Calcium Gluconate Mediated In Situ Gelling of Alginates for Ocular Drug Delivery

Kenneth Reed* and Mary Lankford
Belmont University College of Pharmacy, 1900 Belmont Boulevard, Nashville, TN 37212, USA
Office Phone: 615-460-8126
FAX: 615-460-6537
E-mail: ken.reed@belmont.edu

Abstract

Purpose: Alginates form stronger gels when cross linked by divalent cations (e.g., Ca^{++}) than when in the presence of monovalent cations (e.g., Na^{+}). Previous work indicated that Ca^{++} can be displaced from calcium gluconate (CaG) by monovalent cations that are predominant in tear fluid. The purpose of this study was to determine the in situ gelling properties and drug release rates of alginate plus CaG formulations.

Methods: Alginate solutions containing various concentrations of CaG were manufactured. Viscosities of the manufactured solutions were measured at ambient temperature to mimic patient storage and at eye temperature to forecast in situ gelling potential. The rates of drug release from test solutions were measured by using tryptophan as a model drug.

Results: Alginate formulations were found to form stronger gels under physiological conditions when adequate amounts of CaG were added than when formulations containing only alginate were tested. Additionally, tryptophan was released more slowly when CaG was added to alginate. Alginate plus CaG formulations were found to be shear thinning, which should result in better patient acceptance.

Conclusions: The inactive ingredient CaG can be used to improve the use of alginate for ocular in situ gelation.

Keywords: Ocular, Alginate, In-Situ, Gelation, Release

Introduction

The delivery of drops from an ophthalmic bottle that is squeezed by the patient is the favored presentation for ocular medications. The drop size of marketed products is normally in the range of 25 to 56 µl, with an average drop size of 39 µl (1). Although the eye may hold up to 30 µl of fluid for a short period of time, the volume of fluid is quickly reestablished to 7-10 µl (1). Thus, the majority of administered medication is quickly removed from the front of the eye. Additional physiological processes aid in the rapid removal of ocular medications delivered to the front of the eye. Tear fluid is constantly produced at a rate of 0.5 to 2.2 µl per min (1). Ocular tear flow is an effective protective barrier to foreign objects and fluids, such as aqueous based medications, that are deposited onto the eye. The blinking of the eye occurs at an extremely high shear rate of 4,250 to 28,500 s^{-1} and assists in the removal of debris from the front of the eye (1,2). These processes work together to facilitate the rapid drainage of applied medication to the nasolacrimal duct, at which location the drug can be absorbed into the systemic circulation (1). The result of this rapid drainage, along with a number of other factors resisting the absorption of the drug to its site of action, results in poor bioavailability for simple aqueous based ocular medications (1,3).

The viscosity of a preparation is a measure of its ability to resist flow. It has been demonstrated that both high viscosity hydrocarbon based ointments and hydrogels composed of water soluble polymers resist drainage from the front of the eye. This results in enhanced ocular bioavailability and fewer side effects (3). Ointments have the additional benefit of resisting dilution by the tears, but patients have significant blurred vision when using ointments and their use is messy (3). These properties result in patients commonly using ointments only at night (3).

The use of in situ gelling, or gel forming solutions (GFS), has intrigued numerous investigators due to the potential of administering an ophthalmic medication as a drop that then forms a viscous gel upon contact with the eye. That is, GFS undergo phase transitions from a liquid (sol) to a semisolid (gel) when exposed to eye temperature, physiological pH of the tear, and/or the ionic (cations) composition of the tear fluid. Advantages to the GFS approach include greater patient acceptance while still increasing ocular bioavailability. The GFS approach avoids the disadvantages in the use of high viscosity ophthalmic products that often result in blurred vision, tearing, and deposits on the eyelids (4). It is also possible that the release of drug from the gelled polymer matrix may be

slowed due to the increase in viscosity at the site of action and hence the duration of a drug's action may be prolonged. For the in situ gel approach to be successful, the viscosity of the GFS product must be sufficiently low so as to be free flowing and administered as a drop without the patient having to apply excessive expulsion pressure to the ophthalmic bottle. It is stated in the literature that this initial viscosity should be 5 to 1,000 mPa·S (1). Results generated in our lab indicated that the initial viscosity (23° C and shear rate of 1 s^{-1}) should be about 5.0 Pa·S or less (5). The GFS preparations tested in our lab were free flowing (preparations were shaken due to thixotropic behavior), demonstrated shear thinning flow, and are expected to have significantly lower measured viscosities at the high shear rate encountered when the GFS preparation is expelled through the dropper tip of an ophthalmic bottle (5).

In situ gelling systems (GFS) based upon temperature have the disadvantage of requiring high surfactant concentrations (e.g., Poloxamer >20%), which may cause eye irritation (1), and were found to be unsuitable when used in human volunteers (3). A current trend is to combine a copolymer with Poloxamer in order to reduce the concentration of the Poloxamer in the formulation (1). Alternatively, polyacrylic acid polymers (PAA) may be used to formulate a GFS. In this approach, the GFS formulation is prepared at a low pH (low viscosity) and then the pH is raised when the GFS encounters the tear buffered at a physiological pH (1). The low pH needed for this approach may cause ocular irritation as PAA is an effective buffer. Low concentrations of PAA are needed so as to not overwhelm the ability of the tear fluid to reestablish a physiologically acceptable pH environment. PAA has been combined with other viscosity enhancing polymers in order to reduce the needed PAA concentration (1).

Many studies have been conducted upon GFS polymers in which the phase transition is triggered by ions present in the tear. This approach has a low concentration of any surface active components and enables the buffering of the preparation at neutral pH (4). Solutions composed of the polymer gellan form gels in response to tear cations (3). Gellan is used in the marketed product Timoptic XE® as an in situ gelling polymer which in turn results in improved ocular bioavailability (3).

Alginates are polysaccharides composted of repeating monomer units of β-D-mannuronic acid and α-L-guluronic acid that are referred to as M and G blocks, respectively (6,7). Alginates vary in the sequencing of the M and G blocks with some alginates having a greater percentage of G blocks. Alginates rich in G blocks readily form firm gels in the presence of Ca^{++} ions in contrast to their interaction with monovalent cations (4,7). The formed alginate gels are described as having an "egg-box" structure and can form at low concentrations of Ca^{++} (7-9). The gelation of alginates by calcium is proposed to occur by an initial dimerization step followed by an aggregation of the formed dimers (9). Alginates have been shown to physically entrap numerous molecules and have the potential to delay the release of drugs from the polymer matrix (6,7). Alginates are biodegradable, biocompatible, and mucoadhesive (1,6). Studies have indicated that alginates are non-irritating to the eye and are safe for ocular use (6,7,10,11).

It was reported in the literature that an alginate concentration of 2% (w/w) or greater is needed in order to form a sufficiently strong gel so as to be retained in the eye (12). However, alginate concentrations of 2% or higher were not free flowing (12). Another group found that 1.5% alginate was too viscous to be administered as a drop but demonstrated a human contact time of 31.3 min versus the human contact time of 11.7 min for 0.4% alginate (11). The biopolymer alginate is known to be mucoadhesive and these results indicate that it is well retained in the eye when presented as a strong gel. The results of a comparative study indicated that a lower viscosity alginate solution was retained in the eye for a shorter period of time than gellan and carrageenan, but for a longer period of time than a simple solution (10). The ocular retention results for alginate look very similar to hydroxypropyl methylcellulose (HPMC), although slightly higher (10). Alginate (the salt of alginic acid) has been investigated in the preparation of possible GFS due to its property of forming gels in the presence of cations that are present in the tear fluid (1,3). A high G content alginate formulation was shown to prolong the pharmacological effect of Pilocarpine as compared to a simple solution when administered to rabbit eyes (7). An alginic acid based preparation of Carteolol demonstrated a longer duration of intra ocular pressure lowering than a simple solution (6). It appears that alginate prolongs the release of pilocarpine and carteolol, but is not retained in the eye as well as gellan and carrageenan. It is possible that the relatively low retention of alginate is due to a high concentration of monovalent cations (Na^{+},K^{+}) and low concentration of Ca^{++} in the tear fluid (10,12). The Carteolol glaucoma product Mikelan LA is reported to be long lasting because of increased ocular retention that is due to alginic acid in the formulation (13). The administration of the marketed product Mikelan LA® is reported to cause blurred vision in patients, indicative of gel formation in the eye (13). Perhaps alginate minimally undergoes a phase transition in the eye, but possesses some retention due to initial viscosity and mucoadhesive properties. Overall, the results from the literature indicate that it is well retained in the eye when presented as a strong gel, which makes alginate an interesting candidate for use as an in situ gelling polymer.

Alginates have been used in combination with other polymers to form GFS formulations to improve functionality (3). Balasubramaniam and Pandit found no therapeutic advantage by combining alginate with gellan as compared to gellan alone (2). Their data indicated that the addition of alginate to gellan resulted in formulations with less of a drug burst release effect than expected (2). The addition of 2% HPMC to alginate resulted in preparations that showed a trend of longer ocular retention than alginate or HPMC alone (14). An alginate and HPMC combination formulation worked more efficiently as a Ciprofloxacin GFS when compared to alginate by itself (15). The combination of alginate and Gelrite (a purified form of gellan) was found to slow the release of the drug Matrine to a greater extent and to show longer contact times in humans than alginate or gelrite alone (11). Alginate was found to have greater ocular retention than a comparator solution (14). A GFS composed of alginate and pluronic demonstrated more favorable rheological characteristics under physiological conditions than alginate alone (12).

It is believed that the gellan in Timoptic XE interacts with the majority monovalent cation sodium in the tear to form a gel which resists removal from the eye (16). However, it is known that a stronger gel is formed when gellan complexes with divalent cations (e.g., Ca^{++}) rather than monovalent cations (e.g, Na^{+},K^{+}) (4). Calcium gluconate is used as a source of calcium in parenteral nutrition that enables the co-administration of phosphate without precipitation (17). In previous work, it was demonstrated that the inclusion of calcium gluconate (CaG) with gellan based GFS provides a source of calcium that is displaced by Na^{+} and K^{+} ions present in the tear fluid. This resulted in a stronger formed gel than when gellan was aggregated by monovalent cations that are predominant in the tear fluid (18). Due to the increased strength of alginate gels that are formed in the presence of Ca^{++} rather than Na^{+} and K^{+}, it is of interest to determine if the inclusion of CaG with alginate has the potential to produce a more efficient GFS than the use of alginate alone. Additionally, the release rate of the water soluble amino acid tryptophan, acting as a model drug, will be measured for alginate and alginate plus CaG formulations.

Materials and methods

Materials

Protanal® LF 200 FTS sodium alginate was a gift from FMC Corporation (Philadelphia, PA). Calcium D-gluconate, sodium chloride, potassium carbonate, DL-tryptophan, tromethamine, and lysozyme were purchased from Sigma-Aldrich (Saint Louis, MO). Calcium chloride dihydrate, potassium chloride, and sodium citrate dihydrate were purchased from Fisher Scientific (Fair Lawn, NJ). Magnesium chloride hexahydrate, hydrochloric acid 1N, and sodium hydroxide 1N were purchased from Acros Organics (Morris, NJ). The biotech cellulose ester dialysis membrane tubing (MWCO = 3.5-5 kD) was purchased from Spectrum Laboratories (Rancho Dominguez, CA). The UV/VIS 5500 spectrophotometer was purchased from Shanghai Metash Instruments Co., LTD (Shanghai, China). The Haake ViscoTester 550 rotational viscometer was purchased from Thermo Fisher Scientific (Newington, NH).

Simulated Tear Solutions and Sample Preparations

Calcium simulated tear solution with citrate buffer (CaST) and physiological artificial tear solution (PATS) (18) were prepared to simulate *in vivo* ocular conditions by combining biologically relevant salts – and, in the case of PATS, lysozyme – at the concentrations listed in Table 1. CaST was prepared for use in testing the rate of drug release. A citrate buffer was used in order to provide a stable pH throughout the duration of the experimental drug release runs. PATS was prepared for use in testing viscosity at eye temperature (34°C± 1°C) and contained a carbonate buffer to more closely match the composition of the tear fluid. These solutions were titrated to a target pH of 7.4 ± 0.4 using 1N NaOH and 1N HCl.

Table 1. Composition of Simulated Tear Solutions

Composition of Calcium Simulated Tear Solution (CaST)		**Composition of Physiological Artificial Tear Solution (PATS)**	
Component	**Concentration (w/w)**	**Component**	**Concentration (w/w)**
Sodium Citrate • $2H_2O$	0.121%	Potassium Carbonate	0.171%
Sodium Chloride	0.728%	Sodium Chloride	0.752%
Calcium Chloride • $2H_2O$	0.0985%	Calcium Chloride • $2H_2O$	0.00470%
Potassium Chloride	0.0924%	Magnesium Chloride • $6H_2O$	0.00712%
		Lysozyme	0.207%
1N NaOH and/or 1N HCl	q.s. to pH 7.4	1N NaOH and or 1N HCl	q.s. to pH 7.4

The series of sample gel formulations containing alginate, CaG, and tryptophan included eight or more samples each of eight different sample preparations. Tryptophan preparations were manufactured with a final concentration

of 0.32% (w/w) which is equivalent on a molar basis to Timolol maleate concentration of 0.68%. Additionally, sixteen samples of 0.32% (w/w) tryptophan control solution were prepared. The samples preparations were manufactured according to the compositions listed in Table 2. A 3% (w/w) sodium alginate stock solution was prepared from Protanal® LF 200 FTS sodium alginate powder that was added to water, heated to > 80° C, and stirred with an overhead mixer until the powder went into solution. To prepare the sample gels, sufficient tryptophan powder was gradually added to deionized water, heated to about 60°C, and then transferred to a sonicator bath to aid in dissolution. Measured amounts of 3% sodium alginate stock solution were added to give a final concentration of 1% (w/w). Measured amounts of Calcium D-gluconate (CaG) stock solution (3%, w/w) were added to give final concentrations of 0.25%, 0.28%, and 0.30% (w/w). The sample preparations, including the tryptophan control solution, were titrated to a target pH of 7.4 $\pm$ 0.4 using 0.1M tromethamine and stirred with an overhead mixer for homogeneity. Tromethamine was used as a non-ionic pH adjusting agent to prevent the addition of cations to the preparations.

Table 2. Composition of Sample Gel Preparations.

Solution ID	Tryptophan Concentration (w/w)	Protanal® LF 200 FTS Na Alginate Concentration (w/w)	Calcium D-Gluconate Concentration (w/w)
Tryp-1	0.32%	0.0%	0.0%
Alg-1	0.0%	1.0%	0.0%
Alg-2	0.0%	1.0%	0.25%
Alg-3	0.0%	1.0%	0.28%
Alg-4	0.0%	1.0%	0.30%
Tryp-Alg-1	0.32%	1.0%	0.0%
Tryp-Alg-2	0.32%	1.0%	0.25%
Tryp-Alg-3	0.32%	1.0%	0.28%
Tryp-Alg-4	0.32%	1.0%	0.30%

Viscosity Studies

Viscosities of the alginate-CaG solutions were measured using a rotational viscometer. The viscosity studies focused on the flow behavior of alginate-CaG sample preparations both during patient storage conditions (ambient temperature, 23°C $\pm$ 1°C) and at ocular conditions (eye temperature, 34°C $\pm$ 1°C) (18). The viscosity under ocular conditions was evaluated by mixing alginate-CaG solution with PATS at a 5:1 ratio of 50 g of alginate-CaG preparation to 10 g of PATS (18). The 5:1 ratio is believed to more closely match the administration of a normal drop size (39 µl) of an ocular medication to a basal tear volume of 7-10 µl (1). Due to the thixotropic nature of the sample preparations, the test samples were allowed to rest for at least twelve hours. For each set of viscosity measurements, the sample being analyzed was carefully transferred, with minimal agitation, from the storage bottle to the viscosity vessel in amounts of 37.0g – 37.6g. Viscosity was measured at 12 different shear rates between 1 s^{-1} and 200 s^{-1} under both patient storage and ocular conditions. After acquiring viscosity information from the rested gel samples, the samples were sheared at a high shear rate (1,000 s^{-1}) for 90 seconds and then a viscosity sweep was performed at shear rates (12 steps) between 1 s^{-1} and 200 s^{-1}.

Shear stress (τ)-shear rate ($\dot{\gamma}$) curves were fitted to a power-law fluid model (viscosity = $K\dot{\gamma}^{n+1}$) referred to as the Ostwald-de-Waele relation using the HAAKE RheoWin Data Manager software (Fisher Scientific, Newington, NH). In a power-law fluid, a higher value for the parameter K (flow consistency index) indicates a higher level of viscosity. The value for K is the viscosity in mPa·S when the shear rate equals 1 sec^{-1}. The value *n* represents the flow behavior index, where a value < 1.0 indicates shear-thinning, or pseudoplastic, behavior. K-values from rested (K' or primary K) and shear-thinned (K'' or secondary K) sample preparations were fitted from data collected under both simulated ocular and patient storage conditions. These values are referred to in this work as ET K' and ET K'' (eye temperature of 34° C ±2° C) and RT K' and K'' (23° C). The magnitude of the differences in the K' versus K" values are important in determining the thixotropic nature of the alginate-CaG solutions. An ideal formulation will exhibit enough shear-thinning to be administered from an eyedropper bottle when shaken (RT K'' ≤ 5.0 Pa·S) (5) and become highly viscous upon entering the eye (as evaluated by ET K').

In the packaging condition simulation, RT K' was meant to represent the viscosity during storage in eyedropper packaging, and RT K'' represented the viscosity in a shaken eyedropper bottle. In the ocular condition simulation, ET K' was meant to represent the viscosity upon first entering the eye, and ET K'' represented the viscosity during blinking, which applies a high shear rate.

Curve fit analysis was also performed using the Bingham model which allowed for the calculation of the yield value (Tau) for each gel formulation. XLSTAT (Addinsoft, New York, NY, a statistical software add-on for Microsoft Excel (Microsoft Corporation, Redmond, WA)) was used to perform statistical analysis. Dixon's Q-test

was performed to detect outliers to be discarded, followed by ANOVA and Tukey's post hoc tests, which were performed to detect statistically significant differences ($p \leq 0.05$) in K, Tau, and n values.

Drug Release Studies

The rates of tryptophan transmembrane diffusion from each tryptophan-containing solution were measured and analyzed in a series of release studies. The amount of UV (280 nm wavelength) absorbance of tryptophan in CaST was measured using a UV/VIS spectrophotometer. The absorbance was measured at timed intervals over a period of approximately 30 to 99 hours for each formulation. A calibration curve mapping absorbance at 280nm to tryptophan concentration was generated for each of the three instruments used in the study. The calibration curves were calculated using linear regression for 7 tryptophan concentrations (between 0.016% and 0.32%), were not forced through zero, and were linear in the absorbance region measured in the study. Concentration values in mg/ml were calculated using these calibration curves.

The tryptophan release studies were conducted similarly to those described previously (5,20). Cellulose ester dialysis membrane (molecular weight cutoff of 3,500-5,000 Da) was divided into ten centimeter segments and soaked in deionized water for greater than 24 hours to remove contaminants and preservatives. Thereafter, the dialysis tubing was equilibrated in CaST for at least 24 hours. The molecular weight cutoff was selected to easily allow tryptophan (MW = 204) to diffuse across the semipermeable membrane while restricting the movement of the alginate polymer. Although the molecular weight of calcium gluconate allows it to pass through the dialysis membrane, it does not absorb appreciably in the 280 nm UV wavelength region. For each tryptophan-containing preparation, one gram of formulation was loaded into a ten centimeter piece of dialysis tubing, with each side of the tubing closed tightly with a clamp. Each of the filled dialysis membranes were placed in beakers filled with 100 ml of CaST. The beakers were loaded with stir bars and loosely covered with Parafilm® before being placed on a multi-station stir plate at 1,000 r/min. The absorbance (280 nm) of tryptophan in CaST was measured at regularly timed intervals using dedicated disposable UV cuvettes. After measurement, the samples were returned to their original set up with dedicated disposable transfer pipettes. The total weight of each sample was documented to account for water loss due to evaporation. Deionized water was added to each sample if there was greater than 1% deviation from the original weight.

The release studies were conducted until conditions reached a state near equilibrium, defined as a change in absorbance of less than 0.005 absorbance units per hour. Eight or more experimental runs were conducted for each formulation with at least twelve sampling times for each iteration. Sample absorbance was measured about every thirty minutes over eight hour periods, allowing the samples to rest overnight. Readings were continued the next morning and early evening for up to final absorbance measurement times of approximately 30 to 99 hours. The amount of total tryptophan released was determined by the absorbance values collected at the last time point. The percent total released is defined as: $\% \, Total \; Released = (absorbance_{time} \div absorbance_{final})(100)$

Tryptophan absorbance data from each sample were compiled and analyzed using XLSTAT. Tryptophan release-over-time curves were fitted to a four parameter logistic model which included parameters representing a shape parameter, the absorbance at the initiation of the study, the highest absorbance reached theoretically at infinity (Abs_{∞}), and a "midpoint time" referred to as T_{50} (i.e., the time at which half of the drug has been released). Dixon's Q-test was used to detect outliers to be discarded before continuing analysis. Analysis of variance (ANOVA) was performed, follow by Tukey's post hoc test, to determine statistically significant ($p \leq 0.05$) differences in mean T_{50} values between sample types (i.e. preparations with CaG concentrations of 0%, 0.25%, 0.28%, and 0.3% as well as the tryptophan control solution).

Results and Discussion

Viscosity Studies

Alginate formulations containing CaG were found to be shear thinning (Fig. 1); whereas the alginate only formulations demonstrated more Newtonian type flow (Fig. 2). The amount of shear thinning is described by the average values for *n* (flow behavior index), which is derived using the Ostwald-de-Waele mathematical fit model. If the *n* value is less than 1, then the measured gel is considered to be shear thinning (pseudoplastic flow). The lower the *n* value, the more shear thinning character the gel possesses. The average RT K' (RT primary K) Ostwald-de-Waele *n* values were 0.339 (Tryp-Alg-2), 0.305 (Tryp-Alg-3), and 0.229 (Tryp-Alg-4). All were significantly lower statistically than the *n* value of 0.821 for the alginate only formulation and are reflective of ionic interaction with Ca^{++}.

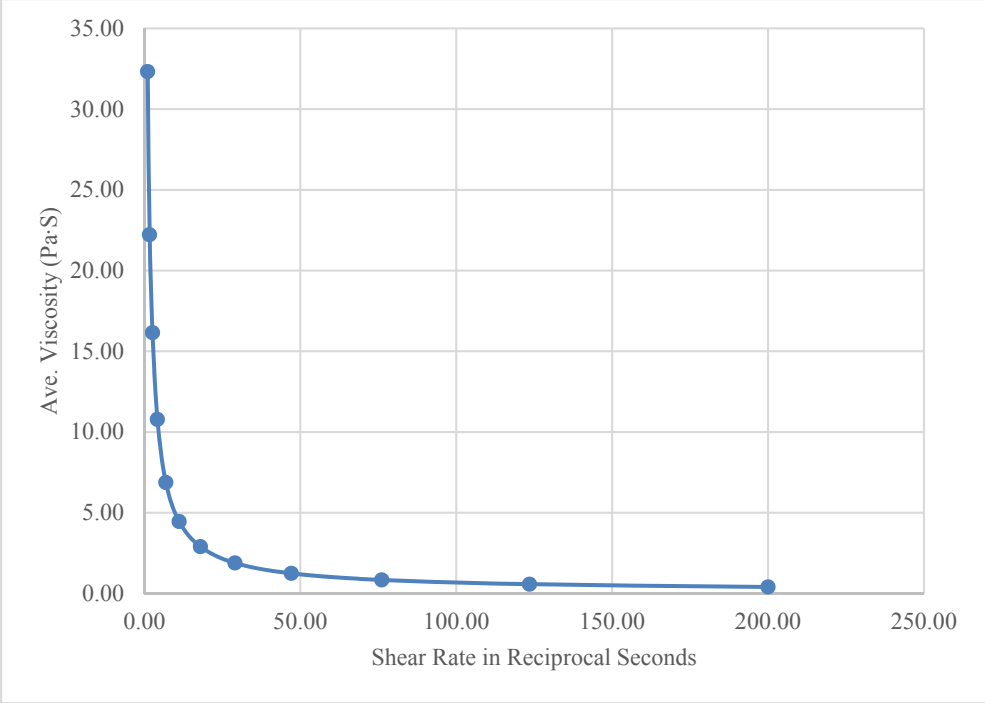

Figure 1. Average viscosities versus shear rate for the Tryp-Alg-3 formulation when mixed 5:1 with PATS, measured at eye temperature, and after sitting at rest for greater than 12 hr.

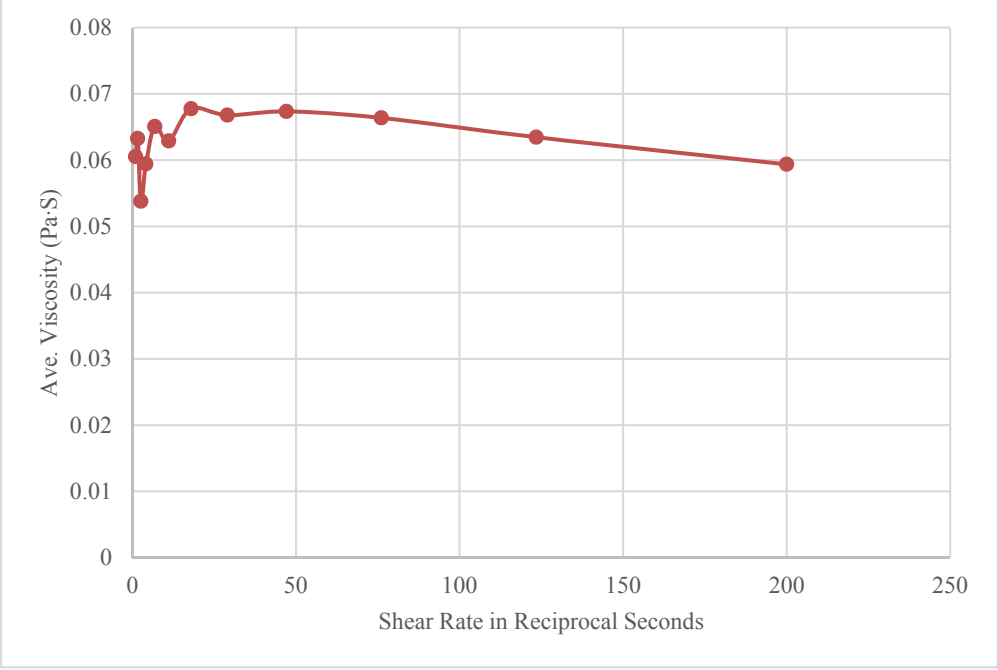

Figure 2. Average viscosities versus shear rate for the Tryp-Alg-1 formulation,when mixed 5:1 with PATS, measured at eye temperature, and after sitting at rest for greater than 12 hr.

The average ET n' (ET primary n) values are given in Fig. 3 for the Tryptophan containing alginate formulation groups. All of the alginate formulations that contained CaG were very shear thinning whereas the alginate only formulation demonstrated nearly constant viscosity with increased shear rates (Newtonian). Shear thinning gels are expected to have their highest viscosity under the low shear rate condition experienced in the cul-de-sac of the eye. The average *n* values under physiological conditions (eye temperature, mixed with PATS) for the 0.25% CaG (Tryp-Alg-2), 0.28% CaG (Tryp-Alg-3), and 0.3% CaG (Tryp-Alg-4) Tryptophan preparations, prior to high shear, were 0.148, 0.148, and 0.199; respectively. A statistical treatment clearly shows a significant ($p \leq 0.05$) difference in pseudoplastic flow behavior of Tryp-Alg-2,-3, and -4 versus Tryp-Alg-1. Tryp-Alg-4 was less shear thinning than Tryp-Alg-2 and Tryp-Alg-3. When only alginate was present in the formulation, the *n* value was 0.945 and is indicative of nearly Newtonian flow ($n = 1$) rather than shear thinning flow. The formulations

containing CaG were much more shear thinning when undergoing gelation than the alginate alone tryptophan formulation (Tryp-Alg-1). The rapid movement of the upper eyelid results in extremely high shear rates (1,3). A shear thinning gel is advantageous for the patient in that a lower viscosity is experienced during blinking. This is more acceptable to the patient than shear insensitive polymer gels that remain at constant high viscosity during the blink (1).

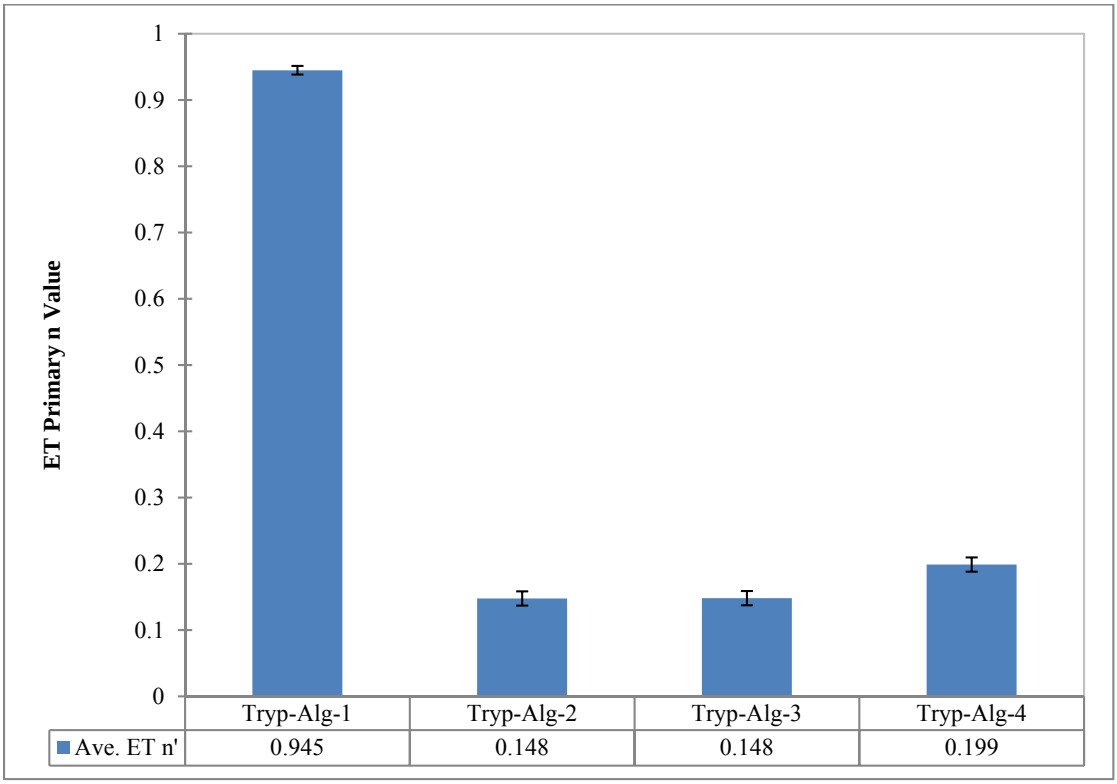

Figure 3. Average (±SE) flow behavior index values of sample preparations measured at eye temperature and before high shear (ET n').

The RT K'values were obtained at ambient temperature (23° C) and prior to the samples being subjected to high shear or mixed with PATS. They are thought to reflect the viscosity of the product upon standing. The RT K" (RT secondary K) values are also generated at ambient temperature, but are measured after sustained high shear.The RT K" values are considered to be indicative of GFS product viscosity characteristics after the patient shakes the bottle. The average viscosities of the different formulation groups, when measured at ambient temperature and after high shear (RT K"), are given in Fig. 4. The RT K'' value is important in determining whether the formulation will be able to be administered from standard eyedropper packaging after agitation. Irrespective of whether tryptophan was present, the average RT K" values increased as CaG concentration was increased. This is likely due to Ca^{++} partitioning between the alginate polymer and the gluconate counter ion. Increased concentrations of CaG result in more available Ca^{++}, which in turn causes increased alginate gelation. Findings from previous research indicated that the force to expel the GFS preparation from the bottle was noted to increase with increasing secondary K values (5). It was noted that RT K'' values of about 5.0 mPas or less allow for administration from a common plastic ophthalmic bottle after shaking and with an acceptable amount of squeeze force (5).The statistical analysis of viscosity results indicates that the RT K" values for formulation groups with 0.28% and 0.3% CaG were larger than those observed for the alginate formulation group with no CaG.

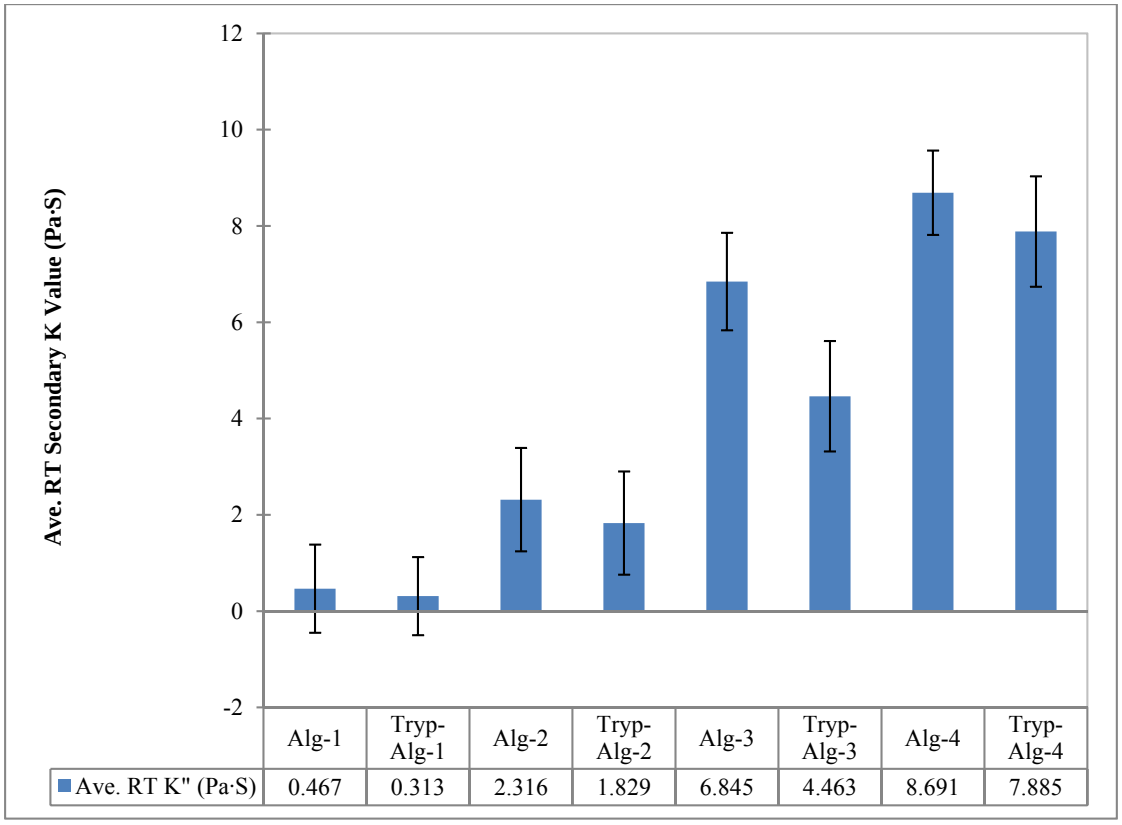

Figure 4. Average (±SE) viscosity of sample preparations measured at ambient temperature and after high shear (RT K").

High RT K' values indicate that the Tryp-Alg-2, -3, and -4 formulations should be shaken vigorously before dispensing from an ophthalmic bottle. The thixotropic properties and low *n* values of these formulations indicate that vigorous shaking by the patient should be effective at making these preparations more easily dispensed. The 1% alginate plus 0.28% CaGformulations (Alg-3 and Tryp-Alg-3) were found to flow freely after shaking, but the average RT secondary K values were above 5.0 Pa·S when tryptophan was not present in the formulation (Tryp-Alg-3 K RT"= 4.4 Pa·S, Alg-3 K RT" = 6.8 Pa·S). It is possible that special packaging will be needed for some alginate plus CaG products, such as using an ophthalmic bottle designed to be more easily squeezed by the patient.

The K value generated by using the Ostwald-de-Waele model corresponds to the viscosity at a shear rate of one reciprocal second. The shear applied in between blinks and in the cul-de-sac area is very low and is given as 0.03 s^{-1} (2). The ET K' (ET primary K) values that are measured at physiological temperature (ET) and mixed with the physiologically based simulated tear (PATS) are considered to represent gel strength when the GFS product is administered to (and located in) the lower cul-de-sac of the eye. The ET K' value is important in determining the extent of gelling (increase in viscosity) once the solution is mixed with tear fluid at eye temperature. The average ET K' and ET Tau' (ET primary Tau) values are given in Fig. 5 and 6, respectively. A sharp increase in both values occurred when the CaG concentration was increased to 0.25%. A further increase in CaG concentration to 0.28% resulted in even stronger gels that appear to level off at 0.3% CaG. Statistical analysis indicates that all formulation groups with CaG formed stronger gels, both ET K' and ET Tau', than the alginate only formulations. The 0.28% and 0.3% CaG plus alginate formulation groups trended to form stronger gels than the corresponding 0.25% CaG formulations.

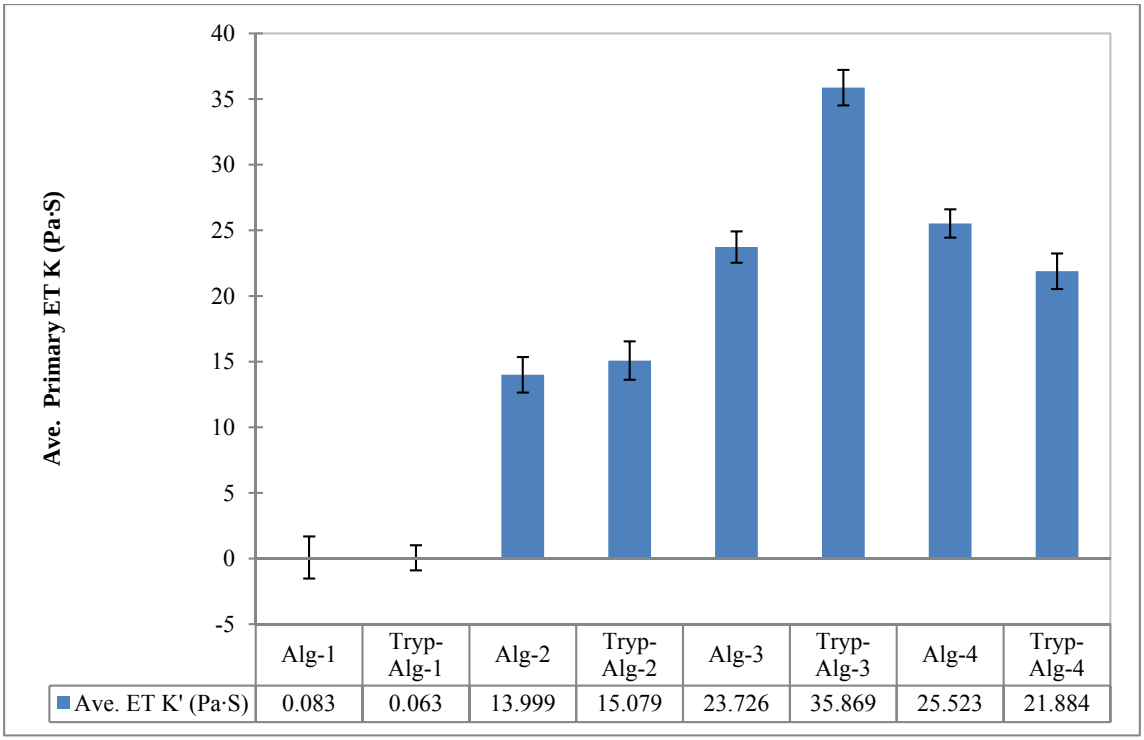

Figure 5. Average (±SE) viscosity of sample preparations measured at eye temperature and before high shear (ET K').

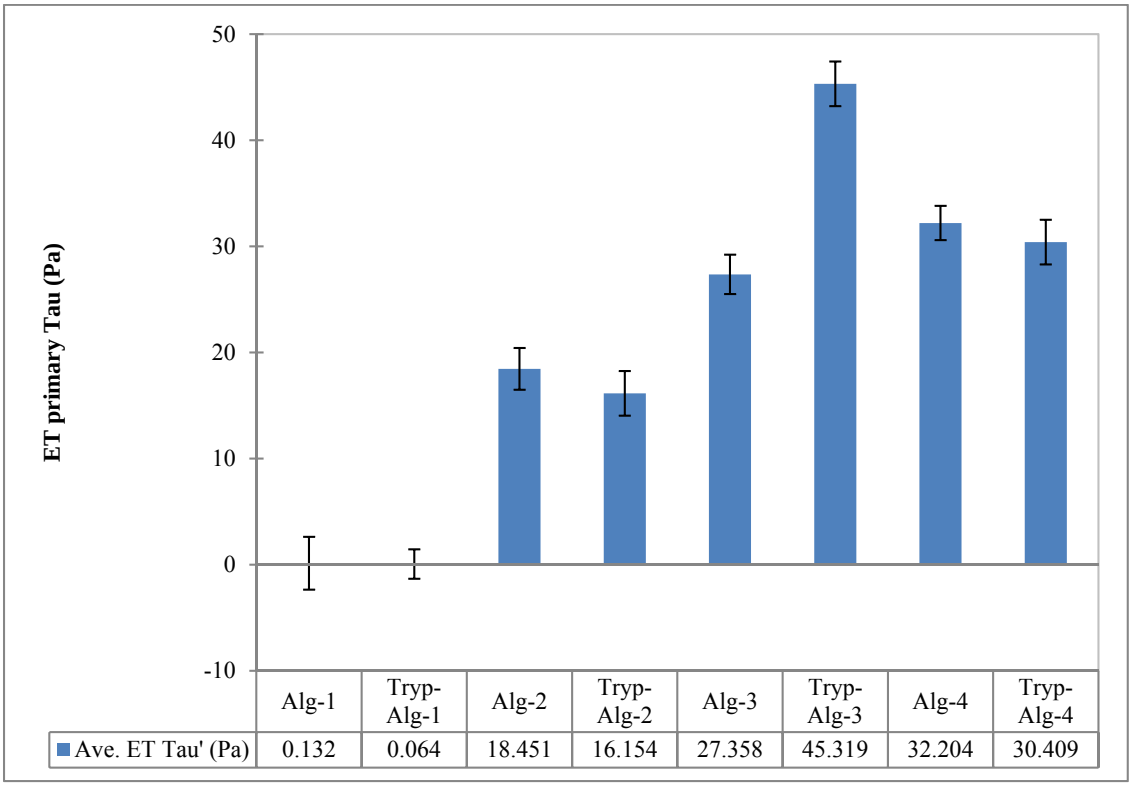

Figure 6. Average (±SE) yield values of sample preparations measured at eye temperature and before high shear (ET Tau').

The yield value is the force needed to overcome the internal force of a semisolid and to initiate flow of the semisolid. The longer ocular retention times observed for aqueous polymer gels is believed to be due to their high yield values (1). The ET K' values and the ET yield values (Tau') are closely correlated to each other and both are expected to be measures of resistance to removal from the cul-de-sac of the eye. Alginate solutions containing 0.28% and 0.3% CaG (Alg-3, Alg-4, Tryp-Alg-3 and Tryp-Alg-4) and mixed with PATS under physiological conditions trended as forming the strongest gels. The Alg-3 and Tryp-Alg-3 GFS had greater average ET K' values (statistically significant) after mixing with PATS than the alginate solutions containing 0% and 0.25%

CaG. Alginate forms strong gels in the presence of Ca^{++}(4,7) but the major cation in the tear is monovalent sodium (16). Our results (ET K' and ET Tau') indicate that the inclusion of CaG with the alginate biopolymer results in CaG functioning as an ion exchange resin to provide a source of Ca^{++}. This available source of Ca^{++} then results in stronger gelation of alginate when mixed with stimulated tear fluid.

All tested preparations were also found to have thixotropic properties. The complexity of the Ca^{++} induced alginate egg box structure appears to rapidly break apart with shear (shear thinning) and then takes time to reform, thus resulting in thixotropic behavior. Due to the thixotropic behavior of the alginate plus CaG formulations, it is expected that the low viscosity induced by an eye blink will last for a period of time before returning to a higher viscosity.

The average ET K' values for formulation Tryp-Alg-4 (0.3% CaG) is lower (statistically significant, $p \leq 0.05$) than the ET K' values for Tryp-Alg-3 (0.28% CaG). Perhaps an optimal amount of CaG is needed in order for the most efficient gelation to occur. It has been shown that an increase in Ca^{++} concentration results in an increase in alginate gel strength until a critical ratio of calcium concentration to alginate concentration is achieved (21). Gel strength was observed to slowly decrease with Ca^{++}concentrations above this critical ratio (21). It was proposed that the decrease in gel stiffness with Ca^{++} concentration greater than the critical ratio is due to interference of the alignment of alginate chains into dimers(21). The critical concentration of Ca^{++} was 22.5 mM for a 1% low molecular weight alginate using calcium carbonate as the calcium source (21). For comparison, 0.28% CaG is equivalent to 6.5 mmol/kg when in the presence of Na^+ and K^+ in PATS. The leveling off or possible slight decrease in the primary K value and yield values when the CaG concentration is raised from 0.28% to 0.3% could be due to calcium being at a higher concentration than needed to meet the critical ratio to 1% alginate. The ET K' and ET Tau' results indicate (statistically significant) that the inclusion of tryptophan with 1% alginate plus 0.28% CaG results in a preparation with increased gel forming potential as compared to the same formulation without tryptophan. Tryptophan has a carboxylic acid group (pKa = 2.83), an indole group (pKa = 5.89), and an amine functional group (pKa = 9.4). The compound L-carnosine is composed of β-alanine and L-histidine joined together and has structural similarity to tryptophan. L-carnosine has a carboxylic acid group (pKa = 2.76), an imidazole group (pKa = 6.78), and an amine functional group (pKa = 9.36) (22). It was reported that the inclusion of L-carnosine with gellan resulted in preparations that had better gel forming capacity as compared to Timoptic-XE (23). It is possible that tryptophan interacts with alginate in a similar manner as to the interaction of L-carnosine with gellan.

It has been reported that patients who were administered a gellan based GFS timolol marketed product had a reduction in optical quality as compared to patients administered an alginic acid based long lasting Carteolol marketed product (13). The difference in optical quality was attributed to the formation of gel fragments on the ocular surface by the gellan GFS that was not seen with the alginic acid based product (13). The package insert of Timoptic-XE® states that transient (0.5 to 5 min) blurred vision may occur and is due to the characteristics of the formulation itself (23). It is possible that the alginate plus CaG GFS will have increased blurring of vision due to stronger formed gels as compared to alginate alone. However, alginates may have better patient acceptance than gellan due to easier spreading during an eye lid blink (4). That is, the formed strong alginate gels may not form gel fragments on the surface of the eye.

Drug Release Studies

The use of dialysis bags to measure the release of drug from a polymer matrix has been performed previously (19,20). The amino acid tryptophan was selected as the model drug because it is safer to work with than pharmacologically active substances such as timolol maleate, it is better for the environment than timolol maleate, it is considered a very hydrophobic amino acid at neutral pH (24), and it has physicochemical properties that are favored for assessing membrane transport (5). The release profile of tryptophan from such an experimental set up is sigmoidal in character and is best fit with a logistic function as used for ED_{50} and LD_{50} data fits (Fig. 7). The T_{50} value is the midpoint of the mathematical logistic fit of the release data. Higher fitted T_{50} values indicate that tryptophan is released at a slower rate from the polymer matrix than T_{50} values that are lower in magnitude. A visual examination of the release data for the solution (Tryp-1) and the 1% alginate plus 0.28% CaG formulation (Tryp-Alg-3) presented in Fig. 7 indicates that tryptophan is released at a slower rate from Tryp-Alg-3 than from a simple solution. The T_{50} values of these same formulations (Tryp-1 T_{50} = 283 min, Tryp-Alg-3 T_{50} = 550 min) are reflective of this observation.

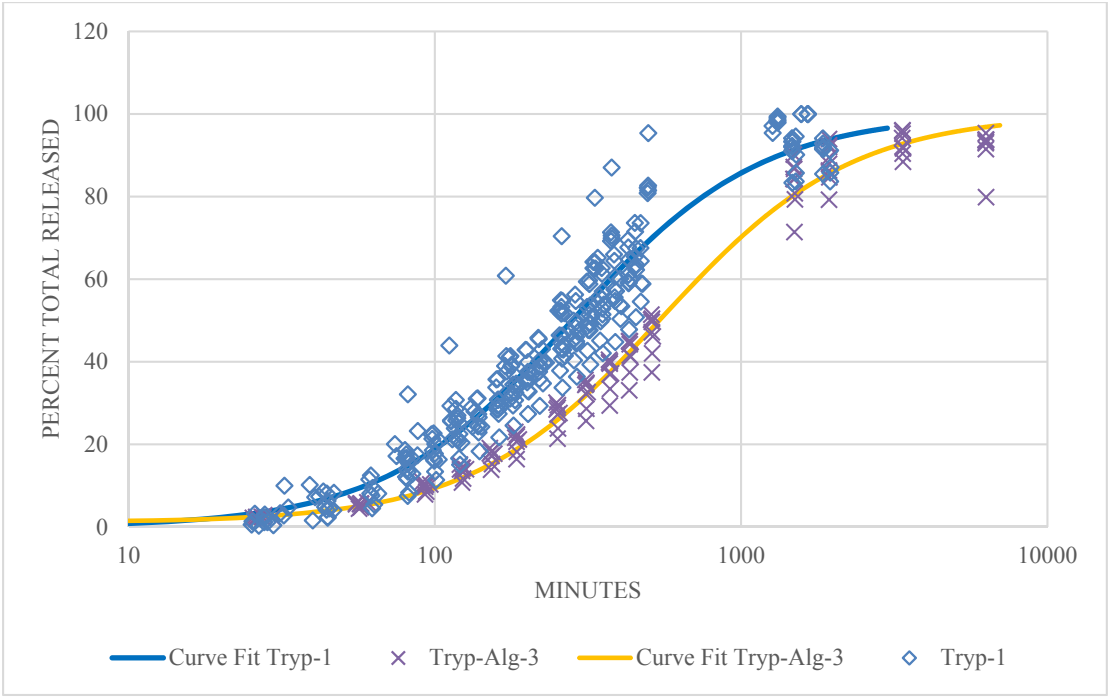

Figure 7. Percent of total amount of tryptophan released versus time for tryptophan simple solution (Tryp-1) and Tryp-Alg-3 formulations. Respective logistic fits are indicated by solid lines.

The average T_{50} values (min) for Tryptophan release (transmembrane diffusion) of the different test formulation groups are given in Fig. 8. The slowest rate of tryptophan release is observed for the 0.28% CaG formulation, as shown by the T_{50} value. The fastest rate of tryptophan release is observed for the alginate formulation that contained 0.25% CaG (Tryp-Alg-2). Statistical analysis of the tryptophan release data indicates that formulations Tryp-Alg-3 and Tryp-Alg-4 demonstrated significantly ($p \leq 0.05$) slower tryptophan release rates than Tryp-1, Tryp-Alg-1, and Tryp-Alg-2 formulation test groups. The alginate solutions containing 0.28% CaG (Tryp-Alg-3) and 0.3% CaG (Tryp-Alg-4) also displayed the highest average ET K' and ET Tau' values. These findings are in agreement with the literature in that drug release rates have been found to decrease with increased gel viscosity (4).

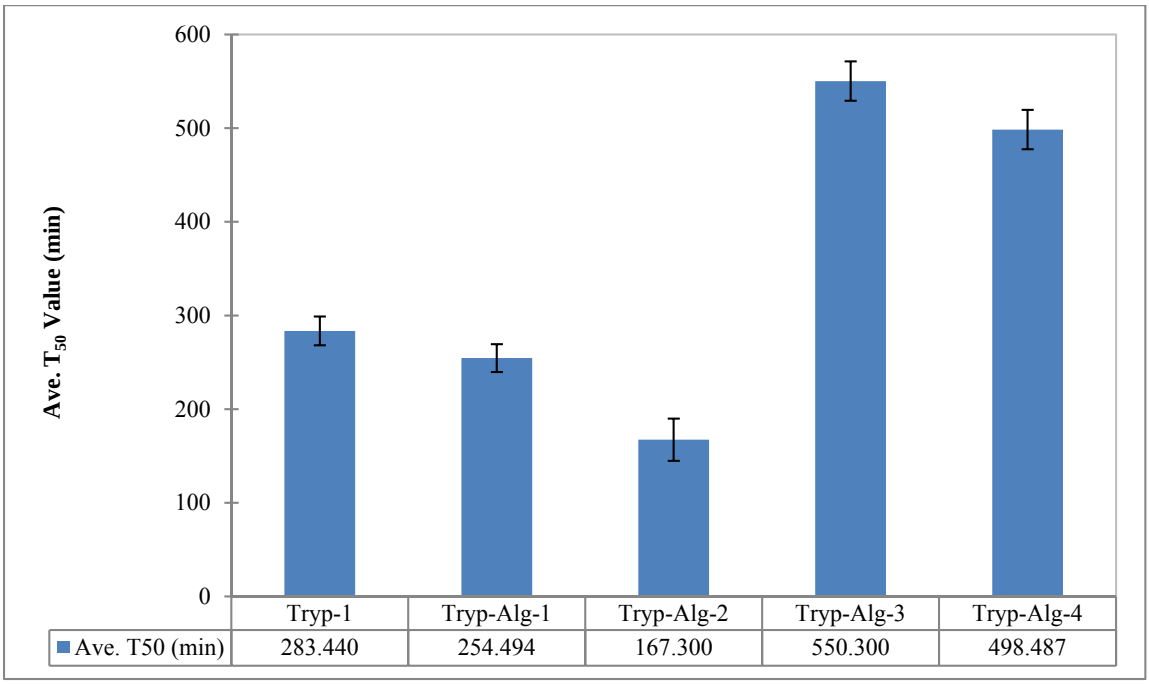

Figure 8. Average(±SE) midpoint (T_{50}) values for tryptophan release from sample test preparations.

The theoretical value for tryptophan concentration at infinity is 0.032 mg/ml. The average final tryptophan concentrations of formulations Tryp-1, Tryp-Alg-2, Tryp-Alg-3, and Tryp-Alg-4 are 0.030 ± 0.002 mg/ml, 0.031 ± 0.003 mg/ml, 0.028 ± 0.002 mg/ml, and 0.034 ± 0.004 mg/ml. The average final concentration for the alginate only formulation (Tryp-Alg-1) was 0.019 ±0.004 mg/mL, even though the last reading was more than 99 hr after the initiation of the release study. It appears that the total amount of tryptophan release from Tryp-Alg-1 was reduced from that observed for the other tryptophan formulations. In comparison, the total amount of carteolol released from an alginate matrix alone appears to have been reduced when compared to the release of carteolol from a solution and hydroxyl ethyl cellulose gel (6). The authors attributed the longer release time of carteolol to an ionic interaction between alginic acid and carteolol (6). The structure of carteolol and tryptophan both have amine groups that should be protonated and positively charged at physiological pH. It is possible that tryptophan does not display total release when only alginate is in the formulation (Tryp-Alg-1) due to an ionic interaction between tryptophan and alginate. The addition of CaG to the formulation (Tryp-Alg-2, Tryp-Alg-3, Tryp-Alg-4) may result in shielding of this ionic interaction, thus making it possible for tryptophan to be more completely released, as shown for formulations containing CaG.

Conclusion

Our data indicates that adding calcium gluconate to alginate can result in a gel forming solution that forms a strong gel under physiological conditions. The formed gel appears to be even stronger than what was found when calcium gluconate is added to Gellan to strengthen gel forming properties (5, 18). The release rate of the water soluble amino acid tryptophan was slowed for those formulations which also demonstrated strong gel forming properties. This formulation approach allows the use of alginate in a gel forming solution formulation without having to use a copolymer.

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