The Effect of Polyvinylpyrrolidone (PVP) on Ocular Gel Forming Solutions Composed of Gellan and Calcium Gluconate

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Abstract - Purpose: This study focuses on the optimization of the ocular in situ gel strength of gellan plus calcium gluconate gel forming solutions both with and without polyvinylpyrrollidone. Additionally, it is of interest to investigate the diffusional release of tryptophan (acting as a model drug) from these same types of formulations. Methods: Aqueous formulations of gellan, calcium gluconate, and polyvinylpyrrolidone were prepared at varying concentrations and were measured for viscosity characteristics indicative of the ability to be administered from common ophthalmic packaging and the ability to form gels upon initial contact with tear fluid. Linear regression and multi-variate, non-linear regression analysis fit equations were used to calculate the highest obtainable ocular in situ viscosities for gel forming solutions (with and without polyvinylpyrrolidone) that can be administered from common ophthalmic packaging. Nonparametric matched pair analysis was used to conduct a statistical comparison of gellan and calcium gluconate formulations both with and without polyvinylpyrrolidone. Results and Conclusions: The data indicates that the addition of polyvinylpyrrolidone to gellan and calcium gluconate preparations increases the in situ gel strength of the formulations while still retaining the ability to be dispensed from an ophthalmic bottle. The diffusional release of tryptophan was found to be significantly slower from gellan and calcium gluconate gel forming solutions when compared to a simple tryptophan solution.

Keywords: Ocular drug delivery, Gel forming solutions, Gellan, In situ gelation, Diffusional release.

Introduction:
Disorders of the eye are common and frequently require pharmacological treatment. The most common method of delivering drug to the eye is by administering pharmacologically active solution directly onto the topical surface of the eye, referred to as “eye drops” [1,2]. This method of drug delivery to the eye is problematic for several reasons, one of which is low bioavailability of around 5% of applied drug [1,3]. Reasons for such low bioavailability of drug are often a result of the anatomical nature of the eye and short retention times of drug at the surface of eye due to blinking, tear dilution and turnover, and rapid drainage through the nasolacrimal duct [1-4].

A proven approach to slow drug removal is to increase the viscosity of the medication [5,6]. Ocular ointment-based drug delivery systems are known to have greater retention times and improved drug delivery due to their increased viscosity and resistance to dilution by the tears. However, ointment formulations have important disadvantages such as difficulty administering drug correctly and eye discomfort or blurred vision, and should be used at night [2,7]. Alternatively, a water-based polymer gel of high viscosity may be used as an effective delivery system. However, large increases in viscosity are often needed in order to observe improvement in clinical effect and the product likely will need to be administered out of a tube, which as with ointments is difficult for patients to use [6].

Because of the disadvantages inherent in ointment and high viscosity gel formulations, research has focused on ‘gel-forming solutions’ (GFS) also referred to as in situ gel systems in the literature [2,4,7,8]. GFSs combine the advantages of drug solution delivery with the advantages of gel delivery of drug, while attempting to minimize the disadvantages of both. GFSs accomplish this because of their ability to be easily applied like an eye drop, but display increased retention time in the eye [2,7,8]. This is possible due to the property of GFSs to shift from liquid to a semi-solid phase upon instillation in the eye. The resulting increase in viscosity allows for more efficacious pharmacological therapy [2,4,7-9]. The chief advantages of increased viscosity of gel formulations is that they remain in contact with the eye surface longer and have greater retention times, potential for a controlled release, and reduction in adverse effects [2,4,7,8]. When the shear thinning GFSs studied here are
subjected to shearing forces, such as an eye blink, their viscosity is reduced [4]. This reduction in viscosity at high shear helps to prevent eye lid drag and discomfort.

Gellan gum is a polysaccharide polymer component used in gels and GFS formulations such as the marketed product Timoptic-XE® [2,4,7,8,10]. Low viscosity gellan solutions are known to increase in viscosity in the presence of both monovalent and divalent tear fluid cations [2]. Efficacy of a drug-delivery method is not only impacted by the physical characteristics of the formulation but also by patient perception and adherence. A randomized, controlled trial demonstrated 70% of patients preferred once a day timolol maleate ophthalmic gel-forming solution over twice a day timolol maleate ophthalmic solution. In this study, fewer adverse events were reported with the GFS and adherence was reported as being higher in the GFS group, likely due to less frequent dosing [10]. Furthermore, gellan gum polymer used in GFSs was demonstrated as a non-irritant to the eye and is suitable for ophthalmic use [7].

In addition to gellan, other excipients may be included in GFS formulations that impact the characteristics of drug delivery. Our lab previously demonstrated improved efficiency of forming stronger gellan gels upon instillation in the eye through the addition of calcium gluconate (CaG) [11]. It is thought that CaG acts as an ion exchange molecule in which Ca ++ is displaced by Na+/K+ in the tear fluid. The ionic interaction of gellan with Ca ++ is known to produce stronger gels than Na + or K+gellan gels [12,13]. The focus of the research reported here is to explore optimal ingredient concentrations of in situ gel-forming properties of gellan plus CaG formulations both with and without Polyvinylpyrrolidone (PVP). Additionally, it is of interest to investigate the release properties of Tryptophan (acting as a model drug) from gellan plus CaG GFS formulations both with and without PVP.

The compositions of the simulated tear fluids (STF) referred to as physiological artificial tear solution (PATS) and citric acid tear solution (CTS) are shown in Table 1.

<table>
<thead>
<tr>
<th>Physiological Artificial Tear Solution (PATS) Composition for Confirmatory Activities</th>
<th>Citrate Tear Solution (CTS) Composition for Tryptophan Release Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Component</td>
<td>Concentration</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>128.7 mM</td>
</tr>
<tr>
<td>Potassium carbonate</td>
<td>12.4 mM</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>0.32 mM</td>
</tr>
<tr>
<td>Magnesium chloride</td>
<td>0.35 mM</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>2.07 mg/mL</td>
</tr>
<tr>
<td>1N NaOH and/or 1 N HCl q.s. to pH 7.4±0.4</td>
<td>Deionized water q.s. to 1000 mL</td>
</tr>
<tr>
<td>Deionized water q.s. to 1000 mL</td>
<td></td>
</tr>
</tbody>
</table>

aPATS and CTS were adjusted to a pH of 7.4±0.4 using 0.1N sodium hydroxide and/or hydrochloric acid.

Studies assessing the gel forming characteristics of sample preparations were conducted using PATS. PATS is formulated to mimic the concentration of electrolytes as found in the tear [14]. Lysozyme is a protein found in the tear and provides antimicrobial protection for the eye by interacting with gram-positive bacteria [14]. It has been indicated that the inclusion of Lysozyme in STF does not affect the ability of gellan to undergo gelation [15]. Data has also been generated that indicates that lysozyme does interact with the polysaccharides alginate and xanthan to increase the strength of formed gels [15,16]. Therefore, lysozyme was added to the composition of PATS for completeness. Potassium carbonate is used as the buffer system in PATS to mimic the carbonate buffer used in tears [14]. The equilibration of carbonic acid with carbon dioxide in the air results in a shift in pH when PATS is exposed to air for a long period of time. PATS was stored immediately after manufacture at 4°C in 100 mL air tight containers with minimal head space. These storage conditions minimize the equilibration of carbonic acid with the atmosphere. Tryptophan release (transmembrane diffusion) studies were conducted using CTS. CTS was formulated (Table 1) with calcium to mimic the divalent composition (Ca ++, Mg ++) of PATS and a citric acid buffer to mimic the carbonic acid buffer found in tear fluid. The use of citric acid enables long term diffusion release studies to be conducted at a stable pH not possible with the carbonate buffer used in PATS.
Materials and Methods:

DL-Tryptophan (Sigma-Aldrich, St. Louis MO, USA), Gellan gum (Spectrum Chemical, Gardena CA), Calcium D-gluconate (Sigma-Aldrich, St. Louis MO), Povidone (Polyvinylpyrrolidone) - Grade K12 (ISP Technologies, Wayne NJ), Sodium chloride (Acros Organics, Fair Lawn NJ), Potassium Chloride (Fisher Scientific, Fair Lawn NJ), Calcium chloride dihydrate, USP (Fisher Scientific, Fair Lawn NJ), Magnesium chloride (Fisher Scientific, Fair Lawn NJ), Citric acid monohydrate (Sigma-Aldrich, St. Louis MO), Tromethamine (Sigma-Aldrich, St. Louis MO).

The gellan + CaG formulation series consisted of fifty-one different preparations that consisted of gellan in a concentration range of 0.5% to 1.1% and CaG concentrations that varied from 0.03% to 0.3%. The gellan + CaG + PVP formulation series consisted of seventy-seven different preparations that had gellan concentrations that ranged from 0.5% to 1.0%, CaG concentrations from 0.02% to 0.12%, and PVP concentrations from 0.02% to 4.0%. The 0.32% Tryptophan + gellan + CaG + PVP formulation series consisted of fifty-two different preparations that were compounded with gellan concentrations that ranged from 0.55% to 1.0%, CaG concentrations from 0.02% to 0.16%, and PVP concentrations from 0.2% to 2.0%. 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%. Tromethamine (0.1m) and/or hydrochloric acid (0.1N) were used to adjust the pH to target values of 7.4 ±0.4 prior to being mixed with PATS or used in Tryptophan diffusion studies. Tromethamine was used rather than sodium hydroxide in order to avoid the addition of Na⁺ to the cation triggered gel forming solutions [15]. Sufficient deionized (DI) water was added to the preparations to give a final batch weight of 100 g.

Experimental preparations were mixed thoroughly with physiological artificial tear solution (PATS) at a 5:1 ratio. Previous work indicated that the ratio of 5 parts sample preparation to 1 part simulated tear fluid (PATS) provides the best differentiation between different polymers used to produce GFS formulations [11]. Other investigators used the ratio of 25.7 (3.6 to 1.0) to measure the strength of the formed gel after mixing GFS with their simulated tear fluid [15]. All samples were set aside for at least 12 hours before viscosity measurements were conducted. It has been reported that gellan solutions are thixotropic and that gellan gel strength was found to recover by 6 hr after induced shear [11,17,18]. Experimental preparations were measured at 23°C±2 (RT) to mimic patient storage conditions. The gel resulting from mixing the GFS preparation with PATS was measured for viscosity at 34°C±2 (ET) to mimic temperature conditions as found at the front of the eye.

Log viscosity sweeps (12 different discrete shear rates from 1 to 200 sec⁻¹) were conducted using a Haake ViscoTester 550. The viscosity sweep was followed with a constant high shear rate of 1000 sec⁻¹ for 90 sec. A second log viscosity sweep was conducted after the sample was subjected to the constant high shear. The log viscosity sweep data was fit using an Ostwald-de-Waele (power) model (τ = kγⁿ, where τ is the shear stress and γ is the shear rate). Higher k values are indicative of larger viscosities and are given in pascal·seconds (Pa·s). K values generated from the first log viscosity sweep were reported as K primary or K'. K values generated from the second log viscosity sweep (after high shear) were reported as a K secondary or K". Drop size and expulsion pressure testing from ophthalmic bottles were conducted in order to determine the highest allowable RT K" value. Two bottles of the marketed product Timolol GFS (Sandoz) were used as comparator samples. The bottles of Timolol GFS were held at a 45° angle to the horizon and ten drops were expelled and weighed on an analytical balance. The pressure needed to expel drops was measured using a force sensor (Venier). The Timolol GFS formulation was then emptied from its packaging. The packaging was washed and dried before being used as packaging for three sample gellan + CaG preparations that differed in composition and viscosity (RT K"). The preparations were measured for drop size and expulsion pressure in the same manner as for the marketed Timolol GFS product.

Tryptophan preparations were tested for Tryptophan transmembrane diffusion characteristics within 14 days after manufacture. The absorbance (280 nm) of Tryptophan in the Citrate Artificial Tear Solution (CTS) receiving fluid was measured at timed intervals using a MetaSpec Pro UV-Vis Spectrophotometer. Concentrations of Tryptophan in experimental samples were determined using a UV absorbance (280 nm) standard curve. Tryptophan concentrations were determined using individual linear best fit (n=7, r²= 0.9971 or 0.9972) calibration curves for three different instruments.

The tryptophan transmembrane diffusion (release) studies were conducted in a manner described previously [19]. Ten centimeter segments of cellulose ester dialysis membrane (molecular weight cut off of 3,500-5,000 Da) were soaked in DI water ≥24 hr to remove preservative and then equilibrated with CTS for ≥24 hr. The molecular weight cut off was selected so as to easily allow the diffusion of Tryptophan (MW = 204) through the semipermeable membrane while restricting the movement of gellan polymer with reported MWs of 55 to 2,200 kDa [20]. The UV absorbance spectrum of CaG and PVP is such that interference with the UV absorbance of Tryptophan at 280 nm is not expected [21,22]. A 10 cm length of the dialysis tubing was removed from the
soaking CTS, one end was closed with a clamp, filled with 1 g of preparation, and the top end was closed with a clamp. Each filled dialysis membrane bag was placed in a beaker filled with 100 mL of fresh CTS. A stir bar was placed in the beaker and then covered with Parafilm®. Each sample set up was placed on a multi-station stir plate at ambient temperature and stirred (1,100 rpm) throughout the duration of the experimental run. The absorbance (280 nm) of Tryptophan in the CTS receiving fluid was measured at regular timed intervals using dedicated disposable UV cuvettes. The Tryptophan in CTS samples were returned to the set up after their measurement by using dedicated disposable transfer pipettes. The total weight was documented for each experimental set up and deionized water was added, if needed (>1% deviation), to compensate for water loss due to evaporation. Since measured samples were not set aside, it was not necessary to dilute the sample set up to original volume with CTS.

Experimental studies were conducted until near equilibrium conditions were achieved (≥30 hr). The amount of total Tryptophan released was defined as the Tryptophan concentration (absorbance) value obtained at the last time point. The theoretical value for Tryptophan concentration at infinity is 0.032 mg/mL and the average of the measured last time point Tryptophan concentration values was 0.031±0.003 mg/mL. Absorbance readings were within the linear range of the Tryptophan concentration versus calibration curve. The Percent Total Released for each time point is defined as the absorbance (concentration) at that time point divided by the absorbance (concentration) at the last time point and then converted to percent. Seven or eight experimental runs were conducted for each sample test preparation that lasted up to at least 1,571 min (26 hr) and no more than 3,123 min (52 hr).

Linear regression (Microsoft Excel) was performed for ET K’ values as the dependent variable (Y) versus the corresponding RT K” values as the independent variable (X). Statistical significance was determined based upon the correlation coefficient value and number of observation pairs [23]. Additionally, the independent variables (RT K” and ET K’) and the predictor (independent) variables of gellan (X1), CaG (X2), and PVP (X3) concentrations were mathematically modeled using multi-variate, nonlinear regression analysis (Addinsoft, XLSTAT). The functions used in the regression analysis were in the form of a polynomial equation raised to the first power (e.g., \( Y = pr1 + (pr2 \cdot (X1)) + (pr3 \cdot (X2)) \)) and raised to the second power (e.g., \( Y = pr1 + (pr2 \cdot (X1)) + (pr3 \cdot (X2)) + (pr4 \cdot (X1)^2) + (pr5 \cdot (X2)^2) \)). The mathematical fitting parameters (coefficients) are referred to as pr1, pr2 etc. The gellan concentration is the X1 independent variable, the CaG concentration is the X2 independent variable, and the PVP concentration is considered the X3 independent variable. Akaike information criterion values were calculated for the nonlinear regression analysis results and the values were used to determine whether the use of polynomial fit equations to the second power justified the loss of overall degrees of freedom. The residuals of the multivariate fits were examined as to whether there were any systematic errors in the mathematical fit of the data.

Numerical values for both RT K” and ET K’ were calculated using the multivariate fit equations (2,600 calculations for each fit equation) with varied concentration of gellan, CaG, and PVP. The values input into the fit equations were independently varied for gellan (0.5% to 1.1% with 0.05% increments), CaG (0.02% to 0.1% with 0.02% increments), and PVP (0.1% to 4.0% with 0.1% increments).

The nonparametric Wilcoxon Matched-Pairs and Signed-Ranks test (Addinsoft, XLSTAT) were used to enable a statistical comparison of the RT K” and ET K’ values for formulations containing PVP versus those without PVP. Matched pairs of formulations were based upon having the same composition of gellan and CaG.

The general form of a logistic function was used for sigmoidal curve fitting of the Percent Total Tryptophan Released versus time curves using the software KaleidaGraph by Synergy Software. The examination of residuals and correlation coefficients indicated that a four parameter logistic curve fit was the most appropriate model for fitting data generated using this experimental design for assessing drug release [19]. The shape and midpoint (T50) fit parameters were allowed to vary while the fit parameters corresponding to the lowest (0%) and highest (100%) amounts were fixed at their theoretical values. The generated T50 values were treated as independent data points and the Dixon test for outliers was applied to all generated T50 values. Outlier experimental runs were discarded in further statistical analysis. One-way ANOVA and the Tukey’s post hoc test (Addinsoft, XLSTAT) were used to determine statistical significance (p ≤ 0.05) for T50 values of the different preparations.
Results and Discussion:

The K value generated by using the Ostwald-de-Waele model corresponds to the viscosity (PaS) at a shear rate of one reciprocal second. The K values generated from the second viscosity sweep (performed after sustained high shear) of preparation samples held at ambient temperature are referred to as RT K". The RT K" values are considered to be indicative of GFS product viscosity characteristics after the patient shakes the bottle. Our data indicated that RT K" values of gellan + CaG and gellan + CaG + PVP formulation series preparations should be 5.0 PaS or less in order for the preparation to be dispensable from Timolol GFS ophthalmic packaging with an appropriate bottle squeeze force. All tested preparations were found to demonstrate shear thinning and thixotropic properties. Tested samples with RT K" values ≤ 5 PaS were also found to be free flowing after having been shaken vigorously for 5 sec. The drop size for the sample preparations were smaller than for Timolol GFS for all tested formulations containing gellan and CaG regardless of viscosity. The force to expel the GFS preparation from the bottle was noted to increase with increasing secondary K values.

The ET K' value is considered to represent gel strength when the GFS product is administered to and located in the lower cul-de-sac of the eye. The ability of the two systems (gellan + CaG vs gellan + CaG + PVP) to efficiently function as gel forming solutions was evaluated by both considering a RT K" value of 5.0 PaS as the highest allowable value and then using mathematical model fit equations to calculate the highest obtainable viscosities (ET K') of the three different GFS formulation series (gellan + CaG; gellan + CaG +PVP; Tryptophan + gellan + CaG + PVP). Initially, a simple linear fit model was used to determine the highest obtainable ET K' value. Each gellan + CaG preparation’s ET K’ value (measure of formed gel strength) was plotted versus its corresponding RT K" value (measure of ease of administration). A positive linear trend exists between the RT K" values and ET K' values of various gellan + CaG preparations that is described by the statistically significant linear regression equation [(ET K') = 1.2253(RT K") + 2.4458], (R² = 0.64). A forecasted ET K’ value of 8.6 PaS was obtained when using a value of 5 for RT K”. The ET K’ and RT K” values for each of the tested gellan + CaG + PVP formulations were also plotted and a statistically significant linear regression equation was obtained [(ET K') = 1.1157 (RT K") + 3.8636], (R² = 0.59). This equation gives a forecasted ET K’ value of 9.4 PaS when using a value of 5 for RT K”. The fit equation for the 0.32% Tryptophan + gellan + CaG + PVP formulation series was [(ET K') = 1.7333 (RT K") + 1.8573], (R² = 0.69, statistically significant fit) and gives a calculated ET K’ value of 10.5 PaS when using a value of 5 for RT K”.

The independent variables (RT K” and ET K’) and the predictor variables of gellan, CaG, and PVP concentrations were also modeled using multi-variate, nonlinear regression analysis. The mathematical functions used in the regression analysis were of the first and second power polynomial forms. The R² values for the second power fit equations were higher than their corresponding (same formulation series) first power fit equations. However, Akaike information criterion results indicated that first power equations were the most appropriate model fits for the three different formulation series rather than second power equations. Examination of the residuals indicated that there was no systematic error when using the multivariate fit equations.

Table 2. Summary of Nonlinear Regression Analysis and Predicted Highest ET K’ Values when RT K” is Restricted to 5 PaS or Less.

<table>
<thead>
<tr>
<th>Sample Series</th>
<th>Nonlinear Fitted Equation (XLSTAT) for Dependent Variable RT K&quot; (PaS)</th>
<th>R²</th>
<th>Nonlinear Fitted Equation (XLSTAT) for Dependent Variable ET K’ (PaS)</th>
<th>R²</th>
<th>Highest Calculated ET K’ Value with RT K&quot; ≤ 5 PaS</th>
</tr>
</thead>
<tbody>
<tr>
<td>No PVP</td>
<td>RT K&quot; = -5.905 + (11.542 X %Gellan) + (5.483 X %CaG)</td>
<td>0.784</td>
<td>ET K’ = -4.075 + (13.771 X %Gellan) + (2.185 X %CaG)</td>
<td>0.505</td>
<td>8.5</td>
</tr>
<tr>
<td>Plus PVP</td>
<td>RT K&quot; = -8.310 + (11.557 X %Gellan) + (39.297 X %CaG) + (0.494 X %PVP)</td>
<td>0.735</td>
<td>ET K’ = -7.149 + (15.620 X %Gellan) + (33.022 X %CaG) + (1.586 X %PVP)</td>
<td>0.530</td>
<td>13.9</td>
</tr>
<tr>
<td>Plus TRYP PVP</td>
<td>RT K&quot; = -7.038 + (13.27 X %Gellan) + (6.398 X %CaG) + (0.246 X %PVP)</td>
<td>0.584</td>
<td>ET K’ = -7.9422 + (22.587 X %Gellan) – (13.376 X %CaG) + (0.034 X %PVP)</td>
<td>0.509</td>
<td>11.1</td>
</tr>
</tbody>
</table>
The first power fit equations are reported in Table 2, along with the highest calculated ET K’ value for that formulation series when RT K” was held to 5 PaS or less. The highest calculated ET K’ value (13.9 PaS) was obtained for the gellan + CaG + PVP formulation series. The second highest ET K’ value (11.1 PaS) was obtained for the Tryp. + gellan + CaG + PVP formulation series. The lowest ET K’ value (8.5 PaS) was obtained for the gellan + CaG formulation series. The relative order of the ET K’ values for the different formulation series remained the same when second power fit equations were used rather than the first power fit equations. Thus, the derived nonlinear multivariate models indicate that the addition of PVP to gellan + CaG gel forming solution formulations results in higher ET K’ values when the preparations’ RT K” values are restricted to 5 PaS or less.

The RT K” and the ET K’ values for the gellan + CaG formulation series and gellan + CaG + PVP formulation series preparations were matched to each other on the basis of identical gellan and CaG concentrations. The use of nonparametric statistical analysis was applied to the matched pairs. The analysis indicated that ET K’ values are larger (statistically significant (p≤0.05)) when PVP is added to gellan + CaG GFS formulations. There was no statistically significant difference (p≥0.05) in RT K” values with or without PVP when all concentrations of PVP were tested. However, if the RT K” and ET K’ values for preparations were only included in the nonparametric analysis when the PVP concentration is equal to or greater than 0.1%, then both RT K” and ET K’ values are larger (statistically significant (p≤0.05)) when PVP is present in the formulation. There were an insufficient number of matched pairs to perform a nonparametric test comparing viscosity values for formulations with and without Tryptophan.

The ET K’ (after sustained high shear) values are reflective of the formed gels viscosity characteristics after being subjected to the high shear of upper lid movement. Shear thinning and thixotropic behavior of a GFS preparation is indicated when its’ ET K” values are much smaller than its’ corresponding ET K’ value. All of the GFS preparations were found to be strongly shear thinning and thixotropic after mixing with the simulated tear fluid (PATS) and are expected to have good patient acceptance [24].

The amino acid Tryptophan was selected as a model drug for transmembrane diffusion (release) studies because 1) it bears some structural similarity to Timolol, 2) it has strong UV absorbance, 3) it possesses low toxicity, 4) it has a low environmental impact, and 5) it is inexpensive. The MW of Tryptophan is 204 as compared to 1) it bears some structural similarity to Timolol, 2) it has strong UV absorbance, 3) it possesses low toxicity, 4) it has a low environmental impact, and 5) it is inexpensive. The MW of Tryptophan is 204 as compared to Timolol with a MW = 316. However; the calculated logP (pH=7.4) and topological polar surface area for Tryptophan was selected as a model drug for transmembrane diffusion (release) studies because 1) it bears some structural similarity to Timolol, 2) it has strong UV absorbance, 3) it possesses low toxicity, 4) it has a low environmental impact, and 5) it is inexpensive. The MW of Tryptophan is 204 as compared to Timolol with a MW = 316. However; the calculated logP (pH=7.4) and topological polar surface area for Tryptophan is 1.09 and 79.11 Å² while 0.97 and 79.74 Å² for Timolol, respectively [25]. LogP and the topological polar surface area are considered to be good predictors of biological membrane permeability. All of the release curves of the tested Tryptophan formulations demonstrated a sigmoidal shape. The appearance of Tryptophan in the receiving solution is sigmoidal in character due to the manner in which Tryptophan diffuses across the dialysis membrane [19]. Examination of the residual plots indicated that there was no systematic error when using the logistic fit equations.

The average T₅₀ and shape parameter values of the individual experimental runs for each preparation were used in conjunction with the initial value of 0% and the time infinity value of 100% to generate representative curves as shown in Figure 1. The T₅₀ value is the midpoint of the sigmoidal release curves and higher T₅₀ values are indicative of slower rates of Tryptophan release. The average T₅₀ values (min) for Tryptophan release (transmembrane diffusion) along with their respective 95% confidence intervals are given in Figure 2. The lack of overlap in the 95% confidence intervals when comparing different formulations is indicative of statistical significance. Tryptophan release was found to be significantly slowed when formulated in a 0.32% Trypt. + 0.7% gellan + 0.06% CaG GFS preparation (T₅₀ = 319 min) as compared to transmembrane diffusion from both a simple 0.32% Tryptophan solution (T₅₀ = 220 min) and a 0.32% Tryptophan + 0.7% gellan GFS formulation (T₅₀ = 233 min). The 0.32% Tryptophan formulations that contained 0.2% (T₅₀ = 233 min), 0.4% (T₅₀ = 244 min), 0.8% (T₅₀ = 248 min) and 2.0% PVP (T₅₀ = 196 min) in addition to 0.7% gellan and 0.06% CaG demonstrated Tryptophan release that was more rapid (statistically significant) than the 0.32% Trypt. + 0.7% gellan + 0.06% CaG (T₅₀ = 319 min) GFS preparation. There is no statistically significant difference in Tryptophan release from the 0.32% Trypt. + 0.7% gellan + 0.06% CaG + 1.0% PVP gellan (T₅₀ = 282 min) GFS preparation as compared to the 0.32% Tryptophan + 0.7% gellan + 0.06% CaG + 1.0% PVP gellan (T₅₀ = 282 min) GFS formulation. The diffusion of Tryptophan from the 0.32% Trypt. + 0.7% gellan + 0.06% CaG + 1.0% PVP gellan(T₅₀ = 282 min) GFS formulation was found to be significantly slowed when compared to the simple 0.32% Tryptophan solution (T₅₀ = 220 min) and the 0.32% Trypt. + 0.7% gellan + 0.06% CaG + 2.0% PVP (T₅₀ = 196 min) GFS formulation. The gellan + Calcium Gluconate + 1%PVP preparation had a T₅₀ of 282 min as compared to the T₅₀ of 233 min for the Tryptophan + gellan formulation. However, the difference was not statistically significant (p value of 0.11).
Previous work by this lab [11] indicates that Tryptophan diffuses more slowly across the dialysis membrane than Timolol. However, the rates of Timolol transmembrane diffusion from a simple Timolol solution and two Timolol gellan GFS formulations were in a similar order to that seen with the T50 values reported here for the 0.32% Tryptophan formulation series. Therefore, Tryptophan may be useful in predicting the relative order of Timolol release rates from gellan + CaG + PVP formulations.

Conclusions:
An ideal GFS for ophthalmic drug delivery should 1) be easily administered in the eye as a consistently sized drop, 2) possess increased retention time within the eye, 3) minimize discomfort and blurred vision, and 4) prolong release rate of drug from GFS [2]. The data indicated that the addition of PVP to gellan and calcium gluconate produces gel forming solutions that form stronger initial gels upon addition to the tear fluid (higher ET K’) than gellan and calcium gluconate alone. The addition of Tryptophan to gellan, calcium gluconate, and PVP formulations still resulted in preparations that form stronger initial gels.

Tryptophan release was significantly slower from a gellan and calcium gluconate preparation than from both a simple Tryptophan solution and a gellan formulation. The Tryptophan release rate for those formulations that had 0.2%, 0.4%, 0.8% and 2.0% PVP in addition to gellan and calcium gluconate were significantly faster than a corresponding gellan and calcium gluconate preparations with no PVP. However, the addition of 1% PVP to gellan and calcium gluconate resulted in a preparation that allowed Tryptophan release that was statistically equivalent to the gellan and calcium gluconate GFS preparation. The data indicates that the concentration of PVP that is added to gellan and calcium gluconate formulations must be optimized both in regards to the ability to gel in the eye and to slow ocular drug release rate.

Figure 1. Tryptophan Release (Average Fit Parameters) for Selected 0.32% Tryptophan Solution and 0.32% Tryptophan Gellan Gel Forming Solutions.
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**References:**


