Release Rates of Timolol Maleate from Carbopol and Carboxymethylcellulose Polymer Gels with Incorporated Calcium Phosphate Nanoparticles.

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Abstract

Purpose. It is of interest to determine whether the release rate of Timolol maleate from Carbopol® 980 and sodium carboxymethyl cellulose gels is modified when varying concentrations of calcium phosphate nanoparticles are incorporated into the gels. *Methods*. Timolol solution, Carbopol® 980 and sodium carboxymethyl cellulose gels with and without varying concentrations of calcium phosphate nanoparticles were manufactured and their Timolol trans dialysis membrane diffusion rates measured. The total amount of Timolol diffused versus time profiles were fit using a sigmoidal curve fit model. *Results*. The 1% sodium carboxymethyl cellulose gel with 1% calcium phosphate nanoparticles was found to have a statistically slower Timolol release rate ($p<0.05$) than its corresponding gel without calcium phosphate nanoparticles. When low concentrations (≤ 1%) of calcium phosphate nanoparticles were incorporated into sodium carboxymethyl cellulose and Carbopol® 980 gels, a trend of slower release rates was observed when compared to their corresponding polymer only gels. Release rates did not directly correlate with viscosity values when calcium phosphate nanoparticles were incorporated into the gels. *Conclusion*. The addition of calcium phosphate nanoparticles to Carbopol® 980 and sodium carboxymethyl cellulose gels appears to be effective at decreasing the release rate of Timolol maleate from the polymer matrix, but this effect is only observed at optimal concentration ratios of polymer to calcium phosphate nanoparticles.

Key words: Ophthalmic drug delivery, ocular drug release, viscous gels, beta blockers, logistic fit

Introduction:

Ophthalmic beta-blockers are frequently used in the initial treatment of open-angled glaucoma[1]. The use of low viscosity ophthalmic Timolol maleate solutions results in rapid removal of drug product from the precorneal area and normally less than 5% of Timolol actually reaches intraocular tissues [2]. Systemic drug absorption occurs via the nasolacrimal drainage duct as Timolol is drained from the precorneal area. Resultant systemic cardiovascular (CV) beta blocking effects may cause bradycardia, heart block, heart failure, and asthma. Thirty-two deaths were reported for the first seven years use of Timolol maleate [3].

Ophthalmic gels may allow for an improvement in Timolol ocular delivery by lengthening the residence time of drug in the eye (increased corneal contact) and decreasing the amount of drug absorbed into the systemic circulation [4]. Timolol release from gels is expected to be slowed as compared to the solution dosage form. These properties are attributed to the higher viscosity of gels as compared to solutions. The polymer carboxymethylcellulose (CMC) is commonly used in pharmaceutical products, is water-soluble, and displays mucoadhesive properties [5]. Kyyronen *et al.* concluded that the use of Na CMC with Timolol maleate improved ocular concentrations by 3 to 9 fold as compared to non-viscous eye drops. They also observed a reduction in the rate of systemic absorption of Timolol maleate by 33%, which mitigated CV side effects that

were seen in the other study group with no polymer inclusion [6]. Carbopol 980 NF (C980) is a high molecular weight cross linked polyacrylic acid polymer that also possesses mucoadhesive properties [7]. Jarvinen *et al.* compared CMC to carbopol in the systemic absorption of ophthalmic Timolol maleate in rabbits. Both bioadhesive polymers showed a similar reduction in systemic absorption (50% decrease in plasma AUC) when administered as equal viscosity solutions [8]. These studies highlight the efficacy of gels in preventing the systemic absorption of beta blocker agents which in turn diminishes the occurrence of systemic CV complications [8]. Additionally, the high water content of gels (hydrogels) promotes a high level of biocompatibility. Schenker *et al.* compared Timolol maleate ophthalmic gel to Timolol maleate ophthalmic solution to determine patient preferences and tolerability in the treatment of glaucoma. The study showed compliance was increased in the gel formulation due to less frequent dosing [9].

The diffusion of drug molecules is slowed when they are dissolved in a water based polymer gel rather than when diffusing from a simple solution. The drug molecules must migrate through a polymer network prior to being released into the surrounding environment. That is, a drug molecule must undergo a tortuous pathway through the hydrated polymer chains that is similar to migrating through a maze. The rate of drug release from a linear polymer matrix is in general inversely proportional to its viscosity [10]. A difficulty may be encountered in that very large unworkable viscosities may be needed to achieve the desired prolonged release of drug.

It is theorized that the incorporation of nanoparticles into a water based gel may block diffusional pathways of drug molecules through the polymer network that acts in addition to the slowing of drug diffusion due to the polymer strands themselves. Nanoparticles have a very large increase in surface area as compared to particles in the micron range or larger. The surface area for the nanoparticles of tricalcium diphosphate (10-80 nanometers in diameter) used in this study is 30-60 m^2/g as given by the vendor. This is as compared to the surface area of 0.44-0.46 m^2/g as given for a commercial tableting grade of dibasic calcium phosphate dehydrate [11]. Thus, the slowing of drug diffusion by particles should be more effective with NPs due to the significantly increased surface area that drug molecules must migrate around.

Calcium phosphate NPs were selected in this study because it is generally agreed that Calcium Phosphate Nanoparticles (CaP NP) are safe [12]. It is also anticipated that many drug molecules can adsorb onto the incorporated CaP NPs. The need for drug to desorb prior to release would result in even more slowing of drug release from the water based polymer gel. The form of calcium phosphate used in this study is tricalcium diphosphate ($Ca_3(PO_4)_2$) because it was readily available in nanometer size. The polymers CMC and C980 were selected due to their excellent safety profile and their use in the enhancement of Timolol activity. Both polymers have the COO^- functional group as part of their structure. The presence of this functional group should facilitate the formation of complexes such as: CaP-$Ca^{2+}\cdots{}^{-}$OOC-Gel [13,14,15]. It is thus anticipated that both C980 and CMC will aid in maintaining CaP NPs in a dispersed state. The use of sodium CMC at 1% and 3% results in gels of a practical viscosity range that are well tolerated by the eye [16]. Concentrations were selected for C980 (0.5% and 1.0%) that produced gels with similar viscosities to the 1.0% and 3.0% CMC gels.

The purpose of this study was to determine the release rates of Timolol maleate from C980 and CMC ophthalmic gels of different polymer concentrations and with the addition of varying concentrations of calcium phosphate nanoparticles. Viscosity properties were measured for the different gel preparations. It was examined whether a correlation could be obtained between measured viscosity properties and Timolol release rates.

Material and Methods

All test samples were manufactured with a final Timolol maleate concentration of 0.68% (w/w). Timolol solution was prepared by dissolving the appropriate amount of Timolol maleate in Dulbecco's Phosphate Buffered Saline (DPBS). The concentrations of polymer and CaP NP used in the gel preparations are given in Table 1. Concentrated solutions (CMC) or slurries (C980) of polymer were prepared and allowed to hydrate for at least 12 hours. Measured amounts of concentrated polymer were combined with Timolol alone or with CaP NP to formulate the target concentrations. One normal sodium hydroxide was used to neutralize C980 gels (pH 7-8) after the addition of other components. Sodium CMC gels were confirmed to be within the target pH range. Sufficient deionized water was added to the gels to give a final batch weight of 100 g. An overhead mixer and a homogenizer (2 min) were used to ensure that gel composition was uniform. The preparations were tested for Timolol transmembrane diffusion characteristics within 3 days after manufacture.

A UV-Visible Spectrophotometer was used to measure the amount of UV absorbance at 295 nm of Timolol maleate standard solutions at eight different concentrations. A calibration curve was generated between Timolol maleate absorbance at 295 nm and concentration. The equation was not forced to a Y-intercept of zero and had a r^2 value of 0.9999. Concentrations of Timolol in experimental samples were determined using the standard curve.

Ten centimeter segments of cellulose ester dialysis membrane (molecular weight cut off of 3,500-5,000 Da) were soaked in deionized water for greater than 24 hr in order to remove preservative and any other water soluble contaminants. The molecular weight cut off was selected so as to easily allow the diffusion of Timolol Maleate (MW = 432.492 Da) through the semipermeable membrane while restricting the movement of polymers and nanoparticles through the membrane. Prior to the experimental runs, washed dialysis membrane segments were equilibrated with DPBS for 24 hours or longer at ambient temperature (23° C). The dialysis tubing was removed from the soaking DPBS, closed at one end, filled with approximately 1 g of preparation, and the top end was clamped. Each filled dialysis membrane bag was placed in a beaker filled with 100 mL of fresh DPBS to elicit sink conditions. A stir bar was placed in the beaker that was then covered with Para film. Each sample set up was placed on a multi-station stir plate at ambient temperature and stirred at 1000 rpm. 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.

The absorbance (295 nm) of Timolol in the DPBS receiving fluid was measured at regular timed intervals using a UV-Vis Spectrophotometer and dedicated disposable UV cuvettes. Timolol/DPBS samples were returned to the set up after measurement using dedicated disposable transfer pipettes. Therefore, the DPBS receiving fluid in each set up was not diluted by adding DPBS in order to compensate for measured samples being set aside.

Timed samples of the DPBS receiving fluid were collected and assayed. Each experimental run was conducted for at least 7 hrs for the Timolol solution and for at least 18 hrs for the gel preparations. Experimental studies were conducted until near equilibrium conditions were achieved. That is, transmembrane diffusion data was collected until it was clear that small differences in Timolol sample absorbance were occurring with large differences in sampling time. Fifteen or more sampling times were performed for each experimental run with at least six experimental runs being conducted for each preparation. The actual amount of gel preparations transferred to the dialysis bag was measured and absorbance readings were normalized to 1 g of gel being tested. The measured amounts of diffused Timolol were expressed as percentages of the total amount of Timolol that can be theoretically diffused (% Theory). Percent theory equals the measured amount of Timolol divided by 0.067 mg/mL and times 100. That is, 1 mL (assuming a specific gravity of 1.0) of 0.68% Timolol Maleate in the dialysis bag is added to 100 mL of DPBS receiving fluid to give a final volume of 101 mL and a theoretical concentration of 0.067 mg/mL after the total release of Timolol.

The amount of Timolol that diffused through the dialysis membrane into DPBS was measured and plotted as % Theory versus the time at which the sample was pulled from the receiving fluid. Linear regression was performed using Microsoft Excel. Non-linear regression analysis (curve fitting) of the plotted data pairs was performed using the software KaleidaGraph. The following form of the power (Peppas) equation was used in the curve fitting of the data: $\%\,Theory = kt^n$ [17]. The general form of a logistic function (KaleidaGraph) was used for sigmoidal curve fitting. The four variable equation used in the sigmoidal curve fitting of the data as defined in KaleidaGraph is: $Y = M1 + \frac{M2 - M1}{1+\left(\frac{X}{M3}\right)^{M4}}$. Y is the amount of Timolol released (% Theory) at X (time in minutes). M2 corresponds to the concentration before the dialysis run begins and M1 corresponds to the concentration at time infinity [18]. The data was initially fit using the sigmoidal equation with all four variables being allowed to vary. The averages of the variable values for M1 and M2 were 98.8 ±5.5 and 1.0 ±0.9 Percent Theory, respectively. The theoretical values for M1 and M2 are 100% and 0% Theory. For the results reported here, the parameters M1 and M2 were held constant at their theoretical values in order to improve the degrees of freedom for the fitting procedure. The parameters M3 and M4 were left to vary in value during the curve fitting process. The M3 parameter is the time at which the midpoint (50% theory) or point of inflection is reached between the lowest (0.0%) and highest amounts (100%) of Timolol release [18]. The parameter M4 is considered a shape parameter and has no direct physical significance [18]. It gives much less information as to how rapidly Timolol is being diffused as compared to M3. In the range of M4 values derived in this study, a larger M4 value reflects a larger time before 90% Theory is attained. The time to reach 75% Theory (T75) was calculated for each individual fit using the equation: $75 = M3\ x\ (3)^{\frac{1}{M4}}$, in which the assumptions of 0% Theory at time zero and 100% Theory at infinity are applied.

The generated M3 values for each individual experimental run were treated as independent data points. Prior to statistical analysis, any outliers for the different preparation's M3 values for each experimental run were detected using Dixon-type tests [19]. Outlier experimental runs were discarded in further statistical analysis. One way analysis of variance (ANOVA) and the Tukey's post-hoc test were used to determine statistical significance (< 0.05) for M3 values of the different preparations. The average M3 and M4 values of the individual experimental runs for each preparation are treated as being representative of the preparation.

Gel viscosities were assessed using a Haake Viscotester 550. Curve-fit analysis was performed using the Ostwald-De Waele and Bingham models as provided in the equipment software. The Ostwald-De Waele model allowed for the viscosity related constant (k) and flow behavior index (n) to be determined according to the

equation:$\tau = k\dot{\gamma}^n$, where τ is the shear stress and $\dot{\gamma}$ is the shear rate. The higher the k value, the more viscous the gel preparation is. Fluids are considered to be Newtonian if n is equal to 1. Fluids are considered to be pseudoplastic (shear thinning) if n is less than 1 and considered more shear thinning as the value for n decreases. The Bingham model allowed for the calculation of the yield value for each gel preparation.

Results and Discussion

The use of dialysis membranes to measure the rate of Timolol release from ophthalmic dosage forms has been previously performed [20]. Technically, the rate of diffusion across a semipermeable membrane is measured with this method. However, the rate at which Timolol is released from the polymer matrix will determine its effective concentration at the polymer matrix interphase with the membrane. Fick's law, when applied to membranes, indicates that a decrease in concentration will result in a decreased rate of transmembrane diffusion when other relevant parameters (surface area, diffusion coefficient, partition coefficient, and membrane thickness) are held constant. Thus, the rate of diffusion reflects the rate of release of the drug from the polymer matrix. This method is especially relevant for ophthalmic dosage forms as Timolol must migrate out of the polymer gel (matrix) and diffuse through the cornea, a semipermeable membrane composed of three layers, to reach its site of action.

The composite of the 1% C980 gel released Timolol concentrations (expressed as % Theory) were plotted versus the square root of sampling. A linear fit of the data did not demonstrate a strong correlation and a better fit was achieved when the power (Peppas model) equation was used (Fig.1). Although the power model more suitably describes the data than the square root of time model, there were systematic errors in which the power model over estimates the amount of diffused Timolol during early time points, underestimates the amount of diffused Timolol at later time points and over estimates the amount of diffused Timolol at time points far along in the diffusion process. A power curve fit of the total data for each formulation (such as: 1% C980, 0.5% C980, etc.) was conducted and the residuals calculated. Systematic errors are clearly seen when the residual values were combined and then plotted versus the time of diffusion (Fig. 2).

Individual % Theory vs. time curves were fit using a sigmoidal (S shaped) function and very high correlation values (r^2) were obtained that ranged in value from 0.96 - 0.99 with an average r^2 value of 0.99. The nearly uniform distribution of all residuals about the zero value indicates the absence of systematic errors (Fig. 3). The residuals are smaller in value and reflect the better fits (higher r^2 values) obtained when the sigmoidal model is used rather than the power fit. The evaluation of residual values and higher r^2 values indicate that the use of a logistic model to fit the data results in an accurate and correct fit of the experimental data.

The diffusion of Timolol through the dialysis membrane in this study is analogous to a reservoir type of transdermal system in which a high concentration of drug is contained in a reservoir (often composed of a polymer gel) positioned adjacent to a rate limiting semipermeable membrane. The membrane surface area, thickness of the membrane, diffusivity of the drug molecule through the membrane material, and temperature in our release studies are constant in the same manner as for transdermal reservoir systems. In a reservoir type of transdermal system; there is an initial time period, or lag time, in which the high concentration of drug in the reservoir equilibrates with the rate limiting membrane and adhesive layer [21]. In our experimental set up, an initial time period occurs during which Timolol inside the dialysis bag equilibrates with the Timolol that resides within the dialysis membrane. The Timolol concentration in the receiving fluid is expected to demonstrate a rapid but limited exponential growth during this time period, which is referred to as time segment 1. Time segment 1 appears to be around 10 min (or less) in duration. It was assumed that the length and diffusion characteristics of the different Timolol preparations during the first 10 minutes would be sufficiently similar to pool the limited data and allow a nonlinear curve fit. A correlation (r=0.66) was achieved when the data collected up to 10 minutes were fit using an exponential growth curve model. The data appears to support the concept of an initial rapid exponential (convex) growth of concentration in the receiving fluid that occurs during a lag time [21].

At the end of 10 minutes, the amount of Timolol inside the dialysis bags remains much higher than Timolol concentrations in the receiving fluid. That is, receiving fluid concentrations can be ignored and sink conditions are in effect during time segment 2. According to Fick's law, a constant rate of diffusion of Timolol across the dialysis membrane should result in a straight line when % Theory is plotted versus the sampling time [21]. Experimental Timolol data pairs were examined visually for signs of linearity. These initial data pairs were then fit using linear regression. Data pairs were added or eliminated in order to optimize the linear fit r^2 value. Excellent linear fits were obtained for all our experimental runs up until about 40% Theory. Therefore, segment 2 of diffusion experimental runs was defined as taking place at sample times >10 minutes and proceeding until that time at which % Theory is $\leq$ 40%.

As the experiment progresses and sink conditions are less well satisfied, the concentration in the receiving fluid becomes relevant in defining the rate of diffusion. Thus, the departure from zero order diffusion becomes more pronounced as the experiment proceeds until equilibrium is reached between the concentration of Timolol inside

the dialysis bag and the concentration in the receiving fluid. Once sink conditions are no longer met for reservoir transdermal systems; first order, rather than zero order, release occurs [21]. For the experimental results reported here, the % Timolol vs sampling time curves started departing from linearity around 40% Theory. Therefore, time segment 3 was defined as all sample data points in which percent Theory is > 40% and is the upper portion of the S shaped curve. It is expected that the % Theory versus diffusion time will demonstrate an exponential rise during segment 3. Percent Theory values were plotted versus the diffusion time during segment 3 for all experimental runs and an exponential rise curve fit was applied. The resultant r^2 values varied from 0.979 to 0.592 with an average r^2 of 0.867.

The fit values from time segments one, two, and three were used to produce curves and linear lines for the different experimental runs. The generated time segment curves were then combined to produce curves that describe the entire % Theory vs. time graphs. Very strong visual agreement was obtained when comparing time segment combination curves to curves that were generated from the logistic (sigmoidal) best fit curve model. It was found in a separate study, using a very similar experimental set up, that the release profile of Leuprolide was tri-phasic in a manner described in this paper as time segments 1,2, and 3 [22]. Other investigators found that a growing exponential equation best described Fickian diffusion under non-sink conditions as was used here to describe Timolol release during time segment three. They also found that the use of the power equation gave systematic deviations from experimental data when diffusion studies were conducted for long time periods [23]. A sigmoidal curve fit of the experimental data thus appears to be in agreement with expected drug membrane diffusion behavior. The average curve fit parameters for the various formulations are given in Tables 2 and 3.

The linear portion of the curve (time segment 2) is a measure of the initial rate of Timolol transmembrane diffusion from the different experimental formulations. Higher slope values are indicative of faster transmembrane diffusion rates of Timolol. However, only a small portion of the total experimental data (≤40% Theory) can be examined using this approach. The M3 value from the logistic (sigmoidal fit) is the time at which the midpoint (50% theory) or point of inflection is reached between the lowest (0.0%) and highest amounts (100%) of Timolol transmembrane diffusion. An excellent correlation (r^2 = 0.8526) was achieved in the anticipated order when the values of the linear slopes were regressed against the average M3 values. It appears that the M3 value adequately describes the Timolol transmembrane diffusion rate for the initial linear portion (segment 2) of the transmembrane diffusion data.

Time segment 3 of the diffusion curve occurs after sink conditions are no longer in effect. The 75% Theory values are in the upper curvature of the S-shaped curve and occur during time segment 3. A strong correlation (r^2 = 0.805) was noted between T75 values and the curve fit variable describing the curvature characteristics of the exponential rise portion (time segment 3) of the diffusion curve. The value for T75 appears to be a good descriptor of the portion of the Timolol diffusion curve which occurs when sink conditions are no longer met. A strong correlation (r^2 = 0.896) was achieved when T75 and M3 values were regressed against each other. Therefore, the M3 value is representative of the transmembrane diffusion process as indicated by its close agreement with measures indicative of Timolol diffusion rates during time segments 2 and 3 (linear slope and T75).

ANOVA results indicated that there were statistically significant differences in M3 values between the various Timolol test preparations (p value < 0.0001). Tukey's post-hoc analysis of individual M3 values was used to determine which test preparations generated Timolol diffusion rates that were statistically significant from each other. Overall, a difference of 66 min in the average M3 value between the pair of preparations being compared is indicative of statistical significance. Timolol diffusion profiles of selected preparations which demonstrated statistical significant differences in their M3 values are shown in Figs. 4,5, and 6. The generated sigmoidal curve and individual data are shown with the log of time rather than time for the X-axis since the variance of the individual experimental data points from the sigmoidal model fits are more easily recognized.

The 1% CMC gel with 1% CaP NP was found to have a statistically slower diffusion rate ($p<0.05$) than its corresponding gel without CaP NP (Fig. 4). The 3% CMC with 1% CaP, 0.5% C980 with 1% CaP, and 1.0% C980 with 1% CaP gels all demonstrated a trend of slower diffusion rates than their corresponding gels which did not contain CaP NPs. The data indicates that the incorporation of 1% CaP successfully reduces the rate at which Timolol can diffuse out of CMC (1%, 3%) and C980 (0.5%, 1%) polymer gels.

Timolol transmembrane diffusion rates were increased when 2% and 5% CaP NP were added to carbomer gels and when 5% CaP NPs were added to the CMC gels. This is in contrast to the expectation that larger amounts of NPs (increased obstruction) would cause enhanced slowing of the Timolol transmembrane diffusion rate. Perhaps the higher concentrations of CaP NP resulted in a higher effective Timolol concentration at the inner membrane boundary. In agreement with Fick's law; this would result in linear portions with larger linear slopes, shorter times to the 50% theoretical transmembrane diffusion value (M3), and shorter times in which linearity occurs. These effects were observed for both the CMC and C980 gels when CaP NPs were incorporated at high (≥ 2%) concentrations.

The measured viscosity curves for each gel preparation are shown in Figs. 7 and 8. The measured rheology curves were fitted by the Ostwald de Waele and Bingham models and the results are displayed in Table 4. The n values were less than one and indicated that all gels, whether CMC or Carbopol based, exhibited pseudoplastic properties. Higher viscosity gels tended to show more pseudoplastic properties and higher yield values. A surface interaction is likely to occur between solid CaP NPs and the carboxylate groups of CMC and Carbopol [13,14,15]. It appears that the interaction is such that the viscosity properties of the CaP-Polymer gels are dominated by polymer properties. This may be a useful finding for ophthalmic products because increased viscosity can result in blurred vision, more difficulty in dispensing the product, and patient discomfort. Additionally, the incorporation of CaP NP did not significantly change the shear-thinning properties of the tested polymer gels. Shear-thinning flow is desirable for patient comfort as the high sheer rates associated with blinking are less likely to result in perceived eye lid drag by the patient.

Expectedly, the measures for viscosity (k)and and diffusion rate (M3) values for gels that contained only polymer were strongly correlated with an r^2 of 0.89 [10]. There was no statically significant correlation between the k and M3 values when the gels containing CaP NPs were included in the regression analysis. There were no discernable patterns in which increased amounts of CaP NPs caused large alterations in the flow properties of the gels.

Conclusion

Sigmoidal curve fits are excellent descriptors of transmembrane diffusion results and provide a straight forward description of the Timolol transmembrane diffusion process. The time to 50% release (M3 sigmoidal fit parameter) values were found to describe drug release rates for various Timolol gel preparations. It appears that CaP NP may be used in CMC and Carbopol gels to decrease drug release rates without significantly increasing the viscosity of the preparation. The slowing of Timolol diffusion out of polymer gel is only seen at lower ratios of CaP NPs concentration to polymer concentration. Higher concentration ratios of CaP NPs to polymer resulted in an increased diffusion rate out of the polymer matrix. Finding the appropriate concentration ratio between polymer and CaP NP may allow for targeted drug release rates from polymer gels.

Acknowledgements:

Carboxymethylcellulose, sodium salt and Carbopol 980 NF were kindly provided by Ashland Aqualon Function Ingredients and Lubrizol Advanced Materials, respectively.

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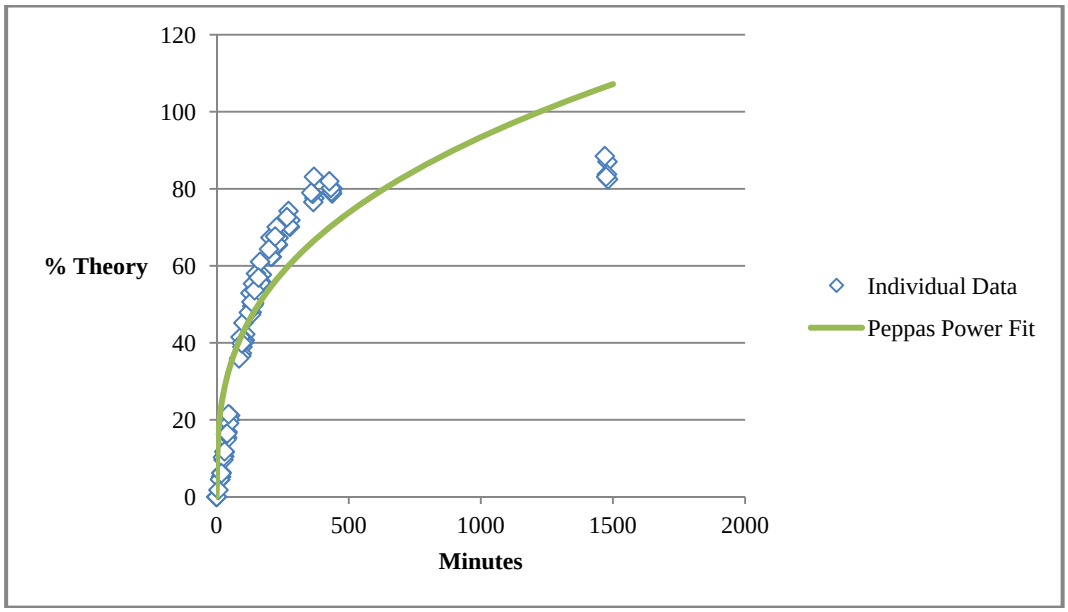

Figure 1: Power Fit for Timolol Release from 1.0% Carbopol 980.

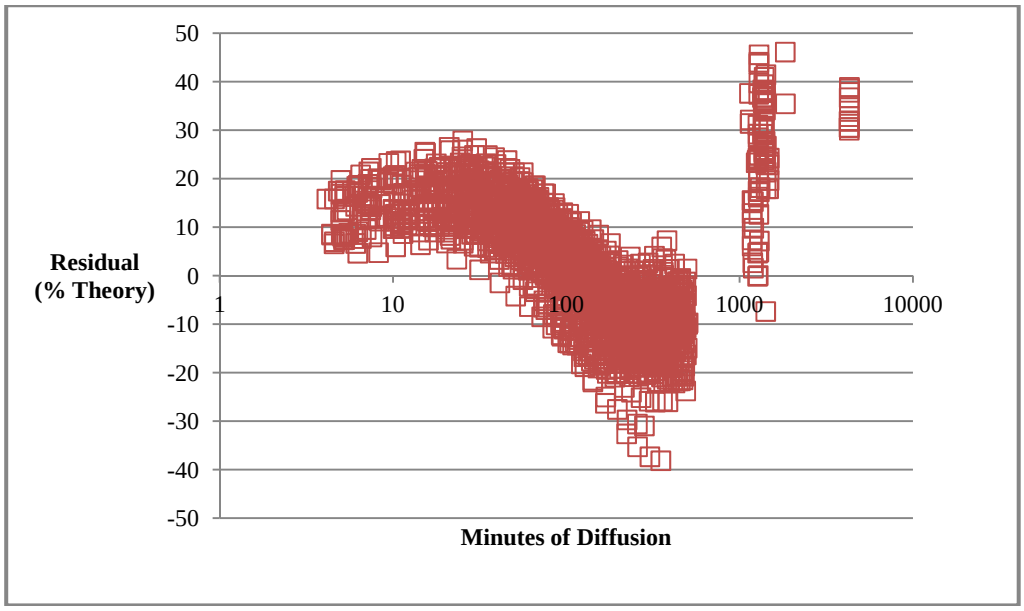

Figure 2: Residual Data from Power Fits for All Experimental Diffusion Experiments.

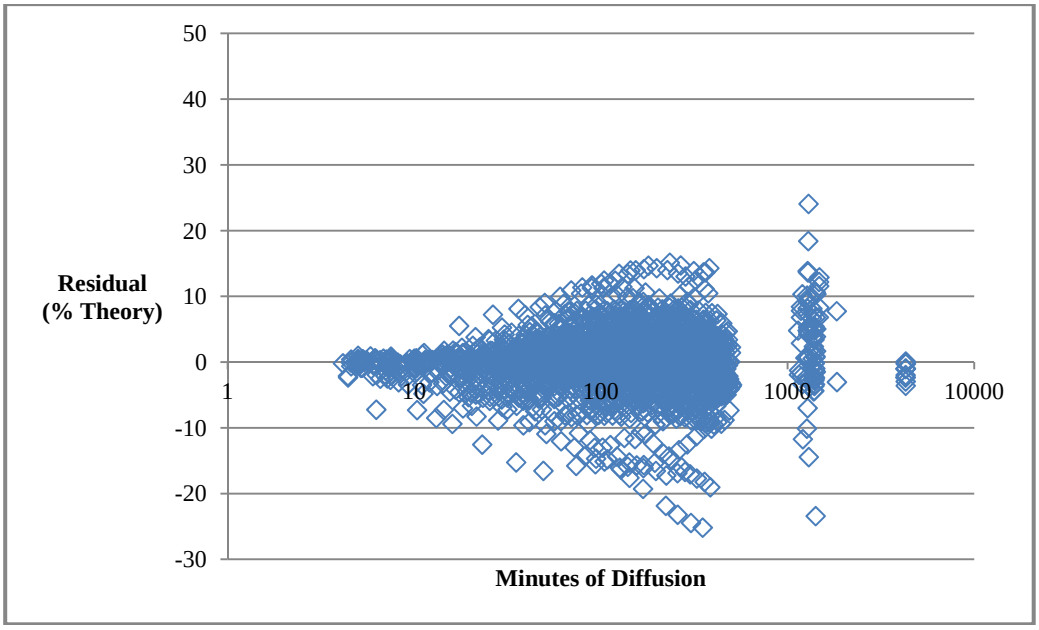

Figure 3: Residual Data from Sigmoidal Fits for All Experimental Diffusion Experiments.

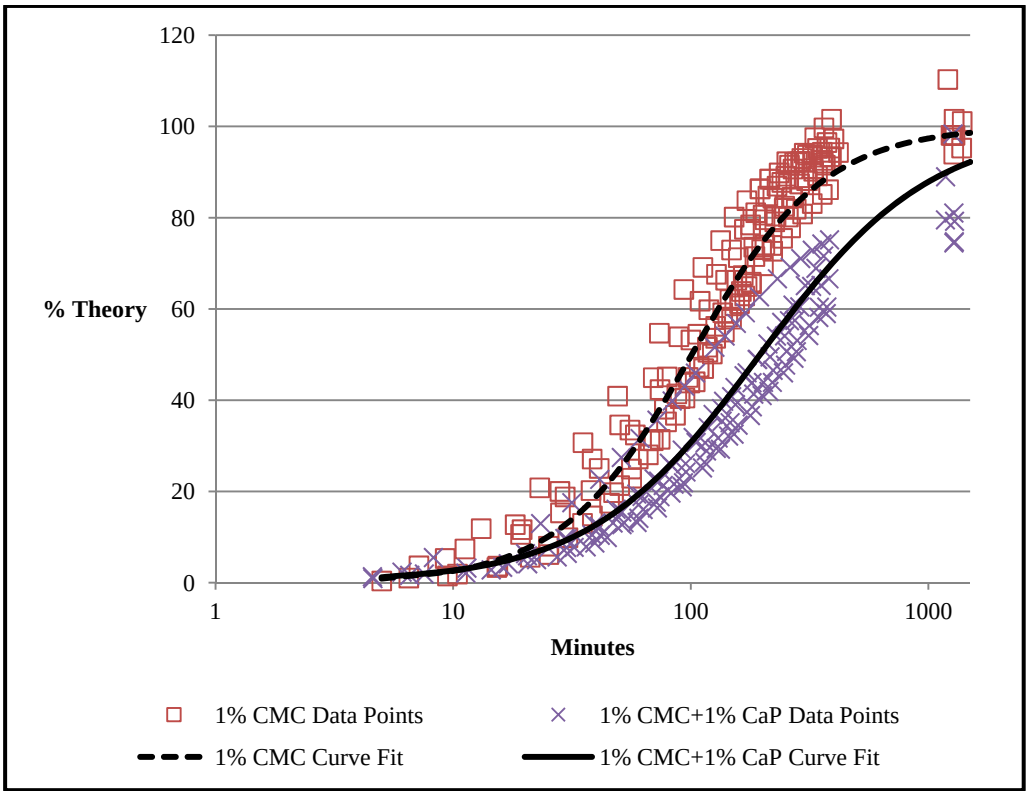

Figure 4: Timolol Release from 1% CMC with or without 1% Calcium Phosphate Nanoparticle Preparation.

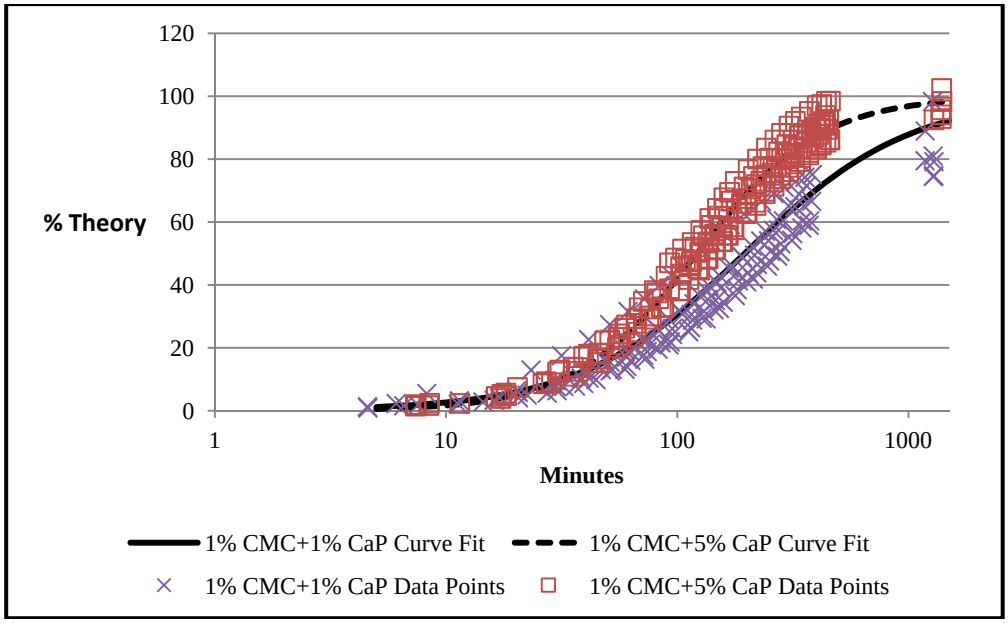

Figure 5: Timolol Release from 1% CMC with 1% or 5% Calcium Phosphate Nanoparticle Preparations.

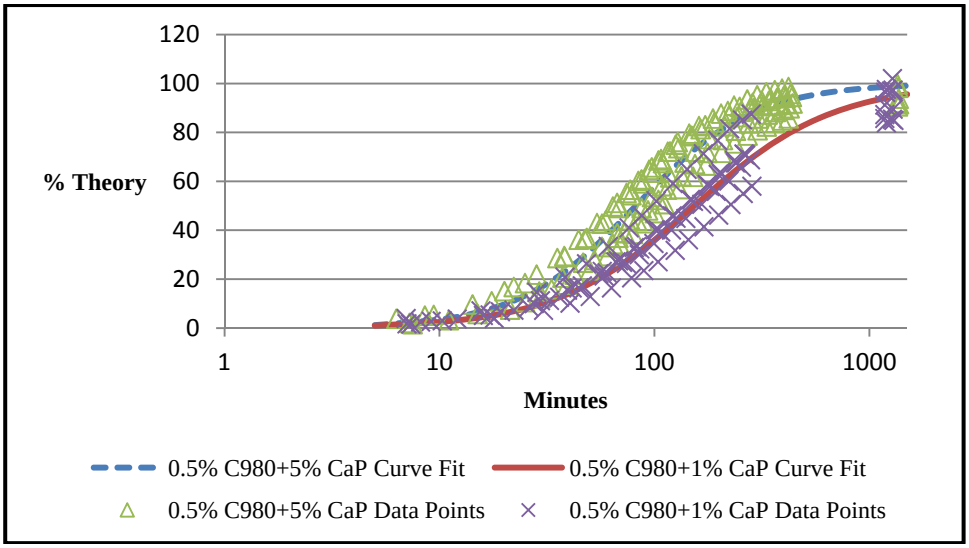

Figure 6: Timolol Release from 0.5% Carbpol 980 with 1% or 5% Calcium Phosphate Nanoparticles.

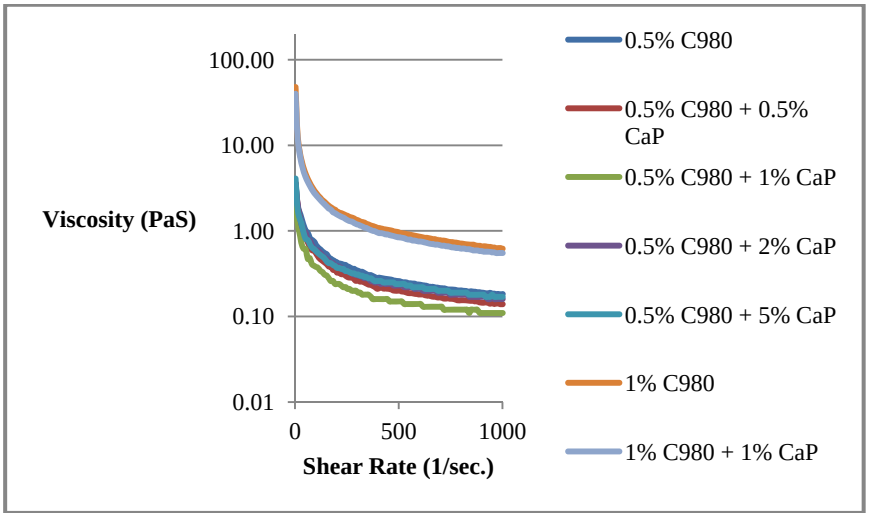

Figure 7: Viscosity Curves for Carbopol 980 Gels.

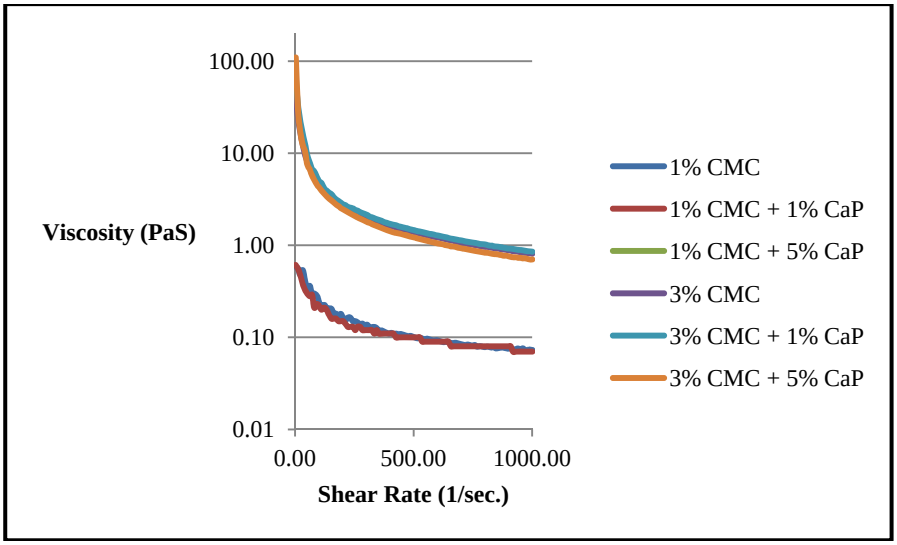

Figure 8: Viscosity Curves of Carboxymethylcellulose Gels.