Thermo-sensitivity and erosion of chitosan crosslinked pNAM hydrogel based eyedrops for application to the inferior fornix

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Introduction:

- Allergic conjunctivitis (AC) affects 36% of the US population [1].
- Topical eyedrop drug delivery to the front of the eye is extremely ineffective but remains the mainstream treatment of AC.
- Less than 5% of topically applied drugs reach the target tissue and administer a therapeutic effect.
- To overcome current dosing issues with topical eyedrops, thermo-responsive hydrogels can be applied to the inferior fornix for sustained drug delivery.
- Chitosan can be degraded by lysozyme, the highest concentration enzyme tear protein and was therefore utilized for controlled degradation.

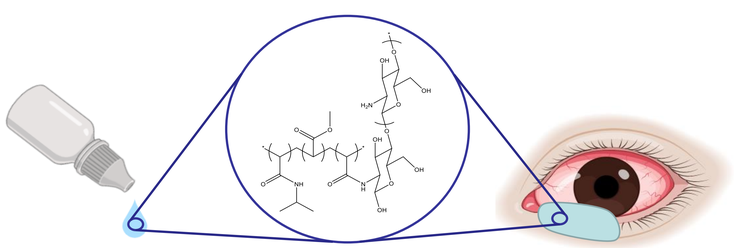

Figure 1: Schematic of thermo-responsive eyedrops composed of chitosan crosslinked pNAM for controlled degradation and sustained drug delivery in the treatment of AC.

Methods:

1. Chemical Synthesis

The base thermo-responsive terpolymer of poly(n-isopropylacrylamide -co- acrylic acid -co- methyl methacrylate) (pNAM) was synthesized by free radical chemistry. Chitosan was incorporated with pNAM either by crosslinking with EDC to produce covalently crosslinked networks (CCN) or by ionic charge to produce polyelectrolyte complexes (PEC). Varying concentrations of chitosan were incorporated to evaluate the impact on hydrogel properties. The full synthetic scheme is visualized in Figure 2.

2. Hydrogel Properties

The extent of hydrogel formation was determined by pH measurement and FTIR analysis. The mechanical properties and lower critical solution temperature (LCST) were determined utilizing a rheometer fitted with a parallel Peltier plate. The swelling properties of the produced hydrogels were determined by gravimetric analysis.

3. Hydrogel Performance

The degradation of the thermo-responsive hydrogels was tested by gravimetric analysis or rheology in order to determine the influence of lysozyme versus swelling. The drug release was quantified by HPLC over a one-week period.

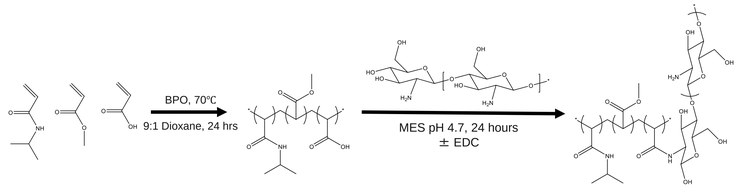

Figure 2: Synthetic scheme of pNAM and CCN-CTS-pNAM or PEC-CTS-pNAM

Result: pNAM Synthesis

The synthesis of the base thermo-responsive pNAM terpolymer was found to highly reproducible between batches (8 total) as shown in Table 1

Polymer Composition (%)			Polymer Size
NIPAAm	AA	MMA	MW (kDA)
80.28 ± 0.07	4.10 ± 0.06	15.61 ± 0.08	61.5 ± 5.3

Table 1:Average polymer composition and molecular weight of pNAM across all batches

Result: Hydrogel Synthesis and Characterization

The physical properties of the produced CCN and PEC hydrogels with varying chitosan concentrations are shown in Table 2. Increasing the amount of chitosan crosslinker resulted in an increase in solution pH and LCST.

Table 2: Physical properties of produced CCN and PEC thermogels

Polymer Network	pH	LCST (℃)
pNAM	4.37 ± 0.2	21.0 ± 0.6
1-CCN-CTS-pNAM	4.94 ± 0.1	24.9 ± 1.1
3-CCN-CTS-pNAM	5.68 ± 0.1	27.8 ± 2.2
3-PEC-CTS-pNAM	5.03 ± 0.0	26.5 ± 2.9
5-CCN-CTS-pNAM	6.51 ± 0.1	29.5 ± 0.5

The mechanical properties of the CCN and PEC was determined by rheology. Frequency sweeps were utilized to compare the change of mechanical attributes with chitosan incorporation as shown in Figure 4. Increasing the amounts of chitosan resulted in higher moduli. Incorporating chitosan by covalent linkages or ionic interaction did not have a significant change on moduli.

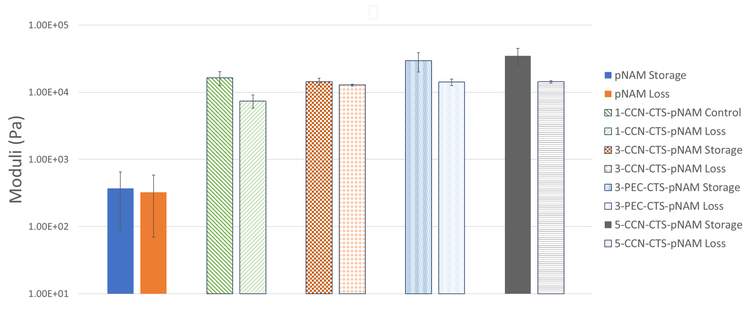

Figure 4: Frequency sweep analysis of the produced CCN and PEC thermogels evaluated at 1 rad/s

Figure 5 shows all of the produced thermogels are highly volume consistent by swelling analysis.

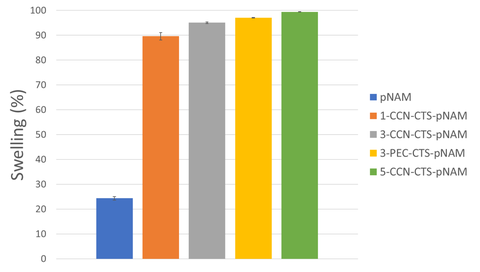

Figure 5: Swelling properties of the produced CCN and PEC thermogels

Result: Degradation and Drug Release Analysis

The degradation of the produced CCN and PEC hydrogels was determined by gravimetric analysis and rheological analysis. It was determined that gel swelling had a significant impact in degradation compared to lysozyme which did not have a statistical impact as shown in Figures 6 and 7. The release of anti-allergy drug Ketotifen Fumarate is shown in Figure 8.

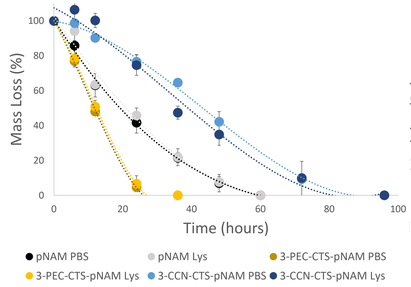

Figure 6: Degradation of CCN and PEC hydrogels incubated with a supernatant containing no lysozyme or the tear concentration of lysozyme.

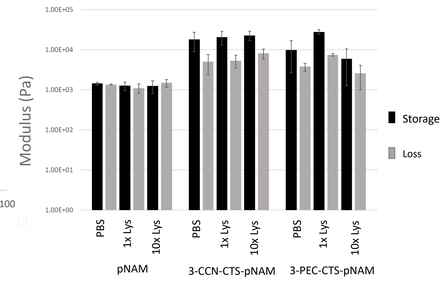

Figure 7: Degradation of CCN and PEC dissolved with no lysozyme, the tear concentration of lysozyme or 10x the tear concentration of lysozyme and incubated for 48 hours before frequency sweep analysis (graph shown at 1 rad/s)

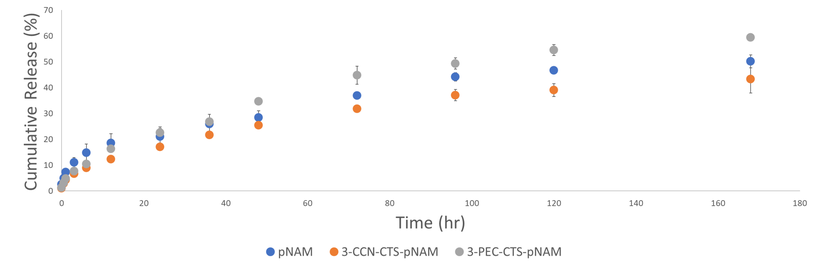

Figure 8: Release of anti-allergy drug Ketotifen Fumarate quantified by HPLC determination over a one-week period.

Conclusions

1. High reproducibility of the base terpolymer pNAM and tunable LCST.
2. Successful incorporation of chitosan as CCN or PEC and determination of increasing pH, LCST and mechanical attributes.
3. CCN and PEC hydrogels are very volume consistent.
4. CCN and PEC hydrogels degrade over a few days where gel swelling is the major factor for degradation.
5. Drug release data shows that the produced gels deliver 40-60% of a standard single dosage of anti-allergy drug Ketotifen Fumarate over a one-week period.
6. Currently completing Live/Dead and MTT assays for determination of *in vitro* thermogel performance.

References

Shaker, M. and E. Salcone (2016). "An update on ocular allergy." Current Opinion in Allergy and Clinical Immunology 16(5): 505-510.

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