Development of an in situ gelling ocular vehicle: A design of experiments (DOE) approach

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Introduction

In situ gelling eye drops have been explored for their promise to extend residence time of topical therapies on the eye.[1-3] Mono- and divalent cations and mucins allow for activation of polymers to form a gel network and provide targets for mucoadhesive polymers. Previous work in our lab demonstrated synergistic interactions between two ion-activated polymers (gellan gum (GG) and carrageenan (CG)) for the development of an in situ gelling ocular drug delivery vehicle (ODDV). The purpose of this work was to expand on this formulation with the addition of the mucoadhesive element, hyaluronan (HA) to optimize an ODDV using a space filling mixture design (SFMD) DOE approach.

Background

Several key physiological factors make the ocular surface a prime target for drug delivery including the presence of cations (Ca^{2+}, K^{+}, Na^{+}) and their crosslinking interactions. The mucins involved in the ocular surface include secreted gel-forming (MUC2, MUC5AC) and membrane-associated (MUC1, MUC4, MUC16) types that are formed in the goblet cells of the conjunctiva. Numerous gelation mechanisms have been identified including temperature and pH but polymers whose gelation mechanism revolves around crosslinking with ions are of interest because of their presence in tear fluid.[3] We explored polymers with ion-sensitive mechanisms as well as viscosity enhancers for the development of a novel combination for an ODDV. Since the factors of a mixture are parts of a whole, they cannot be manipulated independently as in a typical factorial design. The SFMD narrows the experimental design while covering more of the experimental region.

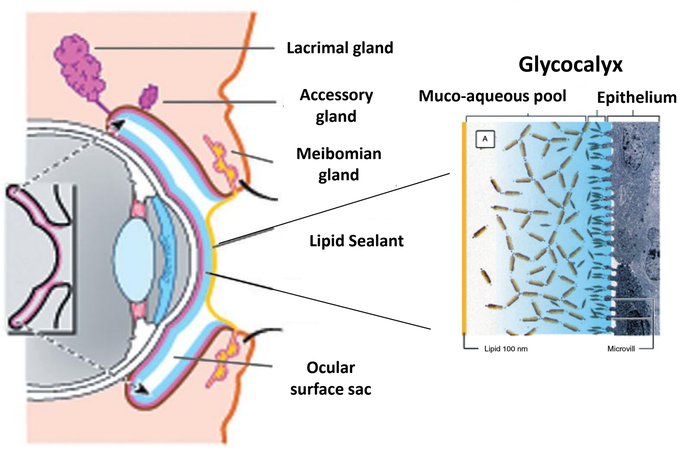


Figure 1: Ocular Surface holds keys to in situ gels. Lacrimal glands secrete ion-rich tear fluid to the surface of the eye providing the basis for gel formation. Gel-forming and membrane-associated mucins in the tear film and corneal epithelium serve as targets for polymer mucoadhesion. (Adapted from Cher I. (2012) *Clin. Exp. Ophthamol*, **6**, 634-43)

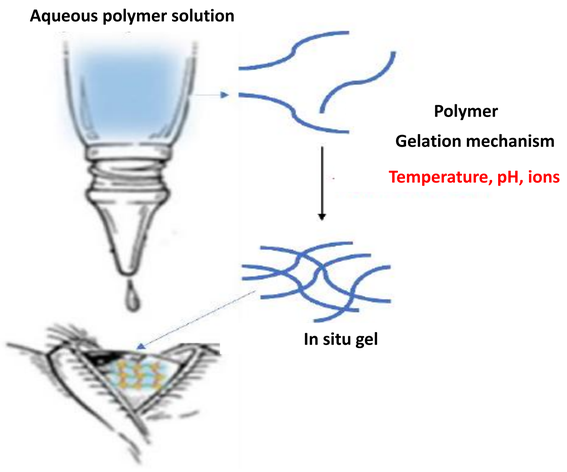


Figure 2: In situ gelation mechanisms. Aqueous polymeric solutions are applied to the eye in drop form. Temperature, pH, or ions allow the formation of a polymer network to form resulting in the production of an in situ gel. (Adapted from Cassano et al. (2021) *Gels*, **7**, 130)

Methods

Sample Preparation:

- %(w/v) solutions of low acyl gellan gum (GG) (CP Kelco), sodium alginate (SA) (Dupont), carrageenan CG-129 (CG) (CP Kelco), and hyaluronan (HA) (Santa Cruz Biotechnology) were made by adding polymer in 10 mL deionized water and heating with stirring between 70 – 90 ˚C for 10 minutes or until polymer was dissolved
- Polymer was then left to cool and set at room temperature overnight.
- Simulated Tear Fluid (STF) was prepared with 6.78 g/L sodium chloride, 2.18 g/L sodium bicarbonate, 0.0084 g/L calcium chloride, and 1.38 g/L potassium chloride. Sodium hydroxide was used to adjust STF to pH 7.4.

DOE:

- The SFMD was built in JMP Pro 18 (JMP, Cary, NC) in which three factor ranges, % GG (0.5 – 0.8), % CG (0.1 – 0.5), % HA (0.1 – 0.2) were set based on previous studies.
- 18 formulations were generated, and rheology was run as described below.

Rheology:

- 1 mL polymer was combined with 1 mL deionized water or STF and rotated on a rotator genie (Scientific Industries, Bohemia, NY) at low speed for 5 revolutions.
- 1.1 mL of sample (polymer with or without STF) were added to a Kinexus rotational rheometer (Malvern Panalytical, Westborough, MA) and a series of rheological sequences were performed.
- Amplitude oscillation performed over a shear strain range [0.100 – 100.00 Pa] at 34 ˚C.

Results

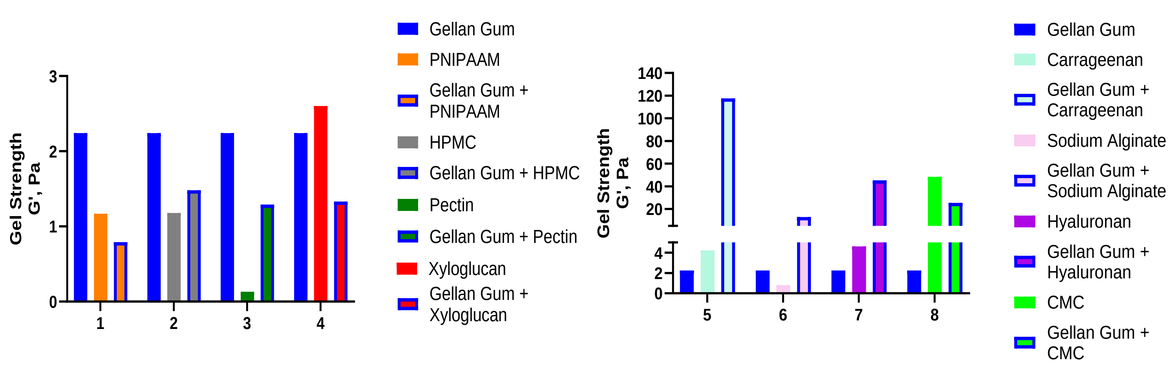


Figure 3: Polymer Synergy Screening. Binary polymer combinations were screened for potential synergy. Amplitude oscillation was performed on singular polymer and polymer combinations in the presence of STF (n = 1).

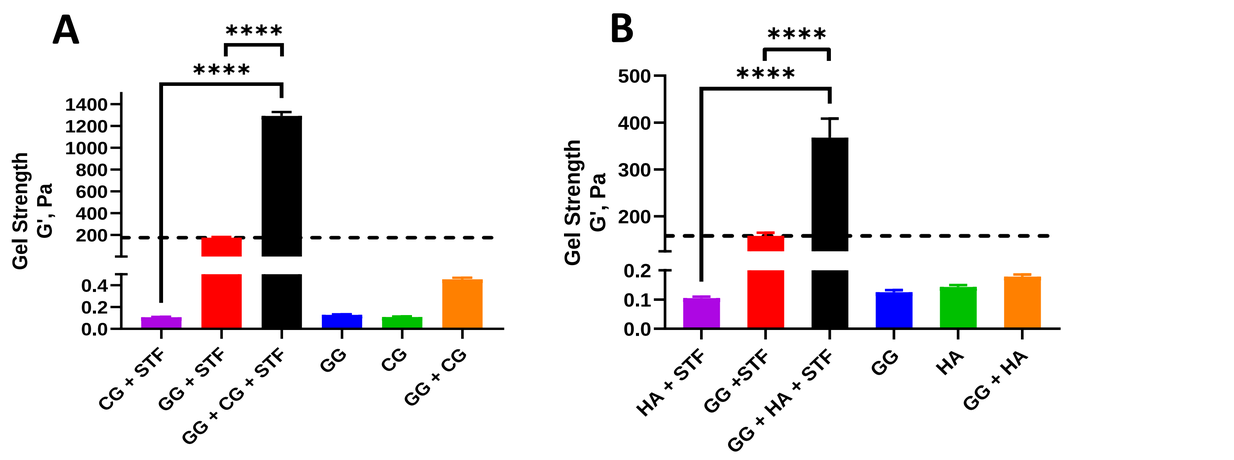


Figure 4: Novel polymer combinations show potent synergism. A. Amplitude oscillation was performed on samples of gellan gum (GG) and carrageenan (CG) with and without STF as singular polymer samples and binary polymer combinations (n = 3). ****, P < 0.0001, Student's t test, GG + CG + STF vs CG + STF, GG + CG + STF vs GG + STF (n = 3). Data shown as mean ± SD. **B.** Amplitude oscillation was performed as in **A** on samples of gellan gum (GG) and hyaluronan (HA) (n = 3). ****, P < 0.0001, Student's t test, GG + HA + STF vs HA + STF, GG + HA + STF vs GG + STF (n = 3). Data shown as mean ± SD. Dashed lines indicate response additivity of Bars 1 & 2 in both panels.

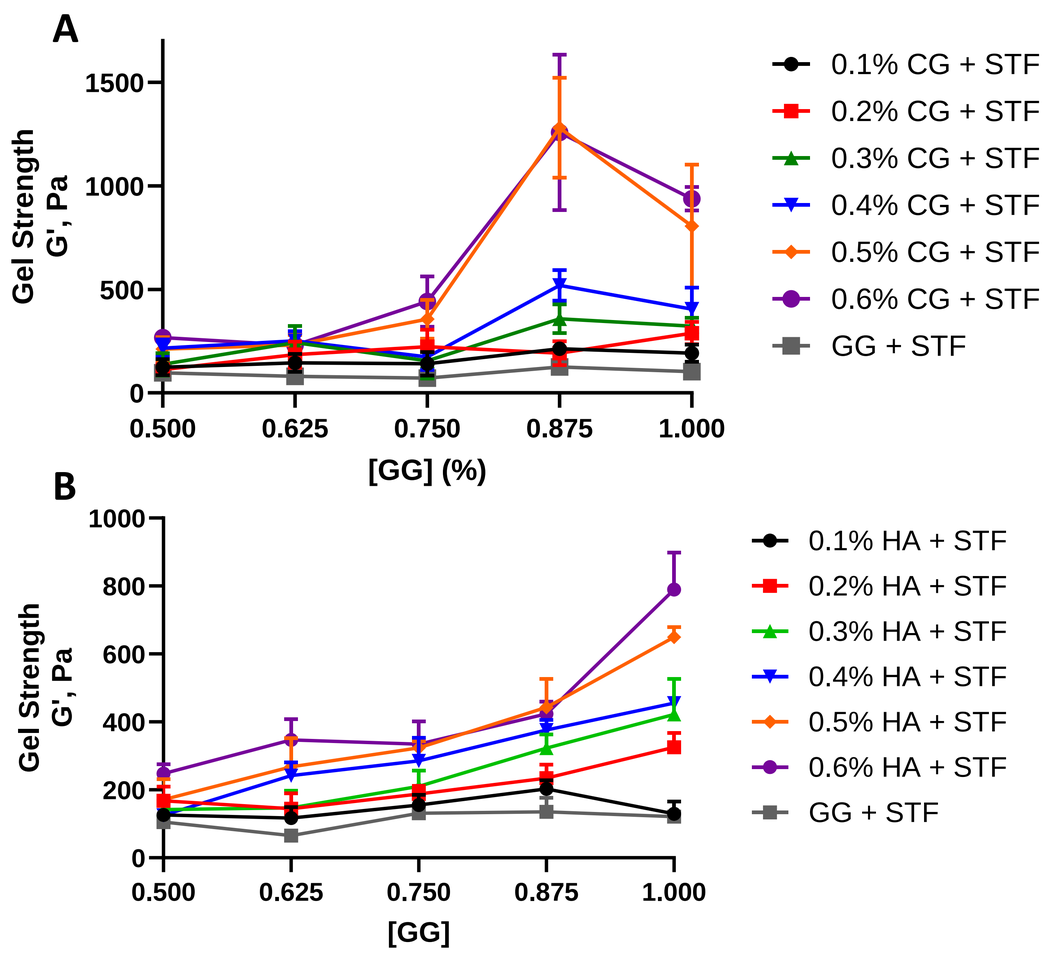
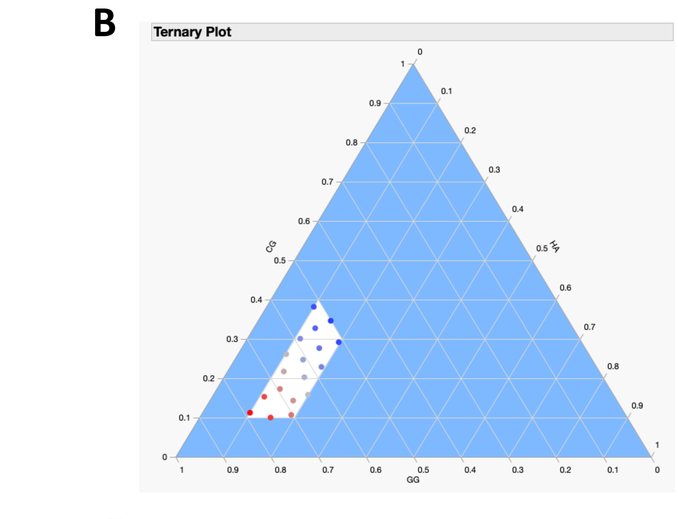
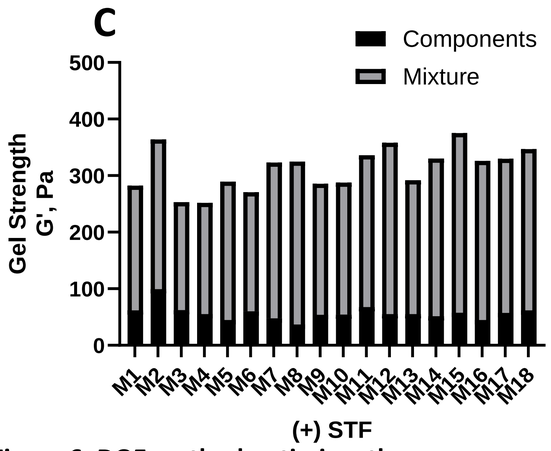


Figure 5: Binary combinations tested for concentration dependent synergy. A. Amplitude oscillation was performed over a range of concentrations of gellan gum (GG) (0.5% - 1.0% (w/v)) and carrageenan (CG) (0.1% - 0.6% (w/v)) with and without STF as singular polymer samples and binary polymer combinations (n = 3). Data shown as mean ± SD **B.** Amplitude oscillation was performed over a range of concentrations of gellan gum (GG) (0.5% - 1.0% (w/v)) and hyaluronan (HA) (0.1% - 0.6% (w/v)) with and without STF as singular polymer samples and binary polymer combinations (n = 3). Data shown as mean ± SD

A

Σ	GG	CG	HA
1	0.750	0.100	0.150
2	0.787	0.112	0.101
3	0.737	0.153	0.110
4	0.642	0.158	0.200
5	0.703	0.107	0.190
6	0.681	0.143	0.176
7	0.628	0.203	0.170
8	0.579	0.229	0.193
9	0.636	0.261	0.102
10	0.694	0.173	0.133
11	0.664	0.217	0.119
12	0.511	0.292	0.198
13	0.500	0.346	0.153
14	0.518	0.382	0.100
15	0.587	0.301	0.112
16	0.542	0.327	0.130
17	0.608	0.247	0.145
18	0.559	0.277	0.164





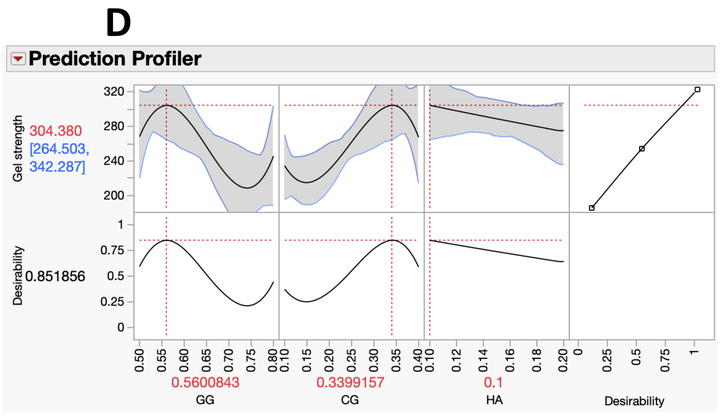


Figure 6: DOE method optimizes three component mixture formulation. A. SFMD generates 18 distinct formulations for testing. **B.** Ternary plot defines the experimental space. Colored dots indicate distinct formulations and fill the concentration gaps typical of classical boundary point mixture designs. **C.** Amplitude oscillation was performed on 18 formulations composed of three polymer mixtures containing gellan gum %(GG) (0.5 - 0.8 (w/v)), carrageenan %(CG) (0.1 - 0.5 (w/v)), and hyaluronan %(HA) (0.1 - 0.2 (w/v)) with and without STF as singular component samples and Mixture (M1-M18) samples (n = 3). Components plotted as the sum of G' of individual polymer samples. Data shown as mean ± SD. **D.** Optimized formulation using analyzed data via a generalized regression model and prediction profiler in JMP Pro 18.

Conclusions

- Potential synergy observed in early polymer screenings was confirmed in two combinations (GG + CG) and (GG + HA).
- Potent synergy was observed at high concentrations of GG + high concentrations of CG or HA.
- Addition of a third polymer to a previously synergistic two polymer combination did not hinder synergy when optimized using a design of experiments approach.
- Nonlinear blending was observed in the mixtures indicating concentration independent gelation supporting the observation of synergistic interactions.
- HA can target gelling mucins in tear film on the surface of the eye, adding to retention of the ODDV on the eye.
- The DOE approach identified an optimal formulation (0.56% GG, 0.34% CG, 0.10% HA) for a three polymer combination that shows promise as an ODDV for prolonged delivery based on the synergistic interactions.

Acknowledgements

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