Characterization of Pluronic F127 for the Controlled Drug Release Vancomycin in the Spinal Column

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Abstract

Pluronic F127 is a unique hydrogel due to its sol-gel transition properties determined by temperature and concentration. By investigating its rheological properties and drug release capability, F127 can be used as a drug delivery system for the spinal column. In this study, rheological measurements including temperature sweeps and frequency sweeps were performed on various concentrations of F127 to characterize its physical structure at different conditions. For temperature sweeps, higher concentrations of F127 showed abrupt increase of viscosity at lower temperature. For frequency sweeps, the fracture point of Pluronic F127 with and without Vancomycin was observed at 18.42 Hz. The calculated flow rate at 18.42 Hz is $(0.53 \pm 0.11) \times 10^{-6}$ m^3/s, which is much higher than the flow rate of human spinal fluid. In addition, Pluronic F127's drug release capability was tested via a flow chamber system where DI water is flowed at a constant rate over the hydrogel with antibiotic additive. UV-Vis spectroscopy was used to characterize the collected flow samples. From the flow test, a slow flow rate of 0.1 mL/min yielded higher release of F127 and Vancomycin as compared to higher flow rates of 0.5 mL/min and 1 mL/min.

Keywords: Pluronic, Vancomycin, hydrogel, drug release, rheology

1. Introduction

Hydrogels are three-dimensional networks formed by polymers that exhibit visco-elastic characteristics widely used in the field of cosmetic, pharmaceutical, and biomedical industries.[1] Pluronic is a triblock copolymer made up of hydrophilic end units, polyethylene oxide (PEO), with a hydrophobic center unit, polypropylene oxide (PPO).[2] Pluronic exhibits unique thermoreversible gelling capability.[3] At low temperature in solution, Pluronic exists as single chain polymers (unimers) (Figure 1a).[4] As temperature is increased, the solubility of the PPO unit is lowered giving rise to formation of micelles (Figure 1b).[3] The hydrophobic blocks form the inner core while the hydrophilic blocks form the exterior corona of the micelle.[5] As more micelles are formed, the coronas of the micelles overlap leading to gel formation (Figure 1c).[5] Pluronic micelles self-assemble into a face-centered cubic (fcc) lattice structure.[6]
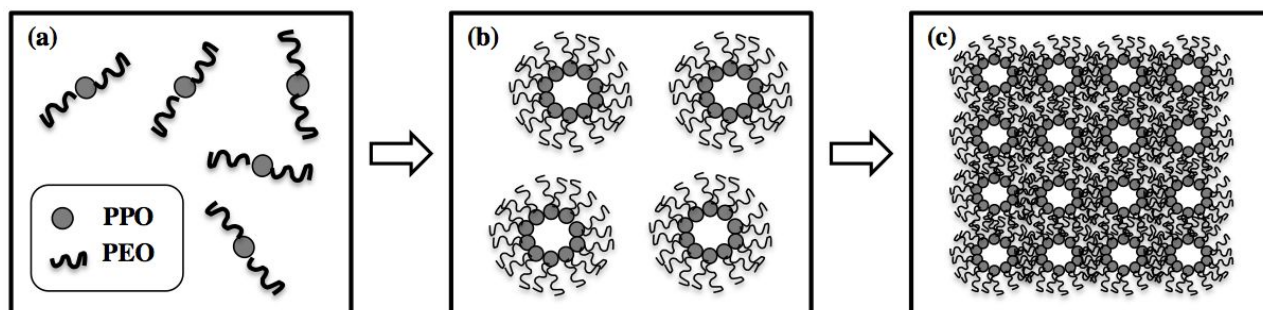
Figure 1. (a) Pluronic unimers in solution, (b) micelle formation, (c) micelles arranged in cubic structure.

Pluronic is biocompatible and FDA approved which makes it a desirable material to have as a drug delivery system. Pluronic is a non-cell adhesive material that can be used as a physical barrier to prevent scar tissue formation. Oh et al. studied the drug delivery of ibuprofen in Pluronic F127/F68 crosslinked with alginate as a physical barrier for adhesion prevention using *in vitro* and *in vivo* studies.[7] Their results showed a stable gel at 30 °C with sustained release of ibuprofen up to 45% of total loading amount. The Pluronic F127/F68/alginate/Ibuprofen gel was shown to be an effective material to prevent tissue adhesion formation. Therefore, it has been proposed to inject Pluronic after spinal surgery to prevent adhesion while delivering antibiotics.

In the spinal column of adults, there is continuous perfusion of cerebrospinal fluid at a rate of 400 to 600 ml per day,[8] which is equivalent to 0.28 to 0.42 ml per minute. Pluronic's sol-gel phase transition is dependent upon the concentration and temperature of the material where Pluronic is a solution under a critical gelation concentration and temperature. Therefore, the gel stability of Pluronic in the spinal column is noteworthy to investigate to determine Pluronic's effectiveness as a physical barrier to prevent cell adhesion.

Vancomycin is an antibiotic used to treat infections such as osteomyelitis.[9] The optimal dosage of Vancomycin as an injectable solution is 5 mg/mL for adults.[10] This dosage can vary by age and weight of patients. Vancomycin does not impede bone fracture healing.[9] Hence, administering Vancomycin into the spinal column after spinal surgery can prevent surgical site infections.

In this study, we investigated Pluronic F127's gel stability under various shear flows simulating cerebrospinal fluid flow to determine the dissolution time of Pluronic. Rheological measurements of Pluronic F127 were conducted to obtain critical gelation temperature of Pluronic at various concentrations and to observe the gel structure at increasing frequency sweeps. We conducted a drug release study of antibiotic Vancomycin to determine the drug elution of Vancomycin and the effects of flow on its release. The drug release study was performed using a flow chamber where DI water was flowed through the Pluronic gel containing Vancomycin at constant flow rates of 0.1, 0.5, and 1 mL/min. Samples collected from the flow chamber were then characterized via UV-Vis spectroscopy.

2. Methods

2.1. *Chemicals and Materials*

Pluronic F127 (MW = 12600 g/mol, Sigma-Aldrich, Saint Louis, MO, USA) and Vancomycin hydrochloride hydrate (CAS-No. 123409-00-7, Sigma Aldrich, Saint Louis,

MO, USA) were used as received without any further purification.

2.2 *Preparation of Pluronic F127 Hydrogel*

Pluronic F127 hydrogels were prepared by mixing together Pluronic F127 in DI water by weight/volume % (w/v %). For preparation of samples, an analytical balance with a precision of 0.001 g was used to weigh the desired amount of Pluronic F127. Samples with varying concentrations of Pluronic F127 in deionized (DI) water were prepared ranging from 1 - 30 (w/v %). Samples were also prepared with the addition of 1% Vancomycin added to the Pluronic F127/DI mixture. All solutions were stirred and kept in a refrigerator for at least one day to achieve homogeneous mixture.

2.3. *Rheology of the Pluronic F127 Hydrogel*

Rheological characterization of samples were performed on a Bohlin Gemini 150 HR Nano rheometer. Temperature sweeps were conducted using samples of varying Pluronic F127 concentrations (20%, 22.5%, 25%, 27.5%, 30% w/v%) in DI using the Peltier cylinder system with the temperature being controlled by the rheometer's cooling system with a water pump. Samples were cooled in a refrigerator and then loaded onto the Peltier cylinder surrounded by the temperature control system. The temperature sweeps were carried out from 17 °C to 60 °C at a constant shear stress of 10 Pa and a strain of 10%. The condition of a constant shear stress of 10 Pa was selected for the temperature sweep after a preliminary test showed a plateau of G' at 10 Pa both at low and high frequencies indicating that the hydrogel maintained its stable network under these conditions. The viscosity (Pa•s) was plotted against temperature (°C) from the data collected in the temperature sweeps. Samples were prepared for frequency sweeps by

placing 3 mL of the hydrogels into polystyrene (PS) petri dishes and placed into a Fisher Scientific Isotemp™ Incubator™ set at 37 °C for a duration of over 10 minutes to allow the samples to gel. Frequency sweeps were performed using the 2 cm Peltier plate spindle under the oscillatory mode of the rheometer. The rotating spindle allowed measurements of elastic modulus (G'), and viscous modulus (G") to be collected following a logarithmic sweep of frequencies. Frequency sweeps of each modulus were evaluated between 0.01 to 100 Hz at a constant temperature of 37 °C, under a constant shear stress of 100 Pa with a set strain of 10%. A constant shear stress of 100 Pa was selected to identify the fracture point of the gel. The moduli were plotted against frequency.

2.4. *Flow Tests for UV-Vis Spectroscopy*

The flow tests were conducted in the Fisher Scientific Isotemp™ Incubator™ set at 37 °C. Prepared samples were loaded into a chamber for hydrogel testing that was created by the Garcia Center for Polymers at Engineered Interfaces at Stony Brook University. The schematic of the chamber can be found below in Figure 2.
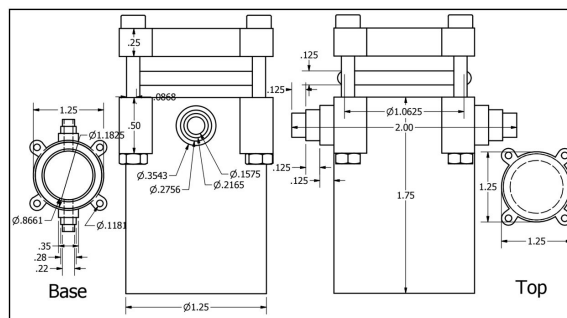

Figure 2. Schematics of version 1.1 of the Tubing Apparatus for Hydrogel Testing. All dimensions are in inches.

A NE-1000 Single Syringe Pump (NE, Ringoes, NJ) was used to conduct the flow test to collect samples for UV-Vis

spectroscopy. A BD 20 mL syringe with a Lver-Lox™ tip (BD, Franklin Lakes, NJ) was loaded with DI and secured on the syringe pump. The chamber was loaded with 13 mL of hydrogel and secured by nuts and bolts. The hydrogel in the chamber sat in the incubator at 37 °C to gel. The syringe pump was then connected to the loaded closed chamber by amber latex tubing with an ID size of 3/16" and a wall size of 1/16" (VWR Scientific, West Chester, PA). The syringe pump was set to mL/min and flow tests of 0.1, 0.5, and 1.0 mL/min were conducted in order to simulate the flow rate range of spinal fluid (0.28-0.42 mL/min). For each single test, three separate samples were collected after 5 mL was collected so that for one test, a sample of 0-5, 5-10, and 10-15 mL were collected separately for UV-vis spectroscopy. UV-Vis of each 5 mL were taken and compared in order to characterize the time dependence of the release. A picture of the running setup can be found in Figure 3.

2.5 UV-Vis Spectroscopy

The UV-Vis spectroscopy of samples were conducted using a Evolution 220 (Thermo Fisher Scientific, Shanghai, China)
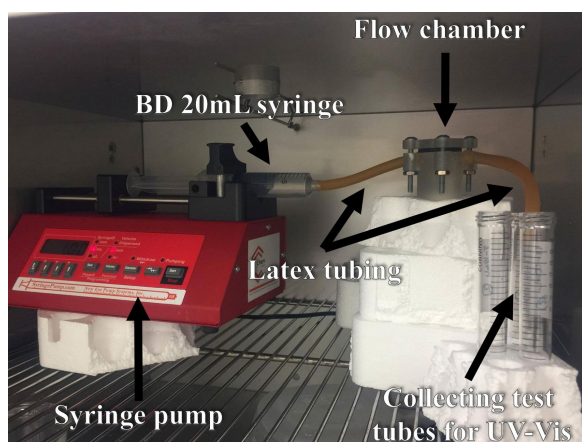

Figure 3. The running setup for the flow test. This setup is conducted in a Fisher Scientific Isotemp™ Incubator™ set at 37 °C.

UV-Vis spectrometer. A blank sample of the DI used in the solutions was used to calibrate the spectrometer. Samples were loaded on optical cuvettes and placed into the spectrometer. The absorbance of the UV-Vis spectrometer was performed between 200-400 nm at room temperature.

3. Results and Discussion

3.1. Gelation Temperature of F127

Rheology measurements were performed for Pluronic F127 aqueous solution for various concentrations: 20, 22.5, 25, 27.5, and 30 w/v %. Figure 4 shows the viscosity as a function of temperature from 18 °C to 60 °C. At low temperature, viscosity slowly increases as the copolymer starts to form micelles.[11, 12] Viscosity increases steeply when sol-gel transition takes place due to associations of the hydrophobic cores.[11, 13, 14] Temperature dependence of gel stability on Pluronic concentration was noticeable. With an increasing concentration of F127, the phase transition temperature decreased. This phenomenon is possibly due to a change in aggregation number with temperature and/or change in the micelle formation process at
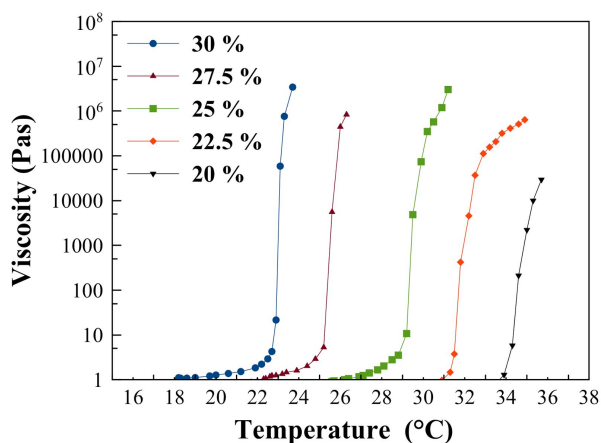

Figure 4. Viscosity as a function of temperature at various concentrations of F127 aqueous solution (w/v %)

different concentration.[15] From the temperature sweeps, we chose the concentration of 30 w/v % to use for following experiments based on gel stability with the lowest opportunity to be liquefied at 37 °C.

3.2. Frequency Sweep on F127 with and without Vancomycin

Frequency sweeps measurements were performed on Pluronic F127 with and without the additive of 1% Vancomycin, and the effects of Vancomycin on the micellar structure of F127 was investigated. Frequency sweeps were conducted within the frequency range of 0.01 - 100 Hz at constant shear (100 Pa) and temperature (37 °C). Figure 5 shows the comparison between the frequency sweeps on F127 with and without Vancomycin. Both F127 systems, with and without Vancomycin, exhibited similar rheological behaviors in the examined frequency range. Regardless of the presence of Vancomycin, elastic modulus (G') was greater than viscous modulus (G") throughout the frequency range. This is associated with the highly entangled F127 gel networks, and reflects the gel behavior of 127.[16, 17] At low frequency ranges (0.01 - 10 Hz), G' for both samples showed low-frequency plateau, and no significant increase was observed with frequency. For the viscous modulus (G"), decrease of G" is observed in both F127 systems. However, decrease of G" for F127 with Vancomycin was observed at lower frequency, which was at 0.01 Hz. Moreover, at this frequency, G' of F127 with Vancomycin was twice higher than that of F127 without Vancomycin. These indicate that the presence of Vancomycin stabilized the F127 at lower frequency and F127 with Vancomycin exists as a stable gel. At frequency of 18.42 Hz, distinctive dips of G' were observed in both systems. The possible explanation is that the frequency of 18.42 Hz was the limit for the material to

maintain its stable elastic domain at 100 Pa in shear. Due to this reason, at frequency higher than 18.42 Hz, the material degraded via breakage of micelle entanglements within shear at 100 Pa. As the micelles were no longer physically bonded at frequency higher than the gel fracture point, different slope of G' were observed due to different forms of micellar solutions.
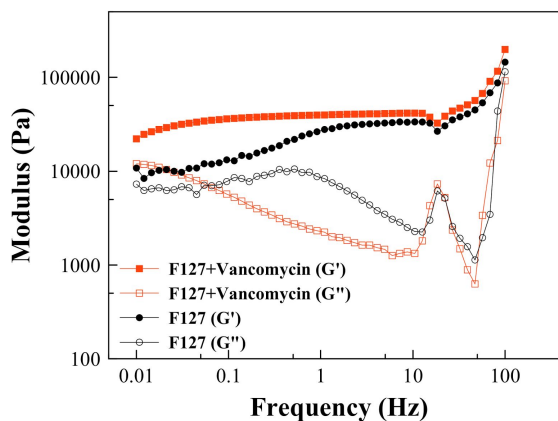
Figure 5. G' (open symbols) and G" (closed symbols) moduli versus frequency for 30 w/v % Pluronic F127 with and without Vancomycin.

The frequency of 18.42 Hz was converted into flow rate, and the theoretical fracture point of F127 in fluid flow was obtained. From the frequency of the rotation in the rheometer, angular velocity of the material was determined as follows:

$$\omega = 2 \cdot \pi \cdot f \qquad (1)$$

In which ω is the angular velocity and f is the frequency of the rotation. By knowing the angular velocity of the material, theoretical linear velocity was obtained from Equation 2 below:

$$v = r \cdot \omega \qquad (2)$$

where v is the linear velocity and r is the radius of the plate in the rheometer (Figure 6). Equations 1 and 2 were combined and rewritten as Equation 3, which gives the linear velocity in terms of the frequency of the rotation:

13

$$v = 2 \cdot r \cdot \pi \cdot f \qquad (3)$$
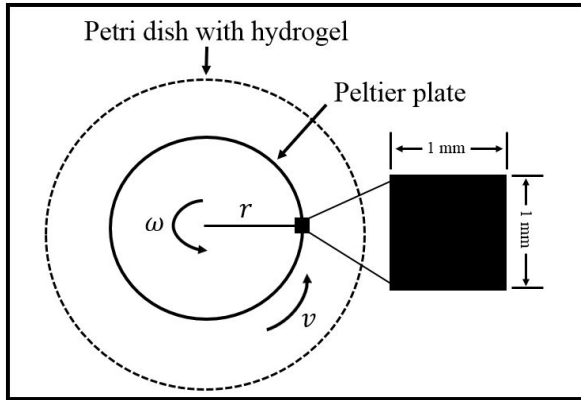
Figure 6. Schematic representation for the theoretical calculation of the flow rate at the fracture point of F127.

Using the Equation 3, the theoretical linear velocity of the material was calculated for the frequency of 18.42 Hz, which is the fracture point of F127 at 100 Pa in shear. According to the Equation 3, the linear velocity of 0.53 ± 0.11 m/s was obtained that corresponds to 18.42 Hz. This velocity is equivalent to the speed at which the outer point on the plate was rotating where the highest amount of mechanical deformation occurs. Assuming that 1×10^{-6} m^2 unit area from the outer point of the plate is moving at the velocity of 0.53 ± 0.11 m/s (Figure 6), approximate flow rate of $(0.53 \pm 0.11) \times 10^{-6}$ m^3/s (= 31.8 ± 6.6 mL/min) was obtained as a corresponding speed for frequency of 18.42 Hz. It is important to remark that the flow rate for the hydrogel fracture point is much greater than that of flow test (0.1, 0.5, 1 mL/min) or spinal fluid flow (0.28-0.42 mL/min).[8] This indicates that Pluronic F127 with and without Vancomycin maintains a stable gel.

3.5. Flow Test of Pluronic F127

UV-Vis for dilutions of 1 - 30 w/v % of Pluronic F127 were taken in order to obtain a baseline for comparison (Fig 8.a).

UV-Vis of Pluronic indicates that a higher and sharper peak corresponds to a higher concentration of Pluronic. Additionally, a broader peak range indicates a less pure sample. Thus, samples with lower concentrations of Pluronic are less pure, this might explain the shift observed in Fig 8.a at concentrations of 15% and less. However, since all concentration samples exhibited a peak around 220 nm, the known peak of Pluronic, we were able to create the calibration curve. Subsequently, UV-Vis of the flow tests at 1 mL/min, 0.5 mL/min and 0.1 mL/min were obtained (Fig 8.c). The time-dependence UV-Vis test showed that for each flow rate, all 5 mL samples exhibited similar curves. Thus we conclude that the release of Pluronic is not time dependent.

A qualitative comparison of the UV-Vis graphs showed that a very minimum amount of Pluronic was released during the flow tests. Therefore, a calibration curve using Beer Lambert Law was created using dilutions of 1 - 15 w/v % Pluronic in order to obtain a more accurate curve fit.

$$A = a(\lambda) \cdot b \cdot c \qquad (4)$$

where A is the measured absorbance, $a(\lambda)$ is a wavelength-dependent absorptivity coefficient, b is the path length and c is the analyte concentration.
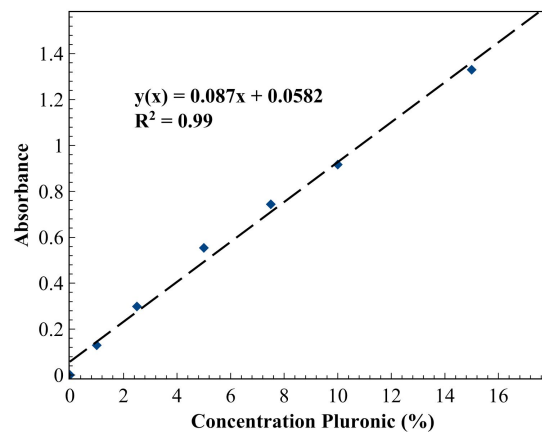

Figure 7. Calibration curve of 1, 2.5, 5, 7.5, 10, 15 w/v % dilutions of Pluronic F127 in DI.

From Figure 7 we can conclude that Beer's Law was obeyed in the specified concentration range. The regression equation for the calibration curve was $y = 0.087x + 0.0582$ having a correlation coefficient (R^2) value of 0.99, and the standard deviation of the y-intercept was found to be 0.0263. A calibration curve with a R^2 value of 0.99 is considered to be linear, therefore using the curve we could reliably find the concentration of pluronic eluted during the flow test experiment. From the UV-Vis of Pluronic F127 dilutions we found that the max absorption peak occurred at 220 nm. Therefore, the absorbance at 220 nm was plotted against each flow rate using the regression equation.
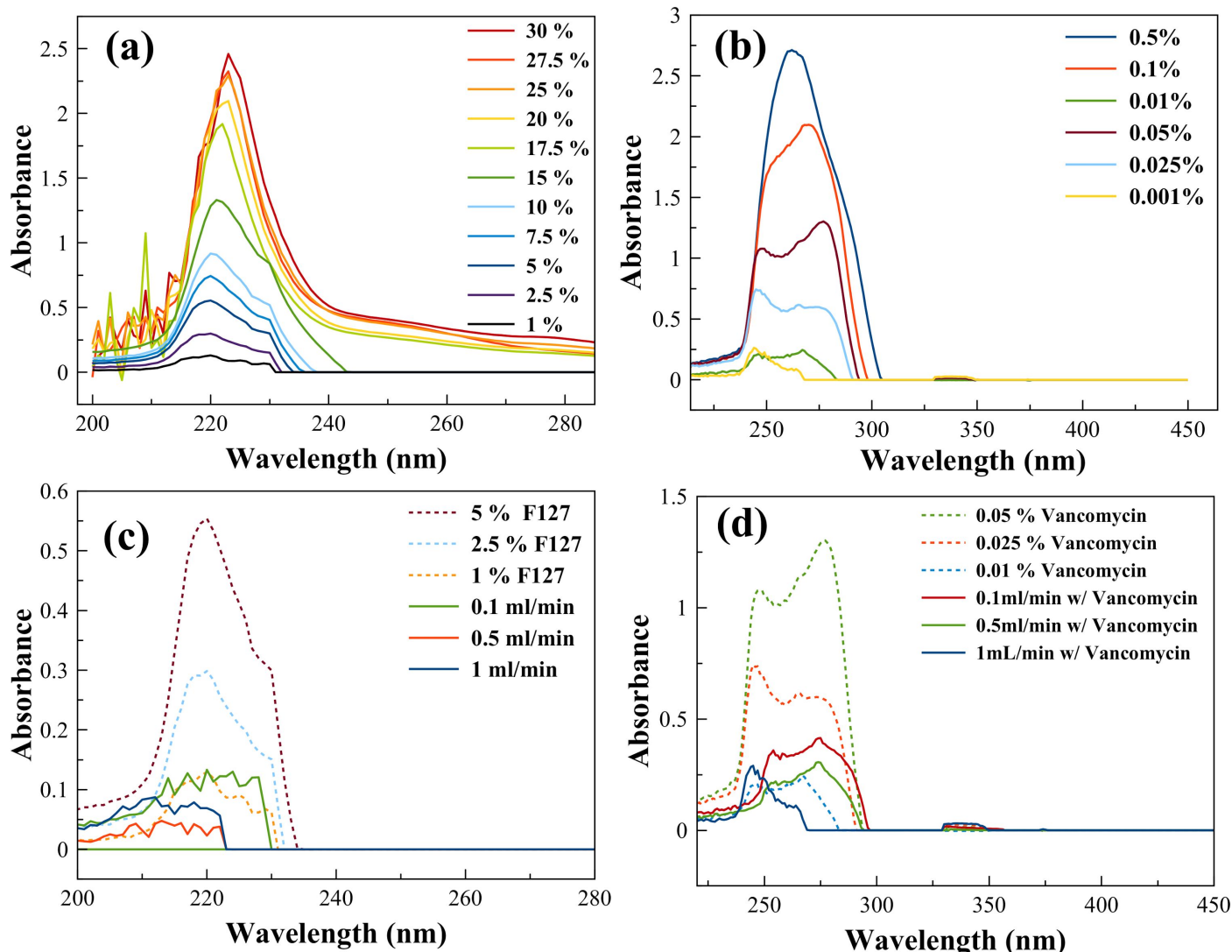
Figure 8. (a) UV-Vis spectrum of 1-30 (w/v%) Pluronic in DI water at 37°C (b) UV-Vis spectrum of 0.001 – 5 (v/v %) Vancomycin in DI water at 37°C (c) UV-vis spectrum of 30% Pluronic F127 in DI water at 37 °C with aliquots of 5%, 2.5%, and 1% Pluronic F127 in DI at 37 °C for reference (d) UV-Vis spectrum of 30% F127 + 1% Vancomycin in DI with flow of 1, 0.5, 0.1 mL/min at 37°C, and UV-Vis spectrum of aliquots of 0.01 %, 0.05%, 0.025% Vancomycin for reference.

15
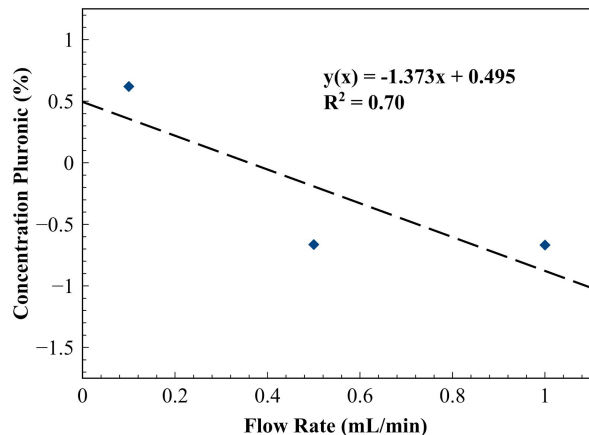
Figure 9. Concentration of 30 w/v % Pluronic F127 released as a function of flow rate at 0.1, 0.5 and 1 ml/min.

When we quantitatively analyzed the data, the calculated concentration of Pluronic released during the flow test was negative due to the fact that the concentrations of Pluronic were very low so there were many impurities that could have interfered with the UV-Vis spectra. Qualitatively, however, we observed that a minimal amount of Pluronic with concentrations far less than 1%, were released under flow. The readable data point at 0.1 mL/min showed that 0.62% Pluronic had eluted. This is because the micelle structure of Pluronic has ample time to disentangle at lower flow rates which allows the solvent of DI Water to degrade the Pluronic gel. Since there was no significant release of Pluronic, we conclude that our hydrogel is stable under flow and a good carrier for Vancomycin at the investigated flow rate.

3.6 Flow Test of Pluronic F127 and Vancomycin

UV-Vis spectroscopy of 0.001 - 5 v/v % Vancomycin were taken as a baseline (Fig 8.b). The same method used to create the calibration curve for pure pluronic was used to create the calibration curve for pure vancomycin. UV-Vis spectroscopy of the flow test (taken at 0.1 mL/min, 0.5 mL/min and 1 mL/min) for 30 w/v % Pluronic F127 with 1% Vancomycin in DI water were also taken (Fig 8.d). The time-dependence UV-Vis also showed that for each flow rate, all 5 mL sample exhibited a similar curve. Therefore, we conclude that the release of vancomycin is not time dependent. A qualitative comparison of the UV-Vis of both the aliquots and Vancomycin flow tests also revealed minimal amounts of Vancomycin elution. Therefore a calibration curve was calculated using 0.01 - 0.1 v/v % Vancomycin for a better curve fit.
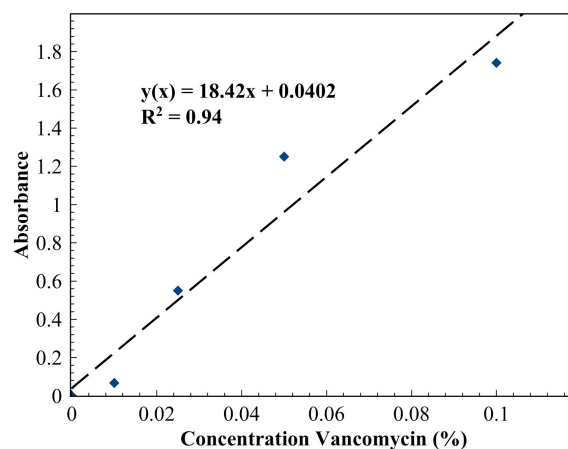

Figure 10. Calibration curve of 0.01, 0.025, 0.05, 0.1 v/v % dilutions of Vancomycin in DI.

Figure 10 obeys Beer - Lambert's Law for the specified range. The regression equation for the calibration was found to be $y = 18.4253x - 0.0402$ having a correlation coefficient (R^2) of 0.94, and the standard deviation of the y-intercept is 0.102. An R^2 value of 0.94 can be considered linear, therefore the calibration curve was used to plot concentration of Vancomycin against the flow rate. Concentration of Vancomycin released under flow was calculated by finding the absorbance at the known absorbance peak of Vancomycin, 280 nm[18], for each flow test and the concentrations were calculated using the regression equation.

For the flow rate of 1 mL/min we calculated a negative concentration, which is

16

theoretically impossible. We attributed this result to the extremely low concentration of Vancomycin that was released at this flow rate. Therefore the concentration was too minimal for UV-Vis analysis. However, since we obtained good results for the flow rates at 0.1 ml/min and 0.5 mL/min we decided to move forward with the quantitative analysis. The slope of concentration vs. flow rate for Vancomycin was calculated to be -0.0213 ± 0.0029 with a correlation coefficient of 0.98.
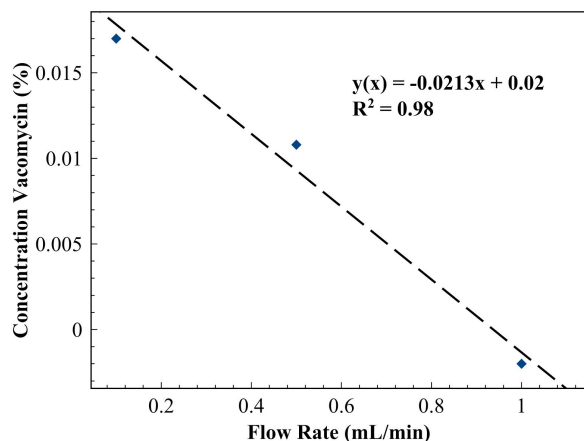
Figure 11. Concentration of 1 % Vancomycin in 30% Pluronic F127 released as a function of flow rate at 0.1, 0.5 and 1 mL/min.

Figure 11 shows small amounts, less than 0.02% concentration of Vancomycin eluting into DI water. Since Vancomycin is hydrophilic, it should have an affinity to release into DI water. However, because there is such a small release we can assume that the Pluronic micelle structure is highly entangled such that Vancomycin is trapped within the micelle branches. As a result, we can confirm that Vancomycin interacts with the hydrophilic corona of the Pluronic micelle structure. Furthermore, we can conclude that there is a decrease in the amount of Vancomycin released from Pluronic solution with increasing flow rate. This phenomenon is likely because at higher flow rates, the flow is such that there is not enough time for the micelle structure to disentangle and thus less

Vancomycin is released. In contrast, at slower flow rates the micelles have enough time to disentangle therefore more Vancomycin can be released from the solution. Qualitatively comparing the rate at which release rate is affected by flow rate for both Vancomycin and Pluronic, we observed similar behaviors. For both Pluronic and Vancomycin, there was a decrease in the amount of material released as flow rate was increased. Additionally, there was a very low release of both Pluronic and Vancomycin at 1 mL/min, indicating that both Pluronic and Vancomycin are resistant to fluid flow and remain stable at this flow rate. Thus, the Pluronic gel with Vancomycin will be retained in the spinal column as the flow rate of spinal fluid is 0.28 - 0.42 mL/min.

4. Conclusion

Pluronic F127 hydrogels possess the ability to serve as a carrier for the drug delivery of Vancomycin to the spinal column area. This is due to Pluronic F127's biocompatibility, FDA approval, and the ability to manipulate the properties so that the release and stability of the hydrogel matrix will be favorable in the spinal column area. The rheological properties of Pluronic F127 were investigated, and flow tests were conducted to study the release of Pluronic and Vancomycin under fluid flow through UV-Vis spectroscopy. From temperature sweeps, the gelation temperature dependence on the hydrogel concentration was observed. As the concentration of Pluronic increased, the temperature of gelation decreased. The temperature of gelation for 30% w/v Pluronic F127 in DI was at 23 °C, while 20% w/v Pluronic F127 in DI was at 34 °C. These values are both below the in vivo temperature of the human body which means that a range of 20% to 30% w/v Pluronic can be injected into a person as a solution at room temperature, and then gels at body temperature. Frequency sweeps were

conducted which showed a thixotropic behavior of the hydrogel as G' and G'' diverged under a constant high shear stress of 100 Pa. Under the influence of a constant shear stress of 100 Pa, an interesting phenomena was observed after 10 Hz which can be attributed to a restructuring of the crystalline structure of the hydrogel matrix. The addition of 1% Vancomycin to the 30% concentration of Pluronic in DI did not alter the rheological properties significantly. This is important because hydrogels are sensitive materials that are affected by both their components, and the environments that the hydrogels experiences. If a significant change existed by the addition of the Vancomycin to the hydrogel matrix, then a further study would need to be conducted to understand the properties of the hydrogel created, and how those properties can be exploited for the controlled release. The flow tests showed that the slower the flow, the higher the release of the hydrogel components, which can be attributed to the duration for the test to occur, and the swelling of the components out of the hydrogel matrix.

These results show the efficacy of a Pluronic F127 hydrogel as carrier to deliver Vancomycin. The Pluronic F127 hydrogel maintains its stable network under the flow conditions of the spinal column area, and elutes the antibiotic Vancomycin. Further studies need to be conducted to establish further parameters for release that mimic *in vivo* conditions such as drug delivery rate of the body, pH, and the effects of enzymatic activity.

5. Acknowledgements

6. References

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