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Gelation Properties and Drug Release Kinetics of a Thermoreversible Hydrogel as a Drug Carrier

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ABSTRACT

Pluronic F-127 (PF-127) is a thermoreversible hydrogel that is a promising candidate for drug delivery. This hydrogel has a unique property that transforms its phase between liquid and gel as the temperature changes. PF-127 can be explored as a potential drug delivery system to treat diseases due to its low toxicity and controllable, sustained drug release. This study aims to determine the optimized relationship between concentration and temperature for successful PF-127 gelation and to characterize its drug release kinetics. PF-127 powder was dissolved in a saline solution to make concentrations ranging from 10% to 30%. The temperature was increased gradually to determine the precise temperature at which each PF-127 solution at a different concentration turned to gel. Concentrations under 16% did not turn to gel at any range of temperature. From 20% to 24% concentrations, PF-127 was a liquid at room temperature, while 26% to 30% concentrations were in a gel state. To characterize the drug release kinetics of PF-127, calf thymus DNA was loaded into PF-127. It showed sustained DNA release over a prolonged period of time as opposed to an immediate burst release. Sustained release kinetics shown in our study is preferable because it may steadily release a drug at an optimal concentration, preventing toxicity from a high drug concentration while maintaining therapeutic potential. The outcomes of this study elucidate a broad application of PF-127 as a potential drug carrier.

Introduction

Pluronic F-127 (PF-127) is an FDA-approved hydrogel that has recently been explored as a promising candidate for drug delivery. PF-127 is a non-ionic triblock copolymer which consists of poly(ethylene oxide), poly(propylene oxide), and poly(ethylene oxide) (PEO-PPO-PEO) (Akash and Rehman, 2015). This thermoreversible hydrogel changes from a liquid state at temperature lower than body temperature to a gel form at body temperature. An effective drug delivery system is an essential prerequisite to treating diseases, and it can be an alternative approach to more complicated interventions such as surgical procedures. Local drug delivery system also has the advantage of preventing systemic toxicity, as it does not work through the vascular system (Nie et al., 2011). Other advantages of PF-127 as a drug delivery carrier include its inherent low toxicity and controllable sustained release kinetics (Escobar-Chávez, 2006). Its unique drug release mechanism may allow it to limit the highly toxic nature of various medical drugs by extending the administration of drug over lengthened times. Therefore, to optimize the drug delivery performance of PF-127, it is crucial to understand the gelation properties and drug release kinetics of the PF-127.

This study aims to determine the optimized conditions of PF-127 in terms of concentration and process temperature for successful PF-127 gelation and to characterize the drug release kinetics of PF-127. We hypothesize that PF-127 has a minimum concentration that is critical to successfully gelate with gelation occurring in specific ranges of PF-127 concentrations and gelation temperature. We also hypothesize that PF-127 will show a model of sustained release over a longer period of time. By testing different concentrations, we will find the optimal range of the PF-127 concentration that allows proper gelation. By changing the gelation process temperature, we will find the critical gelation temperatures for PF-127 solutions with different concentrations.

Materials and methods

Materials

Pluronic F-127 was purchased from Sigma-Aldrich. Dulbecco's Phosphate Buffered Saline (DPBS 1X) was purchased from ThermoFisher. Calf Thymus DNA Solution was purchased from ThermoFisher.

Methods

Optimal concentration for PF-127 gelation

PF-127 powders of 1g, 2g, 3g, 4g, and 5g were mixed with 10mL of PBS solution to create 10%, 20%, 30%, 40%, and 50% w/v concentrations respectively. These PF-127/PBS solutions were mixed for 16 hours at 4 °C using magnetic stirrers in the 20 ml glass vials. To thoroughly dissolve the PF-127 solutions, the temperature was set to 4°C as a universal temperature. These solutions were transferred to an incubator set at 37°C. After 10 minutes, gelation was determined using the tube inversion method (Wang et al., 2016; Peng et al., 2016).

Optimal temperature for PF-127 gelation

Once optimal concentrations of PF-127 were determined as described above, PF-127 solutions with 20% and 30% concentrations were transferred to a water bath set at 25°C, 35°C, and 45°C respectively. Among these temperatures, 25°C and 35°C were selected as they represent room temperature and body temperature, respectively. Successful PF-127 gelation was determined using the tube inversion method.

Correlation between concentration and temperature for PF-127 gelation

To determine the lowest temperature that PF-127 solutions fully gelate, the temperature of the water bath was increased from 18°C to 55°C by 1°C/5 minutes, and ranges of PF-127 concentrations were observed for gelation. The concentrations were tested in 2% increments, starting at 10% up to 30% (i.e., 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, and 30%). PF-127 gelation was determined using the tube inversion method. If no gelation was observed, the temperature was increased by 1°C, and the glass vials were placed back in the water.

Statistical analysis

One-way ANOVA and paired t-test were conducted for statistical analysis. Sample size was four per each concentration group for the test of correlation between concentration and temperature for PF-127 gelation.

Drug Release Kinetics

To simulate drug release kinetics of PF-127, non-hazardous Calf Thymus DNA was loaded into the PF-127 solutions with 20%, 25%, 30% concentrations at 4°C. These groups were transferred into Transwell 24-well inserts with 0.4μm pores at 100 rpm at 37°C, conditions set by the European Pharmacopoeia to simulate transdermal release (Jeong et al., 2018). Samples were retrieved at 2, 4, and 8 hours, and DNA concentrations were measured using an Oligreen DNA Kit and a UV Vis Spec.

Results

Effect of PF-127 concentration on gelation

After the PF-127 powder was mixed in PBS, only 40 and 50% solutions remained not fully dissolved, leaving leftover PF-127 powder in the vials, and noticeable bubbles formed on the top of the solution (Fig. 1).

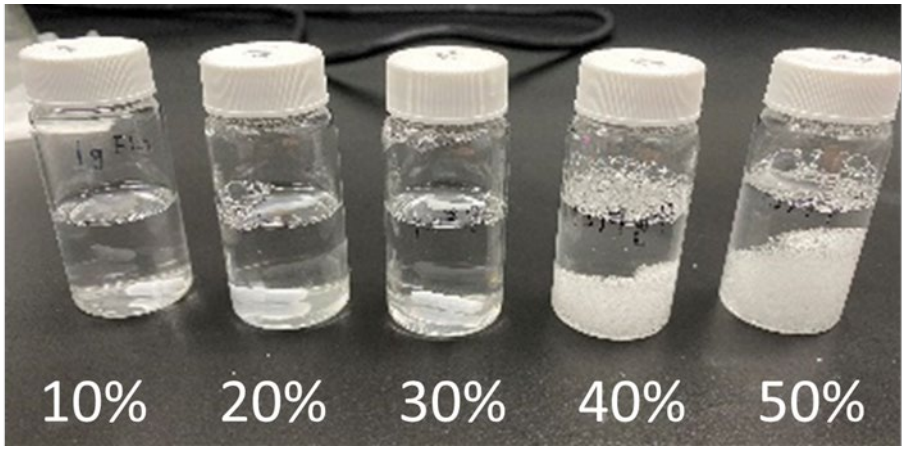

Figure 1. Dissolution of different PF-127 concentrations: 10%, 20%, 30%, 40%, 50% solutions at 4°C

Compared to the 40% solution, more powder and bubbles were observed at the 50% solution. After placing these solutions in the incubator, the 10% solution stayed in a liquid phase, while both 20% and 30% solutions were completely gelated without any bubbles or PF-127 precipitation (Fig. 2).

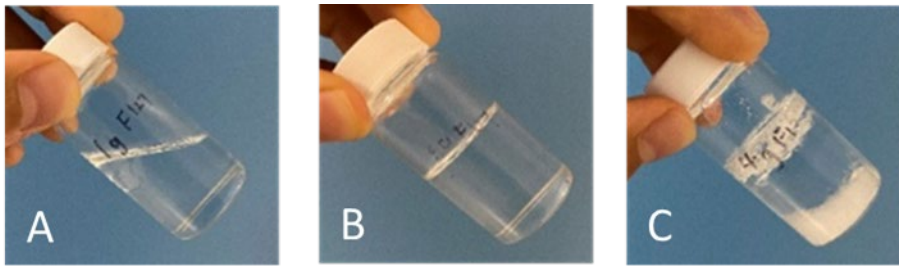

Figure 2. Gelation behavior of PF-127 solutions: 10% (A), 30% (B), and 50% (C) at 37°C

Finally, at the 40% and 50% concentrations, there were bubbles formed on the top surface of the solution and powders remained at the bottom of the vials. The 50% solution had more powder and bubbles than the 40% solution at 37°C. (Table 1)

Table 1. Gelation behaviors of PF-127 with various concentrations at 4°C and 37°C

Concentration	4°C	37°C
10%	Clear solution	No gelation
20-30%	Clear solution	**Gelation**
40-50%	Unable to fully dissolve	**Gelation**, powder did not dissolve

Effect of process temperature on gelation

After the optimal concentrations for the solutions were determined in the previous set of experiments, 20% and 30% concentrations were selected to determine how the temperature affects gelation at each concentration. Those two solutions (20% and 40%) were placed in a water bath for 10 minutes at 25°C, 37°C, and 45°C, respectively. After 10 minutes, gelation was determined using the tube inversion method (Fig. 3, Table 2). At 45°C, both 20% and 30% solutions turned to gel with lots of bubbles near the top of the solutions (Fig. 3A). At 35°C, both concentrations turned to gel with very little to no bubbles (Fig. 3B). At 25°C, the 30% solution turned to gel with no bubbles (Fig. 3C), while the 20% solution did not turn to gel and stayed liquid with no bubbles (Fig. 3D).

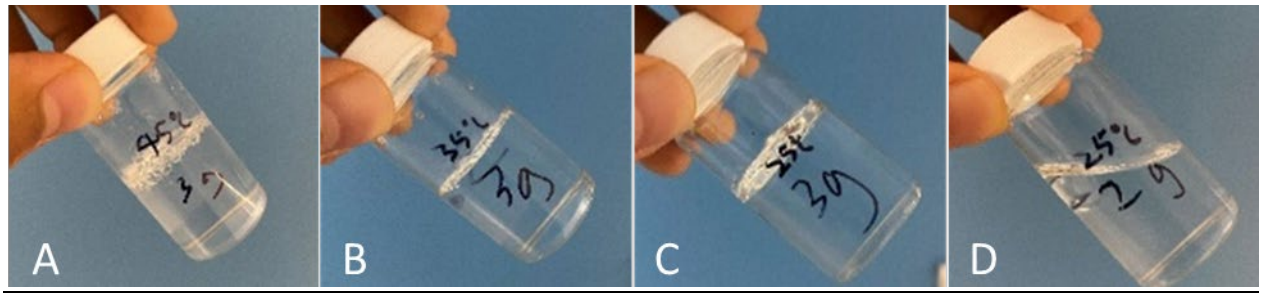

Figure 3. Gelation behavior of 20% and 30% PF-127 concentrations at different temperatures. Figure 3A and 3B show both concentrations at 45°C and 35°C, respectively. Figure 3C and 3D is 30% and 20% concentrations at 25°C, respectively.

Table 2. Gelation behaviors of 20% and 30% PF-127 solutions at 25°C, 37°C, and 45°C

Concentration	25°C	37°C	45°C
20%	Liquid clear solution	Gelation	Gelation with bubbles
30%	Gelation	Gelation	Gelation with bubbles

Correlation between concentration and temperature for PF-127 gelation

With the increased temperature from 18°C to 55°C, gelation did not occur for the groups of concentrations at 10, 12, 14, and 16% of PF-127 solutions, whereas gelation occurred for the groups of concentration that was 18% or greater (20, 22, 24, 26, 28, and 30). Concentrations from 18% to 30% PF-127 solutions represented inversely proportional correlations between the PF-127 concentration and the gelation temperature. Black dots indicate the lowest temperatures when the PF-127 solutions start gelation, as the temperature was increased from 18°C (Fig. 4). Statistical analysis presented that the gelation temperatures for each of the concentration groups (18, 20, 22, 24, 26, 28, and 30°C) were significantly different from each other ($P < 0.05$).

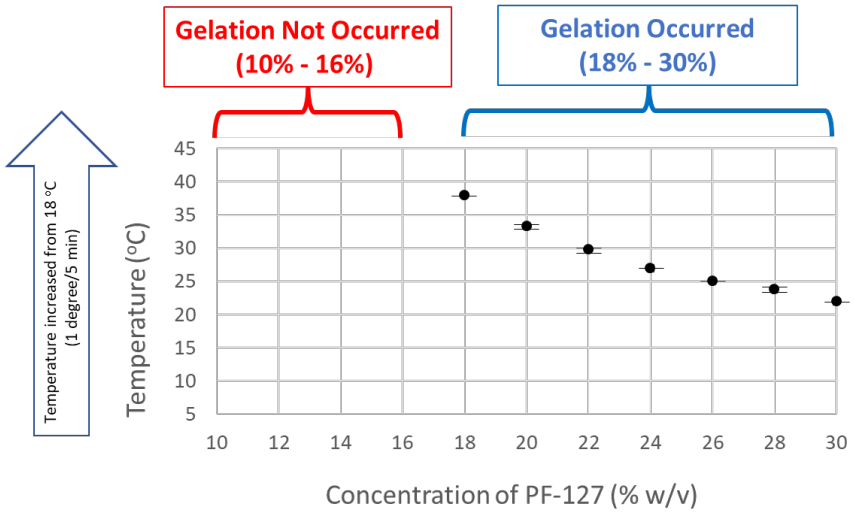

Figure 4. Correlation between concentration and temperature for PF-127 gelation (n = 4 for each concentration group)

DNA release kinetics

Samples of three experimental groups and one control group were taken at 2, 4, and 8 hours to measure the quantity of DNA released. PF-127 maintained the gel state and released DNA in a sustained manner. All experimental groups

showed an increased total percentage of drug released up to 8 hours. The change in concentration showed no significant trend in the amount of released DNA.

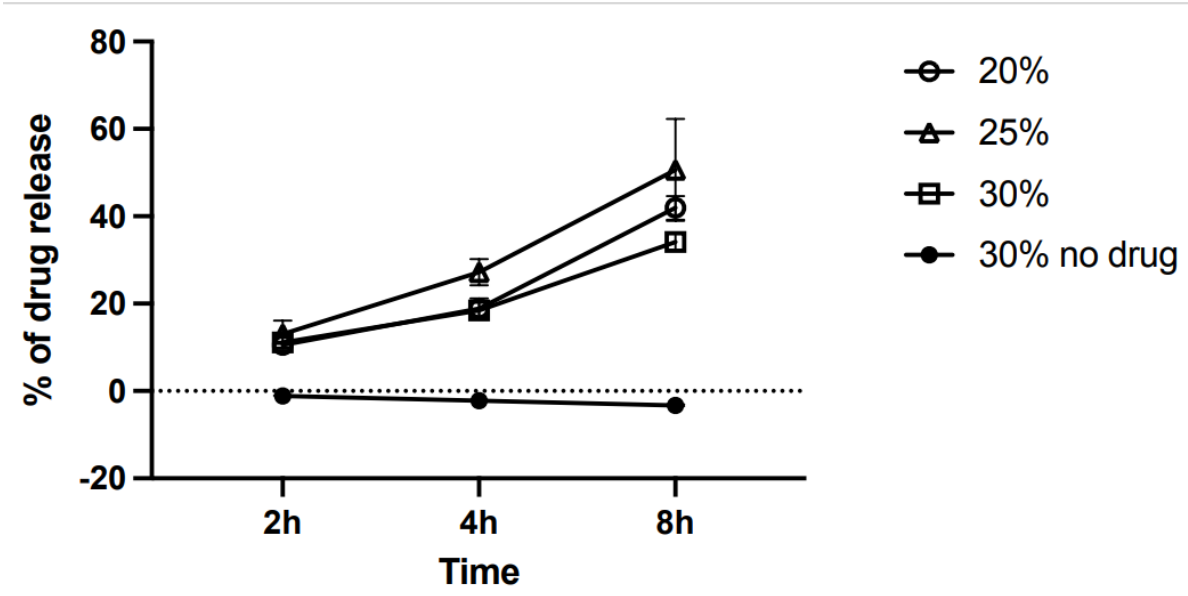

Figure 5. Total percentage of drug (DNA) release for 20%, 25%, and 30% over 2, 4, and 8 hours

Discussion

In this study, we investigated the optimal conditions of the PF-127 concentration and the gelation temperature. Among the concentrations of the PF-127 solutions tested in this study, the optimal concentrations for gelation were 20% and 30%. The optimal temperatures for gelation were 35°C for 20% and 30% PF-127 solutions, and 25°C for 30% solution, respectively. All concentrations equal to or greater than 20% successfully turned to gel at body temperature. We also investigated the temperature needed for gelation over a different range of PF-127 concentrations. As the concentration increases, the lowest temperature that is required for gelation decreases.

The results provide three different trends that occur as the temperature of concentrations increases from room to body temperature. At an 18% concentration, PF-127 will stay a liquid even as it reaches body temperature. From ranges 20% to 24% concentrations, PF-127 will transition from a solution to a gel. In contrast to the 18% concentration, 26% to 30% concentrations of PF-127 will be in a gel state at room and body temperatures. Using this concentration range, future applications of PF-127 will be able to control the phases of PF-127 during administration.

Dumortier *et al.* have conducted a similar study, where they dissolved PF-127 in water instead of PBS. The method for determining the gelation temperature used a rate of 1°C/min, whereas our study used 5 minutes increments to ensure the test for gelation was precise. The authors reported that the temperature required for successful gelation decreased as the concentration increased, which is congruent to the findings of our study. Furthermore, other studies have concluded that concentrations around 14% and under do not have the capability of gelation. The sol-gel transition temperature in our study was around 16 – 18°C, which was slightly higher than that of the previous study. It is notable that their study tested a narrower range of concentrations (10.5% - 18.9%), whereas we extended the concentration range from 10% to 30%. The breath of this study has confirmed and extended the trends of previous studies to a larger range and may widen the possibilities for various drug delivery applications.

The drug release kinetics experiment demonstrates that PF-127 is particularly advantageous in drug delivery due to its ability to prolong the release of potentially toxic medication. Not only does the steady release rate prevent

unnecessarily high amounts administration of toxic drugs in a short period of time, but it also extends the efficacy of the drug by continuing its release in a controlled manner.

The outcomes of this study may also open broader applications for PF-127. For example, a 20% PF-127 solution has potential for injections since it would be a solution in a needle at room temperature. This allows for ease of formulation and administration of the drug (i.e., liquid phase at room temperature vs. gel phase at body temperature). If needed, PF-127 would gelate when entering the body's 37°C temperature. On the other hand, a 30% PF-127 solution would be better suited for topical application to the skin since PF-127 would be in a gel state at room temperature which would aid in controlling the location and spread of the gel (i.e., gel phase at room temperature vs. gel phase at body temperature).

Future work will be done to assess the efficacy of PF-127 as a drug delivery system. This study can be expanded to a cell toxicity test using a cytotoxicity assay to determine whether the hydrogel is harmful to human cells. Then, an in vivo study can be performed on a mouse model to test for toxicity. An in vitro drug release experiment can be performed to test drug efficacy once loaded in PF-127.

Conclusions

PF-127 is a promising candidate material for drug delivery system due to its unique characteristics of thermoreversible gelation along with the controllable and sustained release, drug loading capabilities, and low toxicity. As we hypothesized, PF-127 has a minimum concentration that is critical to gelate successfully. Gelation occurred in specific ranges of PF-127 concentrations and gelation process temperature. The concentration and temperature presented an inverse correlation. Our results elucidate a broad range of possible applications of PF-127. Combinations of different PF-127 concentrations under different processing temperatures will enable to administer drugs in various ways; for example, either injections or topical administrations. From our study outcomes, concentrations from 20% to 24% can be used in treatments that require administration through injections. This range's feature of turning from a liquid solution to a gel will help to keep it liquid in the needle and gelate once inside the body. On the other hand, PF-127 concentrations in the range of 26% to 30% can be applicable to treatments involving topical applications, where the concentration will stay in a gel state during application. This would be advantageous compared to a topical application using a liquid, as gels are easier to apply topically due to their controllable spread. With both injection and topical application options, PF-127 reaps the benefit of maintaining efficacy and preventing toxicity through sustained release when in a gel form. Further studies to prove its low toxicity and efficacy in drug transport will broaden our application of PF-127 as a drug carrier.

Limitations

A limitation of this study was measuring the precise temperature of liquid-to-gel transition since our study only used one method to determine the exact temperature. Although multiple trials were done using the same method to confirm our results, having two different methods to find the temperature of gelation would help to confirm the value. Future research on this topic can solve this limitation by finding the temperature at which the gel turns back into a liquid. In other words, starting at a higher temperature and lowering it by 1°C, the temperature where the solution turns to a gel can be observed and compared to the temperature where the gel turns into a solution. The point of gelation would be confirmed when the two temperatures are equal.

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