Swelling Experiment of Charged PEG-Maleimide Hydrogels

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**Purpose:** Determine the mesh size (ξ) of PEG hydrogels with 100 µM of +3, 0 or -3 charged peptides from 0.1 mg aliquots.

**Charged Peptides:**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Code** | **Peptide Sequence** | **Charge** | **pI** | **MW (g/mol)** | **Volume (µL) for 200 µM (0.05mg)** |
| RGR | GCRGD-RGRGR | +3 | 11.93 | 1089.21 | 459 |
| RDA | GCRGD-RDADR | 0 | 6.33 | 1120.17 | 446 |
| DAD | GCRGD-DADAD | -3 | 3.43 | 993.97 | 503 |

 

**PEG Hydrogel:**

A minimum volume of 1409 µl of PEG MAL is needed for the three tubes of charged peptide aliquots. Plan on making extra PEG, 1500 µl. PEG-MAL and PEG-SHSH are mixed 1:1.

4-arm PEG-MAL (20 kDa) and PEG-dithiol (10 kDa) in 4 mM TEA pH 7

MAL 5% = 3.75 x 10-6 x 4 = = 10 mM



SHSH = 3.75 x 10-6 x 2 = = 10 mM

**Protocol:**

1. Take out 48, 2 ml tubes. Label three sets for the +, -, and 0 charged gels, labels (+, -, 0) 1 – 16 for each set.
2. Place the tubes in the oven set to 70˚C over night or a minimum of 1 hour to remove residual moisture.
3. Take PEG-MAL, PEG-SH and one peptide aliquot 0.1 mg of each peptide. Allow to reach room temperature. Prevents condensation from entering the vial which will degrade the polymer over time.
4. Weigh out PEG-MAL and PEG-SH into separate 2 ml tubes and add 1500 µl of 4mM pH7 TEA to each. Vortex to dissolve.
5. Pipette Volumes from table of PEG-MAL into each of the charged peptide tubes. Vortex and sonicate to fully dissolve. Allow to react for a minimum of 10 minutes. The cystine in the charged peptide sequence reacts with the free maleimides of the 4-arm PEG Maleimide.
	1. PEG-MAL with charged peptide solution is 200 µM so when mixed 1:1 with PEG-SHSH the final concentration will be 100 µM.
6. Place tube rack with hot tubes in a Ziploc bag and seal it; the hot tubes will pull condensation from the air if left out to cool, changing the weight.
7. Weigh all 48 dried cooled 2 ml tubes.
8. Pipette 25 µl RGR PEG-MAL into the 2 ml tube cap coating the entire bottom. Avoid making bubbles.
9. With a new tip, quickly pipette 25 µl PEG-SHSH into the RGR PEG-MAL in the 2 ml tube cap. Reaction occurs in seconds, therefore pipette up and down twice to mix, avoiding making bubbles and take the tip out of the solution. The gel can polymerize in the pipette tip and get stuck.
10. Allow 1 minute to fully polymerize.
11. Weigh tube + gel of all the RGR group before starting the RDA gels to avoid mass loss due to evaporation.
12. Repeat steps 8 to 11 with RDA gels then DAD gels.
13. Add 2 ml PBS to the 2 ml tubes with the gels in the caps. Close the cap and invert the tube. Flick the end of the tube to gather all the PBS to the cap so that it is in contact with the hydrogel.
14. With tape, arrange labels on the benchtop as shown below, then place the tubes cap down on the benchtop in their respective positions until each timepoint is taken.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **+** | **0** | **-** |
| **1** | 1+ 2+ | 1● 2● | 1- 2- |
| **2** | 3+ 4+ | 3● 4● | 3- 4- |
| **3** | 5+ 6+ | 5● 6● | 5- 6- |
| **7** | 7+ 8+ | 7● 8● | 7- 8- |
| **12** | 9+ 10+ | 9● 10● | 9- 10- |
| **24** | 11+ 12+ | 11● 12● | 11- 12- |
| **48** | 13+ 14+ | 13● 14● | 13- 14- |
| **72** | 15+ 16+ | 15● 16● | 15- 16- |

**Time Points:**

1, 2, 3, 7, 12, 24, 48, 72 hr

Two tubes/gels per time point per condition. Therefore 6 tubes per timepoint.

1. Remove PBS from the tube using a 1000 µl pipettor from all 6 timepoint tubes.
2. Take a cotton swab or Kim Wipe to dry the inside of the tube if the gel is still in the cap. Then with a Kim Wipe, carefully dab excess PBS from around the gel in the cap.
3. Weigh the tube + swollen gel.
4. Leaving the cap open, put the tubes in a tube holder in the oven set to 70˚C. Dry gels overnight. Do NOT leave the tube racks in the oven for more than 3 days. Extended time will make them disintegrate. Alternate tube racks if you are running experiments back to back.
5. After gels have been dried for 24 hours, weigh the tube + dried gel.
6. Input weight data into the excel spreadsheet to calculate the mesh size (ξ) of the hydrogels.
	1. Rename the first available sheet as the experiment date. Make sure there is another open sheet in the workbook for the next experiment. You may have to make a copy and add it to the end of the workbook.
	2. The example experiment sheet is locked to preserve the formulas. Password: **UMBCChemEStudent**
	3. On the open experiment sheet, fill in the yellow columns, the formulas will fill in the rest.

**Results:**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Details** | **Swell Time** | **Tube #** | **Mt** | **MtG** | **MtS** | **MtD** |
| DAD | 1 | 1- |  |  |  |  |
| DAD  | 1 | 2- |  |  |  |  |
| DAD  | 2 | 3- |  |  |  |  |
| DAD  | 2 | 4- |  |  |  |  |
| DAD  | 3 | 5- |  |  |  |  |
| DAD  | 3 | 6- |  |  |  |  |
| DAD  | 7 | 7- |  |  |  |  |
| DAD  | 7 | 8- |  |  |  |  |
| DAD  | 12 | 9- |  |  |  |  |
| DAD  | 12 | 10- |  |  |  |  |
| DAD  | 24 | 11- |  |  |  |  |
| DAD  | 24 | 12- |  |  |  |  |
| DAD  | 48 | 13- |  |  |  |  |
| DAD  | 48 | 14- |  |  |  |  |
| DAD  | 72 | 15- |  |  |  |  |
| DAD  | 72 | 16- |  |  |  |  |
| RDA | 1 | 1● |  |  |  |  |
| RDA | 1 | 2● |  |  |  |  |
| RDA | 2 | 3● |  |  |  |  |
| RDA | 2 | 4● |  |  |  |  |
| RDA | 3 | 5● |  |  |  |  |
| RDA | 3 | 6● |  |  |  |  |
| RDA | 7 | 7● |  |  |  |  |
| RDA  | 7 | 8● |  |  |  |  |
| RDA | 12 | 9● |  |  |  |  |
| RDA | 12 | 10● |  |  |  |  |
| RDA | 24 | 11● |  |  |  |  |
| RDA | 24 | 12● |  |  |  |  |
| RDA | 48 | 13● |  |  |  |  |
| RDA | 48 | 14● |  |  |  |  |
| RDA | 72 | 15● |  |  |  |  |
| RDA | 72 | 16● |  |  |  |  |
| RGR | 1 | 1+ |  |  |  |  |
| RGR | 1 | 2+ |  |  |  |  |
| RGR | 2 | 3+ |  |  |  |  |
| RGR | 2 | 4+ |  |  |  |  |
| RGR | 3 | 5+ |  |  |  |  |
| RGR | 3 | 6+ |  |  |  |  |
| RGR | 7 | 7+ |  |  |  |  |
| RGR | 7 | 8+ |  |  |  |  |
| RGR | 12 | 9+ |  |  |  |  |
| RGR | 12 | 10+ |  |  |  |  |
| RGR | 24 | 11+ |  |  |  |  |
| RGR | 24 | 12+ |  |  |  |  |
| RGR | 48 | 13+ |  |  |  |  |
| RGR | 48 | 14+ |  |  |  |  |
| RGR | 72 | 15+ |  |  |  |  |
| RGR | 72 | 16+ |  |  |  |  |