6-R,S) Tetrahydrafolate Preparation

(Blakely, R.L. (1957) *Biochem J.* 65, 331-342 and Curthoys, N.P., and Rabinowitz, J.C. (1971) *J. Biol. Chem.* 246, 6942-6952.)

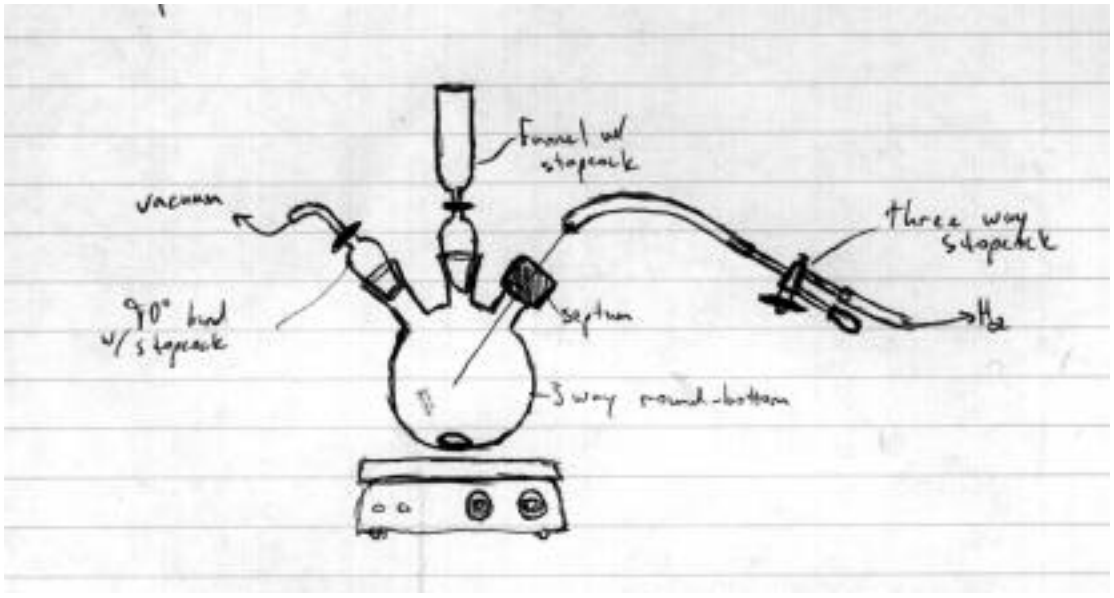
Column Preparation:

1. Measure out 13g DEAE cellulose (Sigma)
2. Mix with 250ml 1M NaCl (makes ~100ml column)
3. Load into column (25x300mm works well)
4. Wash column with 1L water
5. Wash column with 1L 0.5M 2-mercaptoethanol

Apparatus Set-up:

The apparatus for this prep is best described by the drawing. The system needs to facilitate all of the following:

• constant, vigorous stirring
• vacuum (water aspirator)
• pressurized hydrogen
• addition of fluids without the introduction of oxygen
• good ventilation (work in hood)



*be sure to eliminate any leaks (up to 10psi) before beginning prep

1. Place 20ml 50mM $NaHCO_3$ in the round-bottom flask (250ml) with 0.3g PtO_2 (Sigma, P 0804) and stir vigorously for the rest of the prep. (Stirring should be as fast as possible without throwing off stir-bar; this is very important)
2. Place system under vacuum for 2 minutes, and then under ~2psi H_2 for 5 minutes (repeat this sequence 5 times; the Pt should aggregate during this process)
3. While you are purging the system prepare the folate mixture:
   2g folic acid (Sigma, F 7876)
   40ml 0.5M $NaHCO_3$
   60ml water (stir until clear, and keep this as dark as possible)

4. After the system is purged, return to vacuum and add the folate mix under negative pressure through the funnel (make sure no air gets into the system even if you leave behind a couple of milliliters).
5. Cover the flask with foil to keep the reaction dark and return the system to positive H_2 pressure (~8psi). Keep stirring vigorously for 3 hours.
6. Switch back to vacuum and add 10ml 2-ME (concentrate) under negative pressure (no air)
7. Return to 8psi H_2 for 10 minutes
8. Filter the reaction mix through Whatman paper and then mix with 1L 0.5M 2-ME. You can look at spectra here, 1:1000 in 50mM 2-ME. (Remember to keep the reaction as dark as possible at all times)
9. Load all of this solution onto the column and wrap it in foil. Flow rate can be as fast as it will run by gravity.
10. Wash with 0.5L 0.5M 2-ME
11. Elute with 1L 0.2M Tris-Cl pH 7.5, 0.5M 2-ME. Monitor fractions by measuring spectra of 1:1000 dilutions in 50mM 2-ME. Blank against 50mM 2-ME.
12. Pool the fractions with good spectra and high A_{298}.
13. Look at a final spectra and adjust the final concentration of THF to 10mM using 0.2M Tris-Cl pH 7.5, 0.5M 2-ME (Extinction Coefficient at 298 = 30,000 M^{-1})
14. Store under vacuum in dark at 4˚C. Aliquots can be removed and the flask can be re-evacuated many times before the substrate will go bad. Can be used safely up to 6 months.