Gelation Properties of Thermosensitive Hydrogels

Josefin Holmgren
josefin.holmgren@telia.com

under the direction of
Ms. Jenny Fagerland
Department of Fibre and Polymer Technology
Royal Institute of Technology

Research Academy for Young Scientists
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Abstract

Thermosensitive hydrogels have a promising application as scaffolds in tissue engineering, which makes knowledge of their temperature dependence and concentration dependence crucial. The aim of this study is therefore to examine at what temperatures and concentrations Pluronic F-127, Pluronic F-68, PCTC-PEG-PCTC and 90-10% and 50-50% mixtures of Pluronic F-127 and Pluronic F-68, respectively, form gels. Five different samples for each hydrogel or mixture were created with the concentrations 10%, 15%, 20%, 25% and 30%. Every sample was exposed to five different temperatures and whether they had formed gels or not was determined by the test tube inversion method. Pluronic F-127 and the mixtures 90-10% and 50-50% formed gels. The result showed that an increase of Pluronic F-127 decreased the gelation temperature while an increase of Pluronic F-68 led to the opposite. However, no gels were formed with Pluronic F-68 and PCTC-PEG-PCTC.

Contents

1 Introduction

Due to lack of donors and complications in transplantations, the supply of organs and tissue is not living up to the demand, which is leading to the death of approximately 18 people in the U.S each day [1]. A way to counter this is to produce organs and tissue artificially. This field called *tissue engineering* has undergone considerable development in recent years.

To construct new tissue or organs, a scaffold that can fulfill certain requirements is needed. Necessary properties for such a scaffold is biodegradability, biocompatibility and non-toxicity. Furthermore it has to support cell reproduction, so the cells can proliferate and differentiate. The scaffold also needs to have a high porosity, so cells can grow evenly and nutrients and waste products can flow sufficiently throughout the scaffold [2].

Hydrogels are attractive scaffold materials, as they are structurally similar to the extracellular matrix of many tissues [3]. Moreover, many of them have proved to be degradable and non-toxic [4]. A hydrogel consists of a network of polymer chains that are either covalently bonded, ionically bonded or linked through intermolecular, generally hydrogen, bonds. As hydrogels have a hydrophilic character, they swell and form gels under certain circumstances when they come in contact with water [5]. There are different mechanisms that can cause the hydrogel to swell in water, for instance pH change, UV-irradiation or temperature modulation [6]. For biomedical applications, like tissue engineering, thermosensitive hydrogels are especially appealing as the temperatures at which they form a gel are adjustable [7]. They may be injected as fluids, which will subsequently stiffen inside the body due to the change in temperature [6].

When a thermosensitive hydrogel reacts with water in the right temperature the hydrophobic parts of the polymer will attract each other and create micelles. In Figure 1 a micelle is shown, in which the center consists of hydrophobic segments and the outside of hydrophilic segments. The micelles will then aggregate and bind water, hence form a gel. At what temperature a thermosensitive hydrogel forms a gel partially depends on

the structure of the molecule and its mass. The gelation temperature may also be altered through the monomer composition within the polymer (private comm. Jenny Fagerland, KTH, 01-07-13).

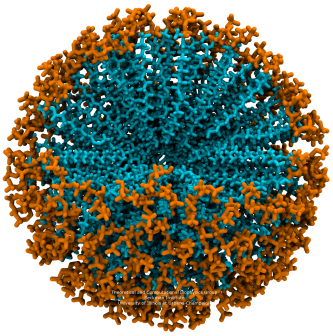

Figure 1: A picture of a micelle, in which the center consists of hydrophobic parts and the outside of hydrophilic parts.

A typical thermosensitive hydrogel is a poloxamer (PEO-PPO-PEO). It consists of two different monomers, PEO(poly(ethylene oxide)) and PPO(poly(phenylene oxide)). In Figure 2 the molecular structure of a poloxamer is shown. PEO is a hydrophilic monomer, whereas PPO is a hydrophobic monomer [8, 9]. PEO and PPO are also non-toxic and degradable [8] (private comm. Jenny Fagerland, KTH, 01-07-2013). Two very common poloxamers are Pluronic F-127 and Pluronic F-68. While Pluronic F-127 contains 70% of PEO, Pluronic F-68 contains 80% of PEO. Pluronic F-127 weighs twice as much as Pluronic F-68 [6]. Studies have shown that Pluronic F-127 is able to form gels at lower temperatures compared to Pluronic F-68 that needs higher temperatures to form gels [10].

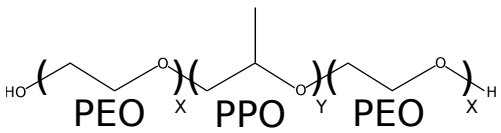

Figure 2: The molecular structure of a poloxamer. It consists of two different monomers, PEO and PPO, where PPO is always in the center.

Another relatively new thermosensitive hydrogel is the PCTC-PEG-PCTC. This hydrogel consists of three different monomers; CL (ϵ-caprolactone), TMC (Trimethylene carbonate) and PEG (poly(ethylene glycol). In Figure 3 the molecular structure of this hydrogel is shown. CL and TMC are hydrophobic monomers, while PEG is a hydrophilic monomer. All three monomers are non-toxic, and have a slow biodegradability [8, 11, 12]. TMC is often used to increase the elasticity in rigid polymers [11]. PEG has the same structure as PEO, but has a lower molecular mass and has been produced in a different way [8] (private comm. Jenny Fagerland, KTH, 01-07-2013). In studies PCTC-PEG-PCTC has shown to create gels at 25°C and above [13]. Scientists hope that this hydrogel will be used for biomedical applications in the future.

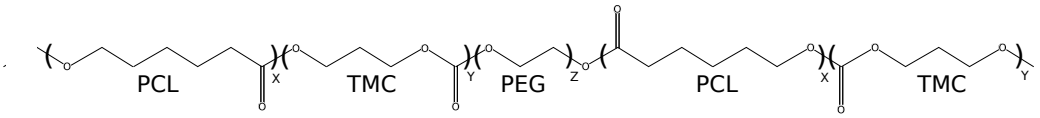

Figure 3: The molecular structure of PCTC-PEG-PCTC. The molecule consists of three different monomers, PCL, TMC and PEG, where PEG is always in the center.

If tissue engineering with thermosensitive hydrogels as scaffolds will remain a promising way to grow organs and tissue artificially, detailed knowledge of the temperatures and concentrations at which gels are formed from their liquid states is important. The aim of this study is to examine at what temperatures and concentrations Pluronic F-127, Pluronic F-68 and PCTC-PEG-PCTC form gels. In addition, two mixtures of these hydrogels will also be tested. Specifically, one 90-10% mixture and one 50-50% mixture of Pluronic F-127 and Pluronic F-68, respectively.

2 Method

To examine when the different hydrogels form gels, five different samples for each substance were prepared with concentrations 10%, 15%, 20%, 25% and 30%, respectively. Each sample was exposed to five different temperatures and whether they had formed gels or not was determined by turning the vials upside down and observe whether the

hydrogel escaped or not, called the test tube inversion method.

Firstly, a proper mass of hydrogel corresponding to the desired concentration was determined and mixed with 3 ml of distilled water in a 5 ml vial. To dissolve the mixtures, the vials containing Pluronic F-127 and/or Pluronic F-68 were put in a beaker comprising ice on a magnet stirrer, as they dissolve easier in lower temperatures. The vials containing PCTC-PEG-PCTC were put on a magnet stirrer in room temperature, 25°C, as they freeze in lower temperatures. The mixtures were then moved into smaller vials (2 ml).

Subsequently, the smaller vials were exposed to five different temperatures, namely 0°C, 25°C, 35°C, 45°C and 65°C. To analyze if gels were formed at 0°C, the vials were put in a beaker full of ice. To observe the vials at 35°C, 45°C and 65°C an oil bath was used. To analyze the vials at 25°C, the vials were left on the laboratory bench. Each sample was exposed to the specific chosen temperature long enough for the temperature of the hydrogel solution to adjust, approximately 5 minutes.

3 Result

Three diagrams were made in Matlab for the observed results. Figure 4 shows the result for Pluronic F-127, Figure 5 shows the result for the mixture 90-10% and Figure 6 shows the result of the mixture 50-50% of Pluronic F-127 and Pluronic F-68, respectively. The hydrogels Pluronic F-68 and PCTC-PEG-PCTC did not form gels at any temperature and are thus not included. Tables 1-5 show the preparations in order to get the right concentrations.

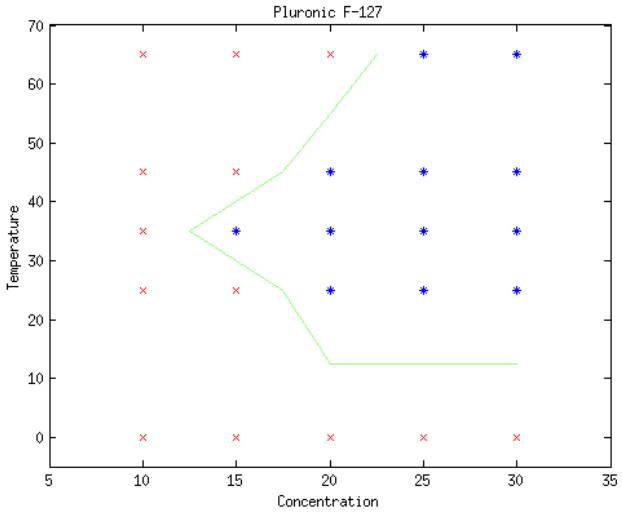

Figure 4: The diagram shows when Pluronic F-127 forms gels. The blue stars indicate gel formation and the red crosses solution. The green line is an approximation of where the sol-gel transition might be according to the obtained data.

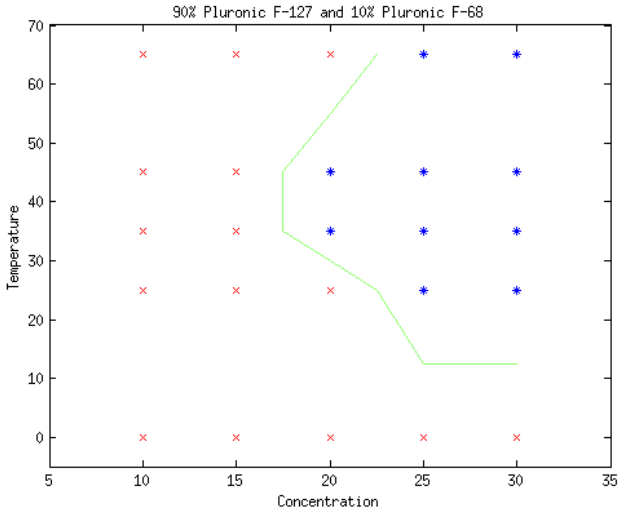

Figure 5: The diagram shows when the mixture of 90-10% Pluronic F-127 and Pluronic F-68, respectively, forms gels. The blue stars indicate gel formation and the red crosses solution. The green line is an approximation of where the sol-gel transition might be according to the obtained data.

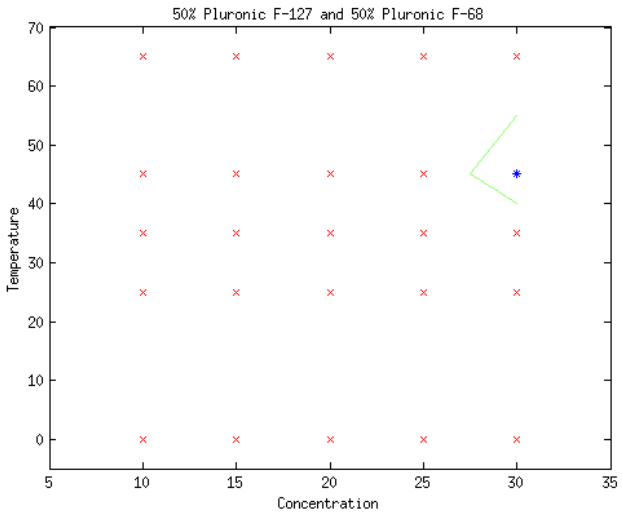

Figure 6: The diagram shows when the mixture of 50-50% Pluronic F-127 and Pluronic F-68, respectively, forms gels. The blue stars indicate gel formation and the red crosses solution. The green line is an approximation of where the sol-gel transition might be according to the obtained data.

Table 1: The preparations for Pluronic F-127.

Conc.	10%	15%	20%	25%	30%
m(Pluronic F-127)	0.33 g	0.53 g	0.75 g	1.00 g	1.30g
m(H2O)	3.0 g	3.0 g	3.0 g	3.0 g	3.0 g

Table 2: The preparations for Pluronic F-68.

Conc.	10%	15%	20%	25%	30%
m(Pluronic F-68)	0.33 g	0.53 g	0.75 g	1.00 g	1.28 g
m(H2O)	3.0 g	3.0 g	3.0 g	3.0 g	3.0 g

Table 3: The preparations for PCTC-PEG-PCTC.

Conc.	10%	15%	20%	25%	30%
m(Pluronic F-127)	0.33 g	0.53 g	0.75 g	1.00 g	1.28 g
m(H2O)	3.0 g	3.0 g	3.0 g	3.0 g	3.0 g

Table 4: The preparations for 90-10% Pluronic F-127 and Pluronic F-68, respectively.

Conc.	10%	15%	20%	25%	30%
m(Pluronic F-127)	0.33 g	0.53 g	0.75 g	1.00 g	1.28 g
m(H2O)	3.0 g	3.0 g	3.0 g	3.0 g	3.0 g

Table 5: The preparations for 50-50% Pluronic F-127 and Pluronic F-68, respectively.

Conc.	10%	15%	20%	25%	30%
m(Pluronic F-127)	0.33 g	0.53 g	0.75 g	1.00 g	1.28 g
m(H2O)	3.0 g	3.0 g	3.0 g	3.0 g	3.0 g

4 Discussion

As seen in the result, both too low and too high temperatures hindered the formation of gels. While the gelation was haltered at low concentrations, increasing the concentration did not seem to hinder gelation within the range of this experiment. When temperatures become too elevated, in general when they approached 40-50°C, the micelles likely aggregated so much that water molecules were pressed out, forming a two phase system instead of a gel (personal comm. Jenny Fagerland, KTH, 04-07-2013). Despite higher temperatures Pluronic F-127 and the 90-10% mixture formed gels and further experiments have to be made at higher temperatures to examine where the upper critical solution temperature is located. However, gelation at higher temperatures are irrelevant for biomedical

applications in the human body.

From the graphs, it was believed that low temperatures also hinder gelation, for example through the decrease of micelle motion which slows down aggregation. On the contrary, another hydrogel, PCTC-PEG-PCTC was observed in its gel-state at 4°C. However, Park et al. showed in their study that PCTC-PEG-PCTC does not form gels at such low temperature, which may indicate the observations of PCTC-PEG-PCTC wrong [13].

An increasing concentration did not seem to hinder the gelation within the range of this experiment. As the solutions contain more micelles, they likely have to bind less water molecules and can therefore aggregate easier (private comm. Jenny Fagerland, KTH, 04-07-2013). However, even higher concentrations should be analyzed if a conclusion should be drawn. At low concentrations the gelation was haltered. The most likely reason is that there were not enough micelles in the solution to bind all the water molecules and aggregate and later form gels (private comm. Jenny Fagerland, KTH, 04-07-2013).

Compared to Pluronic F-127, Pluronic F-68 contains 80% of the monomer PEO while Pluronic F-127 contains 70%. In other words, Pluronic F-68 contains more hydrophilic segments and less hydrophobic segments compared to Pluronic F-127. According to Tarasevich et al., this difference in structure means Pluronic F-68 would need higher temperatures to form gels, which is a plausible reason why Pluronic F-68 did not form any gels whatsoever in these experiments [14]. As Pluronic F-68 contains less hydrophobic segments than Pluronic F-127, more is demanded from the hydrophobic parts to find each other. Therefore Pluronic F-68 most likely needs higher temperatures and concentrations so the hydrophobic segments can find each other and create micelles, subsequently form gels (private comm. Jenny Fagerland, KTH, 04-07-2013).

It is hard to tell if micelles had been formed in the solution, as the solution of Pluronic F-68 was transparent. Nevertheless, the solution of PCTC-PEG-PCTC was white, which indicated that this solution contained micelles. However, differences in monomer structure affect the properties of the respective micelles and because Pluronic F-68 and PCTC-PEG-PCTC are structurally different the opacity of PCTC-PEG-PCTC is not a conclu-

sive evidence that micelles had not formed in the transparent Pluronic F-68 solution. The same applies for Pluronic F-127; as its solution was transparent it can not be concluded if the reason for failed gelation at certain temperatures and concentrations was because micelles did not form aggregates, or because micelles did not form at all (private comm. Jenny Fagerland, KTH, 04-07-2013).

As seen in the results no gels were formed with the hydrogel PCTC-PEG-PCTC. As the solutions *did* contain micelles the micelles did not aggregate. Higher temperatures and concentrations would in this case have helped the micelles to aggregate and form gels. The heat makes the micelles move more and come closer to each other, while higher concentrations make the micelles bind less water molecules, both allowing the micelles to aggregate easier.

When the gelation of PCTC-PEG-PCTC was analyzed in another study, they reached a different result: here the PCTC-PEG-PCTC formed gels at temperatures above 25°C with the concentrations of 15% and above [13]. The difference might be due to that the hydrogel used in this study had been cleaned, which could have influenced the sol-gel transition temperature of the hydrogel.

In the mixture 90-10% of Pluronic F-127 and Pluronic F-68, respectively, gels were formed. If the gelation of Pluronic F-127 (Figure 4) and the gelation of the mixture 90-10% (Figure 5) are compared, small differences can be seen. Higher concentrations and temperatures were needed in the 90-10% mixtures to form gels. This might be due to that the F-68 polymers, which were not able to form any gels, block the Pluronic F-127 micelles trying to aggregate. Due to this, higher temperatures and concentrations are required, in order for the Pluronic F-127 micelles to come closer to each other and aggregate (personal comm. Jenny Fagerland, KTH, 05-07-2013). In the 50-50% mixture of Pluronic F-127 and Pluronic F-68, respectively, gel was formed in one temperature and concentration combination only. The explanation here would be similar: in this mixture even more Pluronic F-68 polymers are available to block the Pluronic F-127 micelles.

A study has shown that a higher molecular mass polymer had a lower gelation tem-

perature compared with a lower molecular mass polymer [14]. This agrees with the result of this study as Pluronic F-127 has a higher molecular weight compared to Pluronic F-68.

The gelation properties of poloxamers have been investigated for a long time. For Pluronic F-127, other studies mostly agree with the result of this study [8, 14, 18]. However studies disagree among lower concentrations [14, 18].

Table 6: The different results among the lower concentrations for Pluronic F-127 from different studies.

Conc.	10%	15%
This study	no gel at any temp.	35°C
Ref. [10]	44°C	35°C
Ref. [15]	no gel at any temp.	no gel at any temp.

In table 6, Ref. [10] and Ref. [15] disagree among the gelation at lower temperatures. Since this study suggests gelation at 15% and not at 10%, it agrees with neither of these studies and further research should be made. More concentrations in the lower range should be examined and the hydrogel should be ensured to have a high purity.

The result of Pluronic F-68 does not agree with previous research, as they show that it can form gels at higher temperatures, around 50 to 70°C [10]. The most probable explanations to the difference might be that this experiment only analyzed five different temperatures, the hydrogel used was two years old and the purity of the hydrogel may have varied between the studies.

The gelation of Pluronic F-127 - Pluronic F-68 mixtures have been investigated before. Li et al. agree with the results of this study, that an increase of Pluronic F-127 in the mixture decreased the gelation temperature and that an increase of Pluronic F-68 led to the opposite [16].

In the future, more research should be done in order to find a way to see if a solution contains micelles. Further studies should also be made concerning the gelation properties of Pluronic F-127, as there is an ambiguousness among the results of the different existing studies. More temperatures and concentrations should be examined for all hydrogels, so that more credible results can be obtained.

To conclude, gels were formed using Pluronic F-127 and the mixtures 90-10% and 50-50% of Pluronic F-127 and Pluronic F-68, respectively. Pluronic F-127 formed gels more easily compared to the others, as lower temperatures and concentrations were needed. The mixtures showed that an increase of Pluronic F-127 decreased the gelation temperature while an increase of Pluronic F-68 led to the opposite. However, no gels were formed using Pluronic F-68 and PCTC-PEG-PCTC. Further studies should specifically examine the gelation of Pluronic F-127 at lower temperatures and generally more temperatures and concentrations for all hydrogels.

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