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Effects of Hofmeister Ions on Gelation of Gelatin and Pluronic Hydrogels

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Effects of Hofmeister Ions on Gelation of Gelatin and Pluronic Hydrogels

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Honors Research Project

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**Executive Summary**

**Background**

The Hofmeister series of ions is a qualitative order of inorganic ions based on their relative effects on protein stability in solution. First documented in 1888, the series reflects the fact that while all other conditions are held constant, the presence of specific ions show different effects on the behavior of proteins and other polymers in solution. These effects impact solubility, denaturing, conductivity, among others. One aspect that has remained unexplored is the effect of specific ions on gelation of physical hydrogels. While salts have an effect on critical solutions temperatures and solubility of hydrogels, the effect on gelation time and gelation rate are undocumented. For this reason, a set of experiments was performed to measure the change in gelation characteristics of thermoreversible hydrogels in the presence of various single charge ions in the Hofmeister series.

Gels are semi-solid materials that contain a network or chemically or physically joined molecules. Physical gels are gels that form through sufficient interactions with the solvent. Gels that are soluble in water are known as hydrogels. Hydrogels have many applications, the most important of which is for drug delivery. Since many hydrogels are non-toxic, they can be used to transport pharmaceutical drugs to specific locations around the body. They can be used to treat wounds, provide healing after surgeries, and slow the release of the drugs for delayed response delivery. Therefore, hydrogels have become of interest in recent years for their various biomedical applications. Understanding the effect of salts on gelation rate can be crucial for precise medical uses.

Gelatin and poloxamer 407 (PF-127) were allowed to gel from their liquid states to a fully-gelled state. The gelation was observed with an NDJ-5S viscometer. The polymer solutions
were mixed to a concentration of 5% by mass for gelatin and 25% by mass for PF-127. Firstly, experiments were performed that used solvents with several ionic concentrations between 0.00M and 0.30M to observe the effect of salt concentration on gelation. Once the effect of salt concentration was known, solutions were prepared to 0.10M with ions from the Hofmeister series of cations and anions. The cations used were Li+, Na+, K+, and Cs+. The anions used were F-, Cl-, Br-, and I-. The results from the viscosity data during gelation of gels in these solutions were analyzed to observe the specific ion effects.

Summary of Results and Conclusions

For gelatin, the increase in salt concentration in solution slowed the rate of gelation compared to de-ionized solutions (Fig. 12). The amount that gelation was slowed was proportional to the concentration. However, there was little additional effect at high concentrations. There appeared to be a limit to which the gelation could be delayed by concentration. Likewise, weakly concentrated solutions did not deviate much compared to de-ionized solvent. For the Hofmeister anions, the gelation data showed a slowing effect proportional to the increased destabilizing effect of the ion based on the Hofmeister series (Fig. 10). The higher destabilizing, or ‘salting-in’, effect, the longer it took for the protein to gel. As for the cations, the results were less conclusive. The ionic solutions slowed gelation over de-ionized solutions but failed to show results proportional to the order in the Hofmeister series. The data did not show significant differences between ions, and more irregularities in data were seen compared to tests with various anions (Fig. 11).

For PF-127, the gelation time did increase as the salt concentration increased (Fig. 15). However, high salt concentration solvents gelled faster than those at low concentrations. This result was unexpected, as gelatin data continued to increase with increased concentration. The unusual result was confirmed when experiments were run with Hofmeister anions. The presence
of the anions in the water solvent slowed the gelation of PF-127. But as the relative destabilizing effect of the ion increased, the gelation rate began to increase, rather than decrease (Fig. 13). What can be concluded from this is that salt solutions have a delaying effect on PF-127 gelation, but as the hydrophobic effects of the ions increase, the gelation rate increased. One explanation could be that the increased hydrophobic interactions help promote micelle formation, which causes the gel to form faster. While this trend was seen with concentration and Hofmeister anions, it was not seen in the cation experiments. The results from the cation experiments are almost indistinguishable from each other, and therefore no conclusions could be drawn about their specific-ion effect on PF-127 (Fig. 14).

**Important Learnings and Future Work**

The results from this study show that the presence of different ions in solution have a relative effect on gelation related to the Hofmeister series. An understanding of effects on gelation is important for applications of hydrogels where gelation time is an important factor in its intended use. The learnings from the report will not only bring awareness of adverse effects of ionic solutes in hydrogel solutions, but also help to provide a basis for engineering the gelation properties using Hofmeister ions as the variable. This can be important for drug delivery or surgical applications, where gelation timing may be critical for material performance. To help expand the knowledge of the relation of the Hofmeister series on gelation, there are some elements that were not able to be investigated in this study. Such examples include seeing quantitative effects of polymer concentration, cooling/heating rate, and other rheological characteristics of hydrogel gelation (such as DMA testing for moduli).
1. Introduction

Polymer gels are an important physical material that exhibit unique properties. Not all polymers in solution form gels, but those that do form a polymer network that have the ability to hold solvent in their structure. Gels that form in water are known as hydrogels. Along with solvent, the free volume of the polymer network can hold other solutes. This makes gels useful for both biomedical and culinary purposes. Many gels can be described as thermoreversible, meaning that their properties and physical state change at different temperatures, and can be continuously changed without negative effects to the material. These polymer solutions transform from a viscous liquid to an elastic solid through the process of gelation. Gelation can include the formation of either physical or chemical polymer networks, and can be induced by changing temperature, composition, or solvent interactions. An example of a change in solvent interaction is with the introduction of aqueous salts to a water solvent. It has been shown that different aqueous ions have different effects on polymers in solution. The ion interactions with water can cause either a salt-in or salt-out effect on the polymer molecules. Salting-in refers to an increase in solubility of non-polar molecules in solution, while salting-out refers to a destabilization of the molecules in water. Salts that stabilize proteins in solution are known as kosmotropes, while salts that destabilize proteins are known as chaotropes.27 A qualitative ranking of the effective stabilizing and destabilizing nature of various ions is known as the Hofmeister series of ions.21 Evidence shows that even when all other solution conditions are held constant, such as temperature, pH, concentration, the presence of different ions changes the properties of the polymer solution.1

The effect of the Hofmeister series on hydrogels has been studied for changes in critical solution temperature3, surface tension and hydration entropy4, hydrophobic interactions16, and
formation of emulsions and foams, among others. The goal of this paper is to expand the knowledge on the effect of the Hofmeister series on polymer gels to include the effects on gelation rate and gelation time. By understanding the effects of various anions and cations on the time-dependent aspects of gelation, one can better control the thermoreversible properties of hydrogels. This can have significant impact for controlling drug delivery. Since drug release rate and delay must be controlled for location-specific medication, the use of different ions and their concentration can be controlled to regulate these release characteristics.

2. Background

2.1 Hofmeister Ions

The Hofmeister series of ions is a qualitative ranking of ions based on their influence on protein stability and solubility. First introduced in 1888, Franz Hofmeister noticed the effects of different ions on the solubility of egg protein. The ions where ordered based on relative activity, and this order of activity has been shown to hold across many experiments and studies.

\[
\text{SO}_4^{2-} > \text{HPO}_4^{2-} > \text{F}^- > \text{acetate} > \text{Cl}^- > \text{Br}^- > \text{NO}_3^- > \text{I}^- > \text{ClO}_4^- > \text{SCN}^-
\]

Figure 1: Hofmeister anions

\[
\text{NH}_4^+ > \text{Cs}^+ > \text{K}^+ > \text{Na}^+ > \text{Li}^+ > \text{Mg}^{2+} > \text{Ba}^{2+}
\]

Figure 2: Hofmeister cations

While these phenomena are well documented, there is no unifying theory that can explain and predict the specific ion effects of the Hofmeister series. Several theories based on hydration radii,
water dipoles, and van der Waals forces have been proposed.\textsuperscript{14} However, each theory only explains several observations, while failing to explain others.

Ion-specific effects on proteins have been studied ever since Hofmeister introduced the effect in 1888. The understanding of these effects has been compounding each year, as research continues to be published. There has been much work in recent years since hydrogels have been found to have many uses with biomedical applications and drug delivery. Here is a summary of what is known. Protein solubility is directly proportional to salt concentration.\textsuperscript{16} These salts dually affect the electrostatic and hydrophobic interactions of proteins. The “hydrophobic” effect was introduced in 1905 to describe colloid sols that coagulate when electrolytes are added in the solution.\textsuperscript{24} Salts have been shown to have lyotropic effects, or the potential to affect the surface tension of the solution. With the ability to affect surface tension and the hydration structure of water, ions change the interactions that contribute to the molecule’s solubility or insolubility. Other factors that affect gelation besides salts are ionic force of the solute, solution pH, molecular weight of the polymer, and polymer concentration in solution.\textsuperscript{19}

\textbf{2.2 Physical Polymer Gels}

\textit{2.2.1 Polymer Gelation}

Gels are semisolid systems that exist in one of two ways. Either they consist of suspensions of small inorganic particles, or they can consist of large organic molecules that can contain liquid within their structure.\textsuperscript{5} Hydrogels are large molecules that are interpenetrated by water. One important characteristics of hydrogels is that they contain ingredients that are water-soluble or dispersible as colloids. Gelation is the process that gels undergo in a polymer system. Gelation occurs when the polymer branching begins to form a network, converting the material
from a viscous liquid to an elastic solid. Since hydrogels are capable of storing water, they are researched for medicinal purposes, as they offer effective forms of drug delivery. Their properties can be developed to control drug release based on heat, time, and location conditions within the body. There are two types of gelation: physical and chemical. Chemical crosslinking of gels forms chemical bonds between polymer chains, whereas physical crosslinking does not involve the formation of chemical crosslinks but uses things such as intermolecular forces and surface interactions to form a bonded network.

2.2.2 Polymer Phase Behavior

The process of gelation can be related to the polymer solubility in a solution. The solubility of the polymer/solvent solution is based on the Gibbs free energy of mixing and is related to solution properties by the Flory-Huggins equation. This free energy is dependent upon the entropic and enthalpic conditions, which are based on solution composition, temperature, volume fraction, molar volume, heat of vaporization, and coordination number.\(^{22}\) Contained within these values is what is known as solubility parameters. A solubility parameter is a factor based on the effects the free energy of mixing due to the polymer/solvent combination. Using this parameter, a function of the temperature versus mole fraction of solute can be generated, where the curve represents the relationship between temperature and composition, which shows regions of solution miscibility. The parabolic curve, known as the binodal curve, shows separate regions of stability where 1 and 2-phase systems can exist. These regions are determined by equivalent chemical potential conditions. The locations that result in a zero second derivative of the free energy of mixing with respect to composition make up what is known as the spinodal curve. Using the binodal and spinodal curves, one can indicate the regions of stable, metastable,
and unstable two-phase systems, indicating the phase make-up of the solution at those conditions. The maximum or minimum temperature where one phase can exist at all compositions is known as the Upper Critical Solution Temperature (UCST), or Lower Critical Solution Temperature (LCST), respectively. Often times, there is a minimum concentration that two phases can exist at any temperature.

Upon heating or cooling into the two-phase region, the polymer begins to separate out into a distinct second phase, which is when the formation of a connected network of polymer branches occurs. Unlike crosslinked polymers, this state of gelation is usually reversible, meaning that adding heat to a UCST-behavior gelled solution at a low temperature will break down the gel into a single solution again. Gelatin exhibits UCST behavior, while PF-127 exhibits LCST behavior. It has been shown that salt concentrations reduce the UCST to a point where the water solvent would freeze before a stable network would form.³
2.2.3 Gelatin

Gelatin is a denatured form of collagen, an animal protein. It is a polypeptide of between 50 and 1000 amino acids per chain.\textsuperscript{27}

![Typical chemical structure of gelatin](image)

As solid crystals, gelatin has a light brown color that is semi-transparent. In solution, gelatin forms a light-yellow color solution that exists as a liquid at temperatures above 40°C and as a weak elastic solid when gelled. The protein is safe for human consumption, as is often used in cooking, as well as in the form of digestible capsules. When in hot solution, the gelatin takes on a randomly-coiled structure of helical proteins. Upon gelation, the polymer forms a triple helix structure that interacts with the polar water solvent and other gelatin chains. The presence of ionic salt creates additional aggregation of the helical chains.
Two factors contribute to the gelation of gelatin. Firstly, van der Waals forces acting between the hydrated shells of the partial collagen-like folds of the molecule create a network with contained water. Secondly, the function groups protruding perpendicular to the gelatin polymer chain create hydrogen bonding with the solvent surrounding the molecule. These together form a hydrated network of reversible chain-solvent interactions. The gelatin used was from both bovine and porcine bones and was supplied by Fluka Analytical®.

2.2.4 Poloxamer 407 (PF-127)

Poloxamer 407, also known as Pluronic F-127 (PF-127) is a bio-reagent polyethylene-oxide-polypropylene-oxide copolymer gel. It is provided as a white powder which appeared to be notably affected by electrostatic forces. Poloxamer 407 was designed as a nonionic surfactant for dermal and transdermal drug delivery systems due to its low viscosity, thermoreversible nature near normal body temperature. It is a synthetic triblock copolymer produced by the condensation of ethylene oxide and propylene oxide. The polymer has the form $E_{100}P_{65}E_{100}$.
It was supplied from Sigma Life Sciences® with an average molecular weight of 12,500 g/mol. PF-127 forms gels at a high enough concentration (critical gel concentration, cgc) that micellization occurs and begins to form a lattice among the micelle regions. Each micelle consists of a hydrophobic center with a hydrophilic polyethylene oxide outside. PF-127 exists as a liquid at temperatures below about 12°C. This is because the hydration layers surrounding the polymer molecule is sufficient to dissolve it. However, an increase in temperature causes the hydrogen bonding among the hydrophilic regions to weaken. With the weakening of the hydrogen bonding, the solvent interactions favor the hydrophobic PPO regions. The switch from hydrogen bonding to hydrophobic interactions causes dehydration of the molecule and leads to gel formation.

2.3 Viscosity of Gels

Polymer gel solutions in one-phase exist as a viscous liquid. At these conditions, polymers in solution do not have adequate conditions to form a polymer network. For example, a
gelatin solution at increased temperature weakens the hydrogen bonds between the polymer and solvent, allowing the chains to move more freely. Therefore, the only resistance to flow stems from single chain-chain interactions and dispersion forces. During the gelation process, the polymer solution transforms from a viscous liquid to an elastic solid. This causes the interactions between the polymer and solution increase. The intermolecular forces now present in the solution require additional energy to overcome for the solution to flow. Therefore, the viscosity of the solution increases proportionally to the state of solution gelation. As the solute/solvent interactions increase in the solution around the measuring device, a higher torque is required to maintain a constant strain rate. This higher shear is due to the increased molecular interactions mentioned previously. Upon a certain gelation state, the physical polymer network no longer yields to a constant strain and begins to cause a physical break in the network. This breakdown of the physical crosslinks cannot be undone unless the state of the solution is changed, i.e. heated back to a liquid and then re-gelled.

3. Experimental Methods

Overall, three set of experiments were performed. The first varied through different cation chlorides. The second varied through sodium salts of various anions. As well, a set of experiments was done with gelatin in solutions of lithium chloride at concentrations of 0.00, 0.05, 0.10, 0.20, and 0.30 molarity. Gelatin solutions were heated to between 35 °C and 50 °C before being cooled in a water bath maintained at 25 °C. The solutions of PF-127 were kept at 3 °C – 4 °C before being allowed to heat up with room temperature air. The air temperature was maintained between 23 °C and 25 °C. The data from the NDJ-5S viscometer was shown in both viscosity (mPa-s) and percent (%) of the maximum viscosity. The maximum viscosity was
dependent upon the type of rotor uses and the rotation speed. For settings used in these experiments, the maximum viscosity that could be measured was 20,000 mPa-s. Once the machine reached this limit, the readout would appear as “OVER”. This point was taken as the end of each test. Therefore, the final viscosity of the gel could not be determined. However, the goal of the experiment was to compare the rates of viscosity change based on the salts present in solution. Therefore, it is more valuable to analyze the gelation rate in the first half of the transition, as well as look at the time required to reach 90% of the maximum viscosity (18,000 mPa-s).

3.1 Materials and Equipment

One obstacle for the experiments was that large amounts of polymer may be needed for the solutions. Some polymers are quite expensive or difficult to make, so using greater than 100 g samples was not feasible. Therefore, tests were run with a control 150 mL beaker, a 100 mL beaker, and two 25 mL vials of different dimensions. This needed to be studied since the heat transfer and surface contact were different depending on the geometries of the containers. The initial tests showed that using the tall and narrow 25 mL vial produced viscosity data similar to that of the 150 mL beaker. Therefore, these 25 mL vials were used for the tests, as they used less material while still giving comparable results. Secondly, qualitative tests were done to determine the best rotor and rotor speed to use. After noticing that the large rotors could not accurately read the high viscosities that occur during the tests, it was decided that the smallest rotor should be used, which is referred to as Rotor 4.
A speed that gave steady and accurate values was determined to be 30 RPM. The NDJ-5S viscometer displayed the viscosity readings on an LCD display. A secondary camera was set up that used Yawcam® software to take photos of the display read-out every 30 seconds. This data allowed measurement of the viscosity at equal time intervals for the duration of the test.

3.2 Preparation of Gel Solutions

The gelatin solutions were prepared at a concentration of 5% gelatin by mass in solvent composed of dissolved salts in de-ionized water. The solution was mixed on a hot plate at 50°C until fully dissolved. As well, the solution was heated to 50°C prior to testing. The PF-127 materials were made to 25% (w/w) in various aqueous salt solutions. The polymer and solvent were mixed in an ice bath between 3 and 4°C until homogeneous. After mixing, the PF-127 solutions were stored in a refrigerator prior to testing. The aqueous salt solutions were prepared prior to the addition of the polymer and stored at room temperature. Several concentrations were prepared based on which experiment was being performed. Although, the salts used for testing the cationic and anionic Hofmeister series are seen in Table 1 below.
Table 1: List of Ionic Salts used to observe the effects of various cations and anions on gelation

| Hofmeister Series: Ionic Salts used to observe effects on Hydrogel Gelation |
|---------------------------------|--------------------------------|
| Cationic Series                 | Anionic Series                 |
| LiCl                            | NaF                            |
| NaCl                            | NaCl                            |
| KCl                             | NaBr                            |
| CsCl                            | NaI                             |

Single charge ions were investigated to maintain the same concentration of ions in solution. As well, it was important to prevent any effects caused by ions with additional electron orbitals.

3.3 Viscosity Measurement

The viscosity was recorded as a function of time with a NDJ-5S Viscometer. The device operates by rotating a stainless-steel rotor at a given rotation speed, designated in rotations per minute (RPM). When the rotor was submerged to the proper limit in the gel, the viscometer was turned on, and it was allowed to operate continuously for the remainder of the test. The rotor is attached to the machine on a pivot that is allowed to rotate freely.
Based on the geometry of the different rotors available, along with their corresponding surface areas, the viscosity can be measured based on the latency of the rotation. A torque is applied to the rotor pivot, so the degree at which the rotor is held back by the viscous solution is proportional to the torque acting on the rotor from the solution, which can be used to determine viscosity. This means that the higher the viscosity, the higher the phase shift between the rotation at zero resistance and the actual rotation of the rotor.
3.4 Experiments

3.4.1 Effect of Concentration on gelation

The goal of this experiment was to determine what kind of effect concentration has on the gelation process, specifically the change in rate of gelation and gelation time. It is well known that salt concentration has an effect on protein stability and solvent interactions. As well, there is a range in which specific concentration effects can be observed. The effects of salts at low concentration may be overwhelmed by unaffected ‘bulk’ water surrounding the remainder of the molecule. Likewise, sufficiently high concentrations may lack the necessary pure water regions to properly show a difference in specific effects. Therefore, the solution concentration was varied for lithium chloride between 0.00M and 0.30M.

3.4.2 Effect of Hofmeister Anions on gelation

Anions according to the Hofmeister series have been shown to have a greater effect on polymers in solution than cations. The ionic radius of anions is much larger than cation, which may interact more with polymer chains. Therefore, it was important to observe the effects of the anion series, as it was the most likely to yield definitive results. The monoatomic anions tested were F⁻, Cl⁻, Br⁻, and I⁻. Each anion was tested at least twice to confirm that the results were reproducible.

3.4.3 Effect of Hofmeister Cations on gelation

While the specific effects of cations has been known to show weaker effects than anions, the presence of cations in solution will still affect the hydrophobic interactions of water and the protein chains in solution. Therefore, a series of monoatomic cations was tested to compare their
relative order in the Hofmeister series to their effects on the gelation of both gelatin and PF-127. The cations tested were Li$^+$, Na$^+$, K$^+$, and Cs$^+$. Just as in all experiments, the solution gelation was tested at least twice to confirm the accuracy of the results.

4. Data and Results

4.1 Gelatin Hydrogel

4.1.1 Hofmeister Anions, gelatin

![Gelation of 5% gelatin by mass in various 0.1M sodium halide solutions.](image)

The figure shows viscosity data for 5% gelatin in ionic solvents. The data shown is the average of at least two individual tests of the same conditions. The results were averaged between the recorded values at the same time during gelation. The concentration of each solvent was kept constant at 0.10M. The plot shows that as ions move along the Hofmeister anion series
towards increased hydrophobic solubility, the total gelation time increased. This is most evident with sodium iodide, where the gelation time was increased by more than double. It should be noted that initial viscosity change rate was similar for each salt. However, the differences between anions was more apparent past about 50% of its gelation time. Based on the data, it appears that ions that have a destabilizing effect on the gel, or salting in effect, slow down the rate of viscosity increase during gelation. If this is true, the reverse should be true as well. As the anions present in the gelatin solution increase protein structure stability, the viscosity change rate should increase. This appears to be the case, as the fluoride ion, the most stabilizing anion tested, showed the fastest increase rate among those anions tests. However, based on the effects of concentration on gelation, the gelation time is likely to increase over pure solvent, regardless of ions present
4.1.2 Hofmeister Cations, gelatin

A similar occurrence appears when for Hofmeister cations and their ability to affect protein gelation. However, the trend does not appear to show a correlation between effect on gelation and the order of the cations of the Hofmeister series. Yet, it is important to remember that the effects of specific cations are less than that of anions. As well, the cation experiments showed a wider range of viscosity results than that of anion tests. There may be other factors on gelation that is seen with the smaller cations that could be contributing to the results seen above. As well, it is possible that the results are within reasonable measurement uncertainty, where the results are not statistically independent from one another.
4.1.3 Salt Concentration, gelatin

Previous experiments show that the upper critical solution temperature decreases as a function of increasing ionic concentration. Therefore, it is known that an increase in the concentration of ions should affect the gelation time as the concentration is changed. The results of the experiment show that the gelation rate is slowed proportionally to the ionic concentration of the solvent. The largest difference in gelation rate was observed between 0.05M and 0.10M concentration. While the overall gelation time increased proportionally, the initial gelation rates are similar below 4000 mPa-s. Only during the late stages of the tests did the differences appear. The reason that there is not much difference between the tests at 0.00M and 0.05M concentration is because there is not ions to have a significant change to the water surrounding the molecule.
As well, the differences between 0.20M and 0.30M are minimal since there is likely not enough unaffected water around the gelatin to show differences.

### 4.2 Pluronic F-127 Hydrogel

#### 4.2.1 Hofmeister Anions, PF-127

![Figure 13: Gelation of 25% PF-127 by mass in solutions of 0.1M sodium halides](image)

The presence of ions in solution also caused a delaying effect on gelation, similar to gelatin. The overall gelation time increased by approximately 50% at this concentration. While the presence of salts does delay the gelation rate, the Hofmeister ions appear to have the opposite effect to that of gelatin, where the increase in hydrophobic solubility (salting-in) decreases the gelation time. The more destabilizing ions of the series cause the gelation behavior to more closely resemble water. This is likely because Pluronic 127 is more non-polar than gelatin, so the increase in hydrophobicity may increase the interactions with the polymer molecule. It is important to note that the delaying effect of gelation did not correspond directly with the
Hofmeister order to anions as was seen in gelatin. Both chloride and iodide ions behaved similarly, but the solution of sodium bromide did not have an effect corresponding to its relative order in the series.

4.2.2 Hofmeister Cations, PF-127

The results of various cations solutions of PF-127 yielded inconclusive yet expected results. The presence of the ions increased the gelation time but failed to show distinguishable differences from each other. This is most evident for the last half of the gelation period, where the viscosity data is almost indistinguishable. The data makes sense because previous data has shown that the relative effect of Hofmeister cations is less than that of Hofmeister anions. Therefore, the slight differences in the data are likely due to experimental error.
4.2.3 Salt Concentration, PF-127

The presence of ions in solution has already been shown to have an effect on gelation rate for gelatin. A similar effect is also seen here with PF-127. However, the results show a significant difference from the gelatin data, in that the increase in salt concentration decreased the gelation time. A higher salt concentration shifted the viscosity data closer to gelation in pure solvent. This shows that the increased ionic interactions around the polymer molecule actually help to precipitate the molecule out of solution. One possible explanation for this is the nonpolar nature of PF-127. An increase in polar regions in the solvent may help promote the less-soluble hydrophobic regions of the molecule.

![Figure 15: Gelation of 25% PF-127 by mass in LiCl solutions at concentrations between 0.00M and 0.30M](image-url)
5. Discussion

5.1 Gelatin

Gelatin is a denatured form of collagen proteins, which forms gels when hydrogen bonding between the molecule and water become sufficient enough to precipitate the polymer from solution. The polar nature of gelatin and its interactions with water can explain what was observed. The anion experiments showed that ions that destabilize the proteins in solution have a delaying effect on gelation of gelatin. The higher the ‘salting in’ effect, the increased time required to reach a fully-gelled state. This is likely because of the effect that the anions have on the structure of the water molecules surrounding the polymer. The change in hydration regions changes the way the polymer interacts with water: for example, increases the time to form hydrogen bonds of the polymer chains with the water molecules. Therefore, the destabilizing effect of ions in solution increases the interactions required to form a polymer network, hence slowing the rate of gelation.

The cation-specific effects on gelatin are less conclusive than the anion effects. The main concern is that the trends seen in the viscosity data do not correlate with the cations’ relative position in the Hofmeister series. It should be noted that the data collected on repeated cation solutions tests showed lower precision than the anion solutions, and cations effects have been shown to be weaker compared to anion effects. Therefore, it is hard to draw a conclusion from the obtained data. The effect from specific Hofmeister cations is indistinguishable from the other factors and error in the experiments.

The concentration study showed expected results. The time required to reach a totally-gelled state was proportional to the ionic concentration in the solvent. The largest change in viscosity data was between 0.05M and 0.10M concentrations. The similarity of results between
0.00M and 0.05M solutions is likely due to a negligible amount of affected hydration regions surrounding the protein. If not enough of protein molecules are affected by ions, then the overall data will not vary much from that with just DI water as the aqueous phase. Likewise, similar results between 0.20M and 0.30M of LiCl in water as the aqueous phase were observed. At high enough concentrations of salt, there is not enough unaffected water regions surrounding the protein to show differences between the two. Therefore, repeating these experiments at even higher ionic concentrations is likely to show little to no change from that of 0.30M, if the trend continues.

5.2 PF-127
PF-127 (also known as Pluronic 127 or poloxamer 407) is a non-polar triblock copolymer consisting of PEO-PPO-PEO blocks. The gelation mechanism of PF-127 is different from that of gelatin, where PF-127 forms micelles at a sufficient concentration of polymer in solution. PF-127 exhibits LCST behavior, while gelatin exhibits UCST behavior. The most surprising result was that as the hydrophobic interactions increased in the solution, the rate of gelation increased. For example, the addition of salts increases the gelation time of PF-127 by about 5 minutes. However, increasing the salt concentration to 0.20M and 0.30M reduced the delay, causing the viscosity data to shift towards the data from the solution using DI-water. This is the opposite trend seen with gelatin. With gelatin, the viscosity change was slowed, while PF-127 shows a faster rate of change.

There are several possible factors that could be contributing to this behavior. The nature of PF-127 is that the hydrophilic regions of the micelle structure exterior dissolve when the temperature is raised and the hydrogen bonding is weakened. The gelation occurs when the hydrophobic interactions with the PPO regions becomes dominant and cause precipitation. It is
possible that the presence of ions in solution acts in two steps. Firstly, the increase in solution ionic strength may promote hydrogen bonding that allows the polymer to remain dissolved. This would explain the delay in overall gelation time. However, once the hydrogen bonding is weakened, the specific ions and ionic concentrations that promote hydrophobicity would increase the hydrophobic interactions cause gel formation. This also explains why solutions containing anions led to higher hydrophobic effects, for example, hence gelling faster than those containing less anions (i.e. with less hydrophobic effects). Another possible explanation could be that the ions delay the weakening of hydrogen bonding, but the increase in hydrophobicity may assist in the formation of the hydrophilic/hydrophobic micelles which form the gel structure.

5.3 Error

The experiments posed several inherent sources of error that should be addressed. The main source of error was likely in the preparation of the gels. The ionic solvents were prepared from solid salts and de-ionized water, so the measurements in both volume and mass may cause inaccuracies, along with human error such as spills. As well, error in the weighing of solid polymer for the gel would have a relatively large effect. If the gelatin solutions differed much from 5% by mass, or the PF-127 solution differed from 25% by mass, the results may not reflect the true effects of the Hofmeister ions.

Another possible source of error was in the viscosity measurements, both in recording and accuracy. The NDJ-5S viscometer could update the measurement read-out every 3 seconds, which often resulted in jumps of more than 20 mPa-s per refresh. Even more so, the recording software used recorded the viscosity read-out every 30 seconds. This showed large jumps in viscosity over one interval. However, it is most likely that the viscosity data did not change at a constant rate between recordings. Therefore, the data may lack small details not recorded
between the 30 second intervals. Along with the viscosity read-out, the viscosity measurements can be affected by things such as irregularities in solution gelation, uneven heat transfer, contamination from the solution or rotor, or misalignment in the viscometer. Irregular solution gelation refers to isolated or unsymmetrical regions where the solution gels at a different rate or time compared to the rest of the solution. This could be caused by a non-uniform polymer concentration or uneven heat transfer. If the heat transfer was not uniform in the, this would cause irregular gelation that would vary between tests. This is a possible cause for differences in viscosity data observed between repeat tests, even from the same prepared solution.

5.4 Future Experiments

During the course of this study, several questions were raised that were not able to be addressed experimentally. The most frequent variable that was addressed was the effect of polymer concentration in solution. Previous research shows that concentration does affect gelation, and it was unknown what kind of effect that deviations in solution concentration had on the gelation time and rate. If this were better understood, it would be possible to better approximate the amount of error it caused. To confirm the results observed with gelatin and PF-127, other sources of gelatin and other synthetic physical gels should be tested for confirmation. Examples include gelatins produced from fish collagen, or PF-127 of different molecular weight. Another factor not investigated in the experiment was pH. Previous studies showed that pH does effect polymer gelation. Though the pH was recorded to maintain consistency, it was not varied to observe its effect on gelation. If salt concentration and ions present can be manipulated to control gelation characteristics, then pH could also be used to control this behavior. Therefore, it would be useful data to collect. If one were to continue with these experiments, it would be good to gain access to other rheology measuring devices such as a dynamic mechanical analyzer (DMA) or rheometer. Besides viscosity, quantities such as loss or storage moduli would be
useful for understanding more details about polymer gelation, and how they are affected by various ions in solution.

6. Conclusion

This project examined the effects of Hofmeister ions, mainly alkali cations and halide anions, on the gelation of a protein - gelatin and a thermo-reversible hydrogel – Pluronic F-127. All the ions delayed the gelation for both gelatin and PF127. For gelatin, the extent of delay of gelation by halide anions was found to follow $I^- > Br^- > Cl^- > F^-$; but the trend was not very obvious for the alkali cations, all four ions (Li$^+$, Na$^+$, K$^+$, and Cs$^+$) lead to similar delays in gelatin gelation. For PF127, the delaying effect of halides was found to follow $F^- > Cl^- > Br^- > I^-$, which is the reverse of the gelatin results. Alkali cations showed no trend for delayed gelation; the results were more identical than those for gelatin. The effect of salt concentration on gelation of gelatin showed a delay directly proportional to the concentration. Low concentrations did not show much change from pure aqueous solvent, and high concentrations did not change much after a certain concentration even as it continued to increase. However, there was a large delaying effect starting at 0.10M concentrations for this experiment. The effect of salt concentration on PF127 was an initial delaying effect similar to gelatin. However, further increasing the salt concentration reduced the delaying effect proportional to the increase in concentration. To better understand these observations, further experimentation should be done into the effects of polymer concentration on gelation. As well, it would be useful to understand the other rheological changes of ionic salts on solution gelation. Therefore, it is recommended to observe gelation with other rheology equipment besides a viscometer.
**Citations**


(2) Zhang, J. Protein-Protein Interactions in Salt Solutions, in *Protein-Protein Interactions – Computational and Experimental Tools*; Intech China: Shanghai, 2012; Chapter 18, pp 359-376.


