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Chem 69a

Lab 5

Supramolecular Nanofiber/Hydrogel Synthesis

The purpose of this experiment was to was to explore the synthesis of hydrogels via nanofiber networks using hydrogelators that are pH and concentration dependent.

Experimental

Materials:

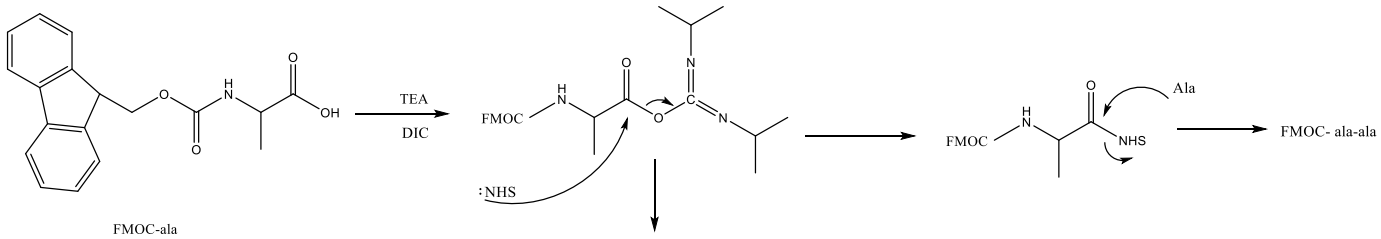
- FMOC-ala
- DIC
- TEA
- DCM
- THF
- Methanol
- FMOC-Y^p
- Sodium carbonate
- Alkaline phosphatase solution

FMOC ala-ala Hydrogelation (Procedure A):

1. Add 69.9 mg NHS and 159.1 mg FMOC-ala to 10 mL THF
2. Add 85 uL DIC to soln and stir over ice for 30-90 min, checking with TLC every 15 min (1:100 MeOH:DCM soln)
3. After white precipitate forms, filter solution through pipette to remove precipitate and save solution. Add 60 mg alanine, 0.167 mL TEA and 10 mL THF to soln and stir for another 2.5 hr at room temperature
4. TLC soln every 30 min to check extent of reaction. Reaction was stopped after 75 min when consecutive TLC plates showed no change in product
5. Filter precipitate out of soln and wash with 10 mL THF, then collect and rotovap soln until solvent is evaporated

   Day 2:
6. Use flash chromatography (50:1 DCM:methanol) to separate compound, spot checking fractions every minute to check for presence of product.
7. Fractions 1-8 were combined and rotovapped to produce oily-gel product
8. 0.0332 g product was produced- 1.2 mL H_2O and 2 uL of 1 M NaOH were added to product
9. pH of solution was monitored as drops of 0.1M HCl were added

10. No gelation was observed; likely due to insufficient amount of product- the concentration was too low for gelation to occur



Phosphatase trigged Hydrogelation (Procedure B):

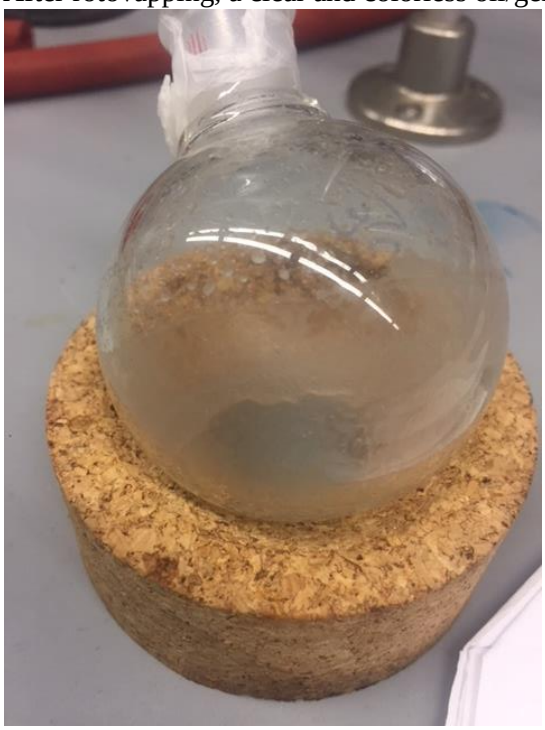
1. Add 8.7 mg Na_2CO_3 to vials A and B, along with 25.5 mg FMOC-Y^p to each vial
2. Add mL H_2O to each vial, sonicate to dissolve solids
3. Add 5 uL alkaline phosphatase soln to vial A, and uL to vial B
4. Heat vials at 37°C for 1.5 hr

No gel was formed in either vial at end of heating- each vial was slightly more viscous with noticeable suspension present, but not solid enough to become gel. This could have been due to an insufficient temperature during heating- vials were initially heated in rotovap water bath, but transferred to water bath on hot plate when rotovap was in use; likely was not sustained at 37°C.

Observations Procedure A:

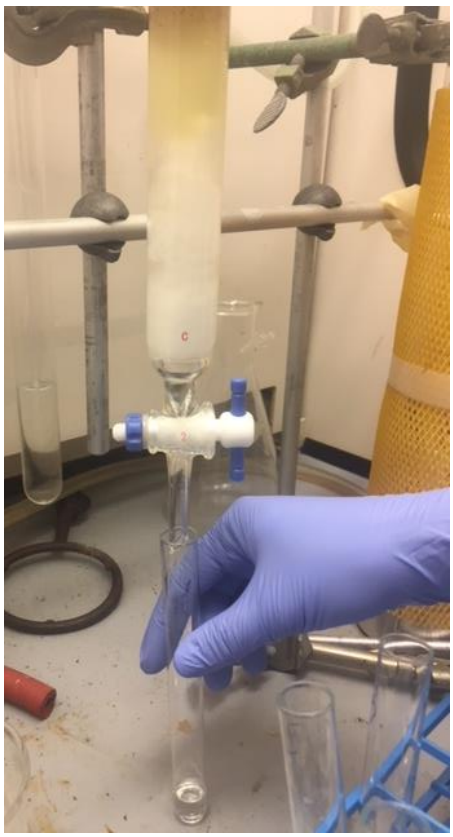
1. Initial reactant appearances
    i. NHS- white crystal
    ii. FMOC- ala – white powder
    iii. THF/DCM/Methanol- clear and colorless liquid
    iv. DIC- clear, colorless, viscous liquid
2. Initial soln was clear and colorless when all reactants dissolved
3. Slight change in soln appearance after first 30 min of stirring (clear with white precipitate present), when TLC showed no difference between plates, soln was filtered and precipitate discarded
4. After addition of alanine and TEA, soln was spotted ever 30 min over 75 min time period to check extent of reaction. Solution was slightly murky and yellow as reaction proceeded
5. When TLC plates showed no difference, soln was vacuum filtered to remove precipitate, producing clear and colorless soln

6. After rotovapping, a clear and colorless oil/gel remained on sides of flask



Day 2:

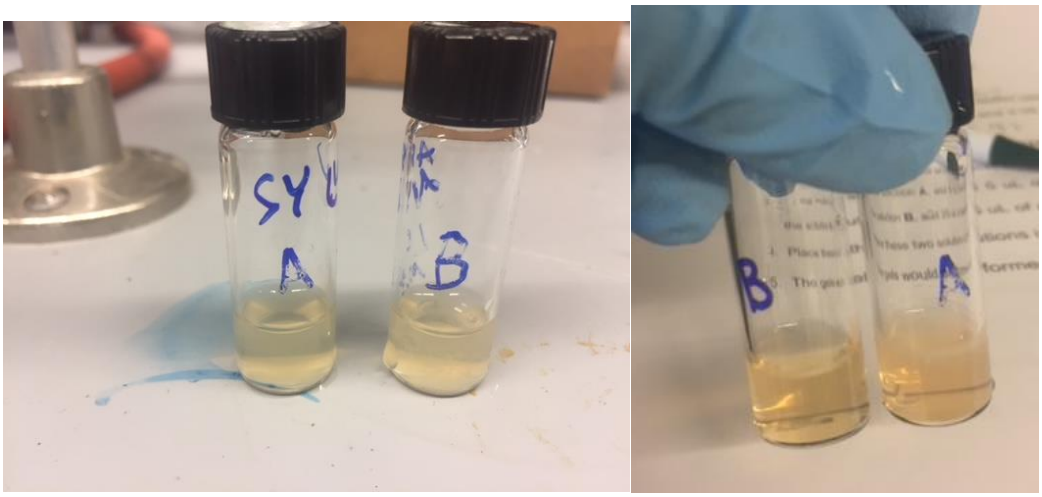
1. When product was filtered on column, a yellow layer remained in column and clear fractions were collected. Fractions 1-8 had product spots under TLC spot test and were combined then rotovapped



2. After rotovapping, clear and colorless viscous soln remained in flask. Addition of NaOH and water created clear and colorless soln. HCl addition produced no change in solution (HCl added until pH of 1 was achieved, as indicated by pH testing paper)

Observations Procedure B:

1. Initial reactant appearances
    i. FMOC-Y^p – brown powder
    ii. Na_2CO_3 – white powder
    iii. Alkaline phosphate solution- clear and colorless
2. Initial solution was clear and light brown after all solids dissolved
3. After hour of heating, each vial was clear and light yellow, small particle suspension was present in vial B but not gel formed in either vial.



Results & Discussion

This lab explored the synthesis of supramolecular hydrogels from self-assembly of organic molecules driven by non-covalent interactions. These hydrogel are responsive to external stimuli including pH, temperature, ionic strength, and ligand-receptor interactions. The first synthesis created a low-molecular weight hydrogelator from FMOC-ala-ala, and then the pH was varied using NaOH to make a basic solution, tuning to acidic pH via introduction of HCl. The FMOC group acts as a base sensitive protecting group for the amino acids that create the gel, so variations in pH must be done very carefully so as to preserve the protecting group. Although our procedure failed to produce a gel, typically these gels result from the formation of entangled fiber networks. Our synthesis likely failed because we did not synthesize enough product to reach a critical concentration of hydrogelator, so not enough fibers were present to produce a network and therefore a gel.

In our second synthesis we attempted to create gels via phosphatase triggered hydrogelation. The FMOC-Y^p undergoes rapid dephosphorylation, followed by formation of aggregates which organize into nanofibers creating a gel network. Unfortunately, this synthesis also failed to produce a gel product; this was likely due to the reaction solution not being maintained at sufficient heat, which catalyzed the aggregation.

Conclusion

Nanofiber/hydrogels have a variety of important medical applications due to their self-assembly in water, which allows them to intrinsically self-assemble in biological processes. Because these gels can be delivered in a minimally invasive way, via syringe, they can easily target many biological mechanisms. For instance enzyme instructed self assembly (EISA) can be used to create molecular nanofibers that selectively inhibit drug resistant cancer cells.