Engineering Ionic Hydrogels to Overcome Charge and Size Barriers in Multi-Protein Co-Delivery

Samantha Wheatley
Advisors: Dr. Jeannine Coburn
Dr. Kevin Crowthers

How does hydrogel composition, specifically **charge**, **stiffness**, and **crosslinking density**, affect the individual and **co-delivery** release rates of **model molecules** with different molecular weights and charges?

Background

- Hydrogels are soft, water-rich polymers that mimic natural tissue and are used for controlled drug delivery (Peppas & Huang, 2004)
- Properties, such as charge, stiffness, and crosslinking, strongly influence how they interact with immune environments (Zulfiqar et al., 2022).
- Despite the availability of suitable synthetic and natural polymers, achieving predictable and controlled release remains a challenge (Guo et al., 2025)

Hypothesis

- Molecular weights will govern release
  - → Dextrans of lower molecular weight will release rapidly due to increased diffusion
  - → Dextrans of higher molecular weight will be retained longer due to reduced diffusion
- The interaction of the dextrans within the hydrogel will have an impact on the release
- The properties of the hydrogels, such as charge and polymer concentration, will be vital to the regulation of the individual and co-delivery of the dextrans

Methodology

Hydrogel Preparation:

- FITC and/or TR dextran incorporated prior to gelation
- Alginate hydrogels (2-5% w/v) ionically crosslinked with calcium chloride

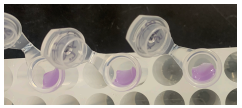

Figure 1. Alginate hydrogel transferred to centrifuge tubes after being formed in centrifuge caps with TR dextran

Release Setup:

Hydrogels incubated and samples taken at 1, 2, 4, 24, 48, 72, and 168 hours

Figure 2: Collecting samples by pipetting the surrounding PBS. Within the PBS, there is released dextran. To measure it, the solution will be pipetted into a black-well plate.

Quantification:

- Fluorescence of each samples measured at the defined timepoints

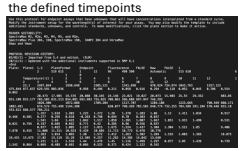

Figure 3: The fluorescent readings from the plate reader for the 72 hour read

Materials

- Alginate (2%, 3%, 4%, 5% concentrations)
- Calcium Chloride (CaCl2)
- Phosphate-Buffered Saline (PBS)
- Fluorescein isothiocyanate(FITC)-dextran (150kDa)
- Texas Red (TR) Dextran (40kDa)

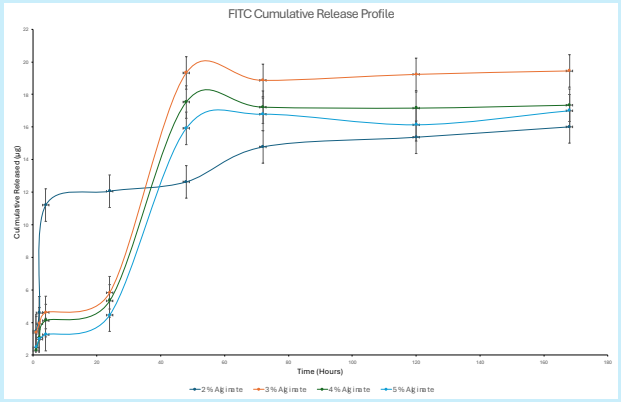

Figure 4. Compiled FITC-dextran individual release over 168 hours from alginate hydrogels with different concentrations

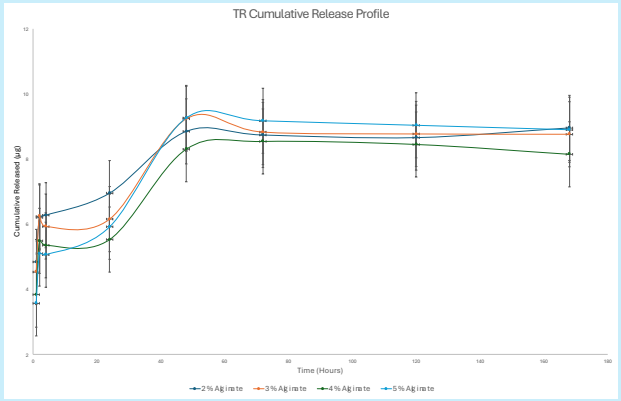

Figure 5. Compiled Texas Red-dextran individual release over 168 hours from alginate hydrogels with different concentrations

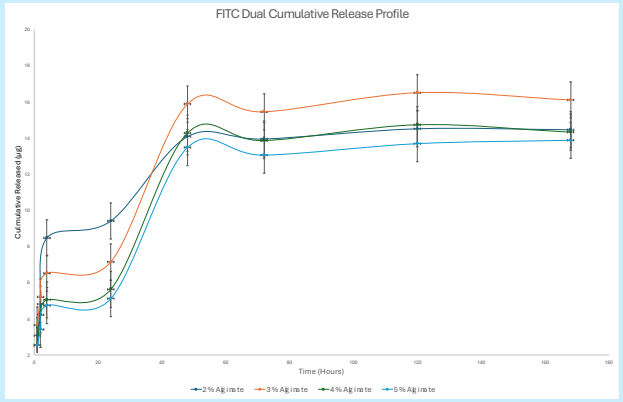

Figure 6. Compiled FITC-dextran dual release over 168 hours from alginate hydrogels with different concentrations

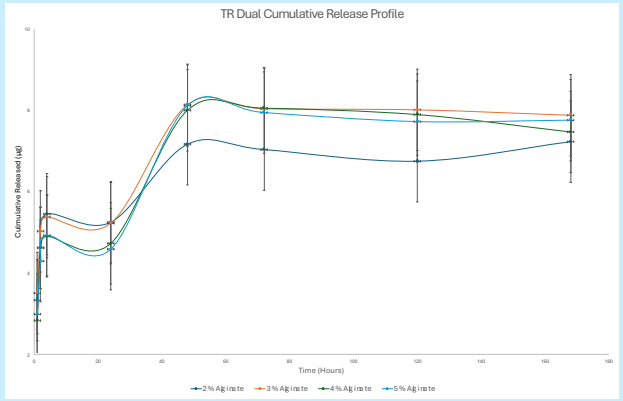

Figure 7. Compiled Texas Red-dextran dual release over 168 hours from alginate hydrogels with different concentrations

Analysis

Release Profiles:

- Analyzed over a 168-hour period
- Assessed burst and sustained release
- Behavior was compared across all alginate concentrations (2%, 3%, 4%, and 5%) to evaluate network density

Dual Release Comparison:

- Individual and dual-loaded hydrogels were compared to assess the effect of co-delivery on molecule transport
- The FITC-dextran and TR-dextran release profiles were analyzed separately to evaluate baseline release behavior to compare to the dual release

Results

Alginate concentration determines dextran release

- 3% alginate gels exhibited the highest cumulative release across both molecules
- Higher alginate concentrations, 4% and 5%, reduced the release, consistent with increased crosslinking density

All releases follow biphasic kinetics

- All formulations have an initial burst followed by a sustained release
- Both loading conditions also have an initial burst followed by a sustained release
- All releases plateau between 40-60 hours

Co-delivery reduced cumulative release without changing release profiles

- Dual-loaded hydrogels released an overall lower cumulative amount of both FITC-dextran and TR-dextran compared to the individual releases
- The overall behavior and shape of the release curves remain similar across loading conditions

Future Steps

- Expand molecular weight range
- Further tune hydrogel properties for programmable multi-molecule release
- Transition from model dextrans to bioactive proteins
- Further explore other hydrogels (e.g., chitosan) that can be programed to handle multiple molecules

References

Peppas, N.A., & Huang, Y. (2004). Hydrogels and drug delivery. *Advanced Drug Delivery Reviews*, 56(11), 1531-1532 https://doi.org/10.1016/j.addr.2004.10.015

Zulfiqar, H., Lee, S., & Kim, S. H. (2022). Hydrogels for protein delivery in biomedical applications. *Journal of Controlled Release*, 343, 598-613 https://doi.org/10.1016/j.jconrel.2022.01.015

Guo, A., Cao, Q., Fang, H., & Tian, H. (2025). *Recent advances and challenges of injectable hydrogels in drug delivery. Journal of Controlled Release*, 385, 114021. https://doi.org/10.1016/j.jconrel.2025.114021