Preparation of Solutions for Ca/EDTA Tutorial

Solutions Needed:

MES Buffer	10 mM MES, pH 5.6
EDTA	10 mM MES, 0.4 mM EDTA pH 5.6
Calcium Chloride	10 mM MES, 5 mM CaCl2, pH5.6

Final volumes of 10 ml MES Buffer, 10 ml EDTA and 1 ml $CaCl_2$ is plenty for training.

10 mM MES, ph 5.6: (MW MES: 195.2 g/mol) 1.925 g/l

0.4 mM EDTA: (MW: 292.24 g/mol) 0.117 g/l

5 mM $CaCl_2$: (MW: 110.98 g/mol, **anhydrous**) 0.555 g/l (if you don't have anhydrous make sure to include the water for your own calculations)

*Please check my math to be on the safe side!

*Check what type of $CaCl_2$ you have (anhydrous, dihydrate, etc.). Keep in mind that $CaCl_2$ is hydroscopic.

Note:

- Buffer matching is key for a successful ITC experiment. Prepare the EDTA and $CaCl_2$ solutions with the **same MES buffer** - not just the same composition, the same batch! If the buffers are not matched large heats of dilution will mask the desired observation.
- pH is important. Be sure your buffer concentration is high enough to compensate for any pH effects during titrations. *After* the solutions have been prepared, carefully check their pH. If they differ by more than 0.05 pH units, then adjust one of the solutions so they match. Make sure that you rinse the pH electrode well. You don't want to contaminate your solutions with the electrode storage solution.
- Concentrations are very important to get good results, be extremely careful making your solutions. Accurate determination of binding parameters is only possible if concentrations are known precisely.