Protocol– Magnetite synthesis

- Materials:
  1. Iron solution- Fe_3Cl and Fe_2Cl with a ratio of 2:1 (0.66 M:0.33 M), filtered and sparged with N_2. Notice; do not use iron powders that were oxidized!
  2. 0.1 M NaOH- needs to be freshly prepared, filtered and sparged with N_2.
  3. Peptide/protein sample- it is better to dissolve your sample in water, different buffers have an effect on the magnetite shape and size. The peptide/protein final concentration in the magnetite solution (13 ml in total) is 100 µM.
- Organize the titration system:

  In our lab, the N_2 system has four pipelines which number one is the one which is closest to the main gas faucet. The first pipeline is usually for the 0.1 M NaOH solution, the second for the iron solution, third for the synthesis process and the forth for the titration column contain 0.1 M NaOH (See figure 1 and 2).

  Figure 1:

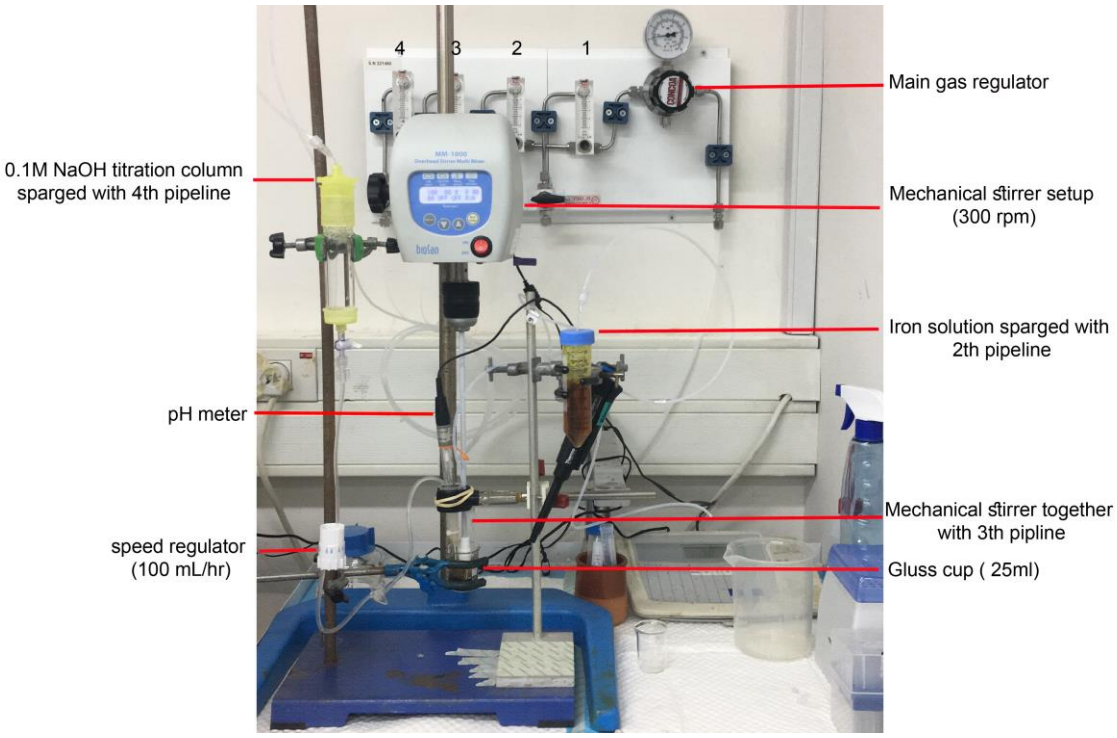
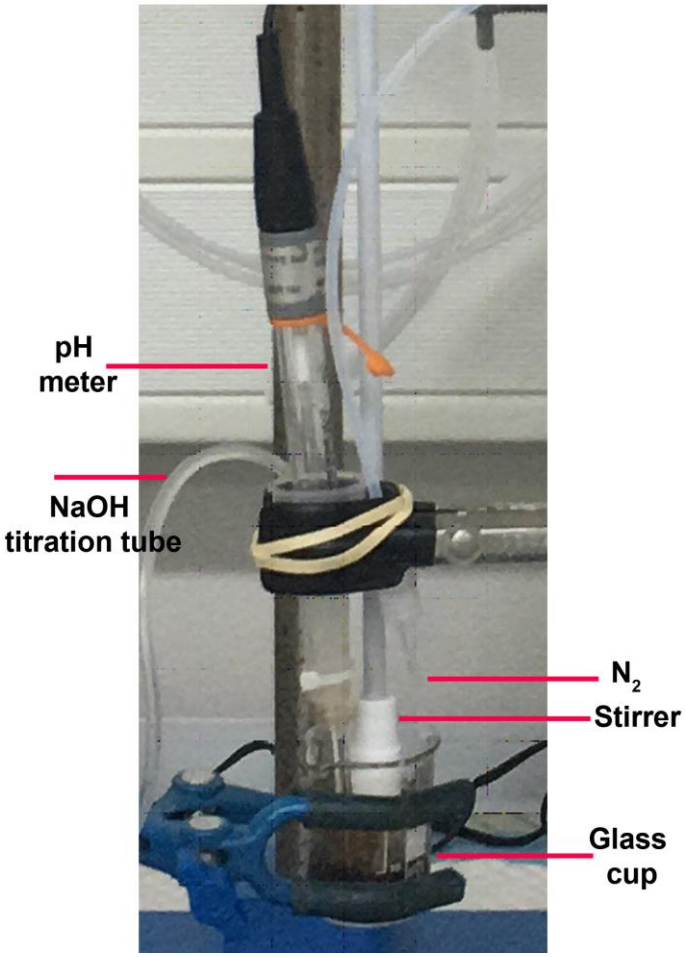


Figure 2:



- Magnetite synthesis protocol:
  1. Open the mechanical stirrer setup and set the stirrer speed at 300 rpm. Be aware that the stirrer is almost touching the cup bottom.
  2. Insert the pH meter to the cup, carefully without touching the stirrer, while its tip tach the bottom of the cup.
  3. Insert the pipeline 3 with special plastic tip and open the gas regulator. This is very important, in order to create an anaerobic environment.
  4. Add 200 µl from the iron solution.
  5. Start titrating NaOH- adjust the speed regulator on 100 mL/hr and open the column valve.

In case of co-precipitation with <u>peptide/protein</u> you will need to do the following step:

5a. At pH 5 add the peptide/protein for final concentration of 100 µM (total volume according to this protocol is 13 ml).

6. Stop the NaOH titration when the pH rises up to pH 9. Continue the N_2 sparged and stirrer for another 10 min. At this point, the solution needs to be black and magnetic. In order to check if your sample is magnetic. Take a small sample (100 ul) from the solution and use a strong magnet to see the movement of the particles **(In case that the solution color is brown but magnetic, apparently you got Maghemite)**.
7. Move the magnetite solution to clean tube and sild with silicon cap; keep the sample in a Gas-pak chamber (BD GasPak™ EZ Container Systems BD 260671).

- **Cleaning the system:**
  1. The pH electrode should be washed with mQ water and should be stored in 3 M KCl. Every few rounds of magnetite syntesise the pH electrode needs to be incubating for 10-15 min in 0.4 M HCl solution in order to remove the iron solution that stack on the electrode surface.
  2. The plastic stirrer and the cups will be washed with liposol and water and sprayed with 20% ETOH. Let it dry on your bench. For deep cleaning the plastic stirrer and cups are needed to be incubated in 0.4 M HCl solution for O.N and then washed with liposol and water.

Troubleshooting:

- Make sure all your solutions are freshly done, filtered and sparged with N_2 for at least 15 min before you start synthesis.
- Make sure the speed of the stirrer is ok and that the stirrer is clean
- The glass cup is clean and dry before you start your synthesis.
- The pressure of the N_2 isn't too low; make sure the big and small gas regulators are open to the maximum.
- Make sure the NaOH in the column is sparged during all the synthesis process.
- Make sure your pH meter is calibrated.