Abstract
This research project was an exploration of how different surfactants affect the crystallization of acetylsalicylic acid in different solvents. Aspirin crystals were dissolved in two different solvents, polar ethanol and non-polar hexane. The crystal structures that formed were then analyzed using a microscope to determine the specific effects of each factor. Studying these results allowed for a prediction of the forms the crystals will take given certain circumstances. Four different surfactants were added to these solvents to determine their effect on the formation of aspirin crystals. The results of this research indicate that Poloxamer 188, Poloxamer 338, and Poloxamer 407 all had different effects on the crystal size and shape. Tween 80 was found to promote crystal growth on all planes and also increase the solubility of aspirin in ethanol.

1. Introduction
Aspirin has been experimented with throughout history: willow bark, whose active ingredient is acetylsalicylic acid, was prescribed by the Chinese for many ailments as early as 500 BC (“The Manufacture of Aspirin”). The aspirin crystal was isolated for the first time in 1828 by Johann Buchner and since then has been used extensively both in medicine and research of crystallization (Bellis).

Aspirin is a drug that is familiar to people around the world. Aspirin belongs to the salicylates drug family, which consists of colorless, crystalline organic carboxylic acids. Another name for aspirin is acetylsalicylic acid (ASA). Aspirin is very important in society because it can be used for many different purposes. The most common uses of aspirin are against minor pains, aches, and fevers. However, aspirin is also used for blood-thinning and as long-term low-doses to prevent heart attacks (Aspirin).

By definition, crystallization is the process in which solid crystals form from a homogeneous solution. Crystallization is also referred to as a chemical liquid-solid separation technique. Crystallization is based on the principles of solubility: solutes tend to be more soluble in hot liquids rather than in cold liquids. The process consists of allowing a hot saturated solution to cool to the point where the solute is no longer soluble in the solvent. Crystals are then formed from a pure substance (“Crystallization”). There are two events that lead to crystallization: nucleation and crystal growth. Nucleation is the step where the solute molecules start moving in the solvent to create clusters (“Crystal Growth”). The crystal growth shows how the crystals grow in different conditions. The purpose of this experiment was to examine the way that different additions to the solvent affect aspirin crystallization.

2. Background Information
Polar solvents cause aspirin crystals to grow along the x-direction (1 0 0) and y direction (0 1 0)\(^1\). Therefore, aspirin crystallizes into rectangular plates when dissolved into ethanol, a polar solvent. However, non-polar solvents inhibit the crystal’s growth in the x direction and facilitate its growth in the y direction (Saue, 1982). As a result, hexane, a non-polar solvent, causes the aspirin to crystallize into needles. In both cases, growth in the z-direction (0 0 1) is minimal.

Hydrogen bonds maintain a large role in the shape of an aspirin crystal (Tomassone). A hydrogen bond is the attractive force between the hydrogen attached to an electronegative atom of a molecule. In order to create a hydrogen bond, an electronegative atom is bonded to a hydrogen atom. Because the positive charge of the nucleus in an electronegative atom is much stronger than that of hydrogen, the electrons of the molecule are more attracted to the electronegative atom. The electrons spend more time with the electronegative atom and it develops a partially

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1 Danesh et al, 2000
Glassby and Ridway, 1968
Watanabe et al, 1982
Tomassone et al, 2006
negative charge, while the hydrogen develops a partially positive charge. A hydrogen bond results when the positive charge of the hydrogen is attracted to the negative charge of another atom or when the electronegative atom’s negative charge is attracted to the positive charge of another atom or molecule.

Because of its attractive forces, hydrogen bonds stabilize the surfaces of crystals (Tomassone). The shape of the resulting aspirin crystal depends on the ability of a plane to compete for hydrogen bonds. Paired molecules (hydrogen bonded) are less affected by the addition of a solvent. An aspirin crystal can be seen as a double layer system with the layers parallel to the (1 0 0) plane in which aspirin molecules are linked by hydrogen bonds between their carboxyl groups. The aspirin molecules on each of the layers are arranged in a zigzag pattern. The molecules form parallel rows along the y-direction and are also parallel to other molecules in the same row (Tomassone et al, 2006).

The interaction forces of the solvent with the faces of the crystal affect the stability of those faces: a strong binding of the solvent to the crystal impedes the growth of that specific face (Tomassone). Accordingly, the polarity of the solvent in which the aspirin is dissolved is important to consider because its polarity will affect the interactions between the crystals and the solvent. Having both polar and non-polar bonds, the (0 1 0) face is the most stable; it is least affected by the addition of ethanol and grows the fastest. Aspirin, a hydrophilic material, reacts favorably with the ethanol, which is also polar. Consequently, the lattice structure of the (1 0 0) plane, though affected by the addition of ethanol, is not completely disrupted by its bonding, and the (1 0 0) plane is able to grow. However, the (0 0 1) face, populated with hydrophobic chemical groups, is completely disrupted by ethanol molecules and shows the slowest growth speed. (Tomassone). As a result, the growth of the (1 0 0) and (0 1 0) planes cause the crystals to form into rectangular plate.

Because the non-polar hexane causes an unfavorable reaction with the polar hydrogen bonds of both the (1 0 0) and (0 0 1) planes, their lattice structures are disrupted. Only the (0 1 0) plane’s structure is uninhibited and, as such, continues to grow, forming the needle shape.

To study the effect of surfactants on the shape and size of crystals, small amounts of surfactants were added. It is expected that depending on the interaction of the surfactant with the different faces of the crystals, surfactants can act as growth quenchers or shape modifiers. A surfactant consists of both hydrophobic and hydrophilic groups, allowing it to be marginally soluble in both polar and non-polar substances (“Surfactant”). Poloxamer 188, Poloxamer 407, Poloxamer 138, and Tween 80 are all surfactants considered in this study.

3. Experimental Design

Crystallization of Aspirin in Ethanol and Hexane

The primary ingredients utilized were aspirin, ethanol, and hexane. The aspirin was powdered and dissolved. Ethanol (ethyl alcohol) serves as a polar solvent, while hexane is a non-polar solvent. Both solvents are liquids at room temperature, making experimentation with them relatively easy. Additionally, complex poloxamer molecules of three different molecular weights were used as solid surfactants, and Tween 80 was used as a liquid surfactant. Since most of the chemicals involved in the experiments were dangerous to inhale, all of the procedures were done in a hood, a safety device used to get rid of harmful fumes.

The first batch of solutions made consisted of aspirin dissolved in ethanol, with various surfactants added. The first solution (Solution 1) began with 100 mL ethanol and 10 g aspirin. Next, aspirin was added in small increments until the saturation point had been passed such that there was some solute remaining out of solution. The aggregate effect was 25 g aspirin in the mixture, with no additional substances. The solution was then stirred and heated until all of the aspirin dissolved, which occurred at 42°C. At this point the stirring was ceased and the solution taken off the heat. A small sample, about 1 to 3 mL, was placed on a microscope slide. Once the sample was on the slide, the solution rapidly cooled to room temperature and much of the solute fell out of solution, forming aspirin crystals.

Crystallization of ASA Crystals in the Presence of Surfactant

A similar process was utilized for the next four solutions (numbered 2 through 5). Each was made with 100 mL ethanol and 25 g aspirin, as this was observed in the previous solution to be an appropriate ratio: too little aspirin and no crystals will come out of solution, even at room temperature; too much aspirin will cause the ethanol to boil before everything can be dissolved. Additionally, crystalline structures formed from solutions of the same concentration provide for a reliable comparison to one another and to the control.

These four solutions, however, also included surfactants, the study of whose properties was an integral part of the experiment. 2 g of Poloxamer 188 were added to Solution 2. Solution 3 was augmented by 0.5 g of the same Poloxamer 188, so that a comparison might be made between varying ratios of surfactant to solute. Solution 4 received 2 g of another surfactant, Poloxamer 338. Solution 5 also received 2 g of a poloxamer, Poloxamer 407.
The same stirring and heating process was used for Solutions 2, 3, 4, and 5 as was used for Solution 1. The solutes, both the aspirin and the poloxamer, in these solutions fully dissolved at 38°C, 39°C, 38°C, and 39°C, respectively. A microscope slide was made for each solution as it was taken off the hotplate. On each slide, noticeable white aspirin crystals began to form.

Later, three more aspirin-in-ethanol solutions were created. Instead of using a powdered surfactant, like the poloxamers, a liquid surfactant was used: Tween 80, or polyoxyethylene (20) sorbitan mono-oleate. The intent was not only to study the role that a very different surfactant might play in crystal formation, but also to provide a model situation where varying levels of a surfactant additive (and thus varying ratios between surfactant and aspirin in solution) might be compared. Thus, Solution 11 contained 3 mL Tween 80, Solution 12 contained 10 mL Tween 80, and Solution 13 contained 20 mL Tween 80. All three solutions had, in addition to the Tween, 100 mL ethanol and 25 g aspirin.

Again following the same stirring and heating process as before, the solutions were saturated. The temperatures at which this occurred were 62°C for Solution 11, 50°C for Solution 12, and 45°C for Solution 13. The clear correlation between Tween 80 added and the temperature at which saturation was reached is evidence supportive of the supposition that incrementally increasing surfactant levels will proportionally change crystal properties. After reaching these points the solutions were removed from the heat and samples were taken for the slides. As expected, white crystals formed on the slides as the thin layer of solution cooled.

In addition to the study of aspirin crystals formed from ethanol solutions, observing crystals of aspirin made from hexane solutions was important to forming well-founded conclusions. Solutions 6 through 10 were dedicated to this purpose.

It was known that aspirin is not as soluble in hexane as in ethanol because hexane is non-polar, while both aspirin and ethanol are polar substances. Therefore, the next five solutions were started. The first of these with 200 mL hexane and 0.5 g aspirin – a far lower concentration of aspirin than was used for ethanol. This solution was heated and stirred, just as was done to all the others samples. However, significant clumps of solute were observed indicating that a large amount of solute was still not dissolved. It was determined that a small amount of a co-solvent should be added to help dissolve the crystals entirely, so as to avoid having large seed crystals in the mixture. Already knowing from experience that ethanol dissolves aspirin well, ethanol was added at 1 mL increments. After a total of 3 mL ethanol was added and at 62°C, all of the aspirin was observed to have dissolved. Following the standard procedure for the project, the solution was taken off the heat, and a small amount was placed on a slide. The same white crystalline growth of aspirin could be observed.

In order to draw valid comparisons between hexane solutions and ethanol solutions with the same poloxamer surfactant, it was desired that the ratio of mass between surfactant and aspirin in hexane match the already-established 0.08 ratio in ethanol. To do this accurately, an amount of aspirin greater than the one used for Solution 6 was needed. It was decided that 5 g aspirin should be used. Also, 20 mL ethanol, in addition to the 200 mL hexane, was used for Solutions 7 through 10 in order to accommodate the dissolution of all the aspirin.

Solution 7 was made with only the 200 mL hexane, 20 mL ethanol, and 5 g aspirin. To Solution 8 was added 0.4 g Poloxamer 188, to Solution 9 was added 0.4 g Poloxamer 338, and to Solution 10 was added 0.4 g Poloxamer 407. Thus the hexane Solutions 8, 9, and 10 were analogous to the ethanol Solutions 2, 4, and 5, respectively, in terms of the poloxamer used and the ratio of that poloxamer to the aspirin. (See Table I)

Solutions 7 through 10 were stirred and heated. The powders in Solution 7 dissolved at 57°C. Solutions 8, 9, and 10 reached their boiling point at 60°C with almost all of the aspirin and poloxamer dissolved. At these points, these four hexane solutions were removed from the heat and slide samples were taken from each one. As mentioned earlier, aspirin crystallization was then observed on the microscope.

It is worth noting that after the preparation and sampling of Solution 9, a phase split was observed. The ethanol collected in an immiscible puddle at the bottom, sinking below the hexane due to its higher density. The next day, the phase split remained evident, and no needle-like crystals could be seen in the main flask. However, crystals were observed in all the other solutions created up to that time, after they were given the chance to settle and cool. The needles are expected for aspirin in a hexane solution, and they were observed in the other hexane solutions. Deciding that the results for this particular solution would not offer much conclusive evidence or insight, another solution, Solution 9F, was prepared in the same manner and with the same composition. The new solution showed no phase split, and furthermore, produced visible needle-like crystals.

As with any scientific experiment, this experiment contained errors, both random and systematic. All measurements have some error associated with them, so the amounts of the substances varied in actuality from the stated quantities. This discrepancy may have been further compounded by the evaporation of the liquid solvents, especially while being heated near their respective boiling points. Impurities in the
substances used or in the environment certainly may have impacted crystal growth, as well.

Replication of the results hinges upon replication of the conditions in which the crystals were allowed to form. For example, the temperature of the air in the room and the size of the sample placed on the microscope slide directly impact the rate at which the solution cools and thus affect the rate of crystal growth. This rate may impact the size and morphology of the crystals.

Figure 1: ASA crystal crystallized from ethanol (Solution 1)

It is expected that the analysis of the data is accurate and truly representative of the underlying chemistry involved in crystal formation. However, it must be pointed out that only a small number of solutions were prepared and studied. A greater number of crystal samples may reveal more information.

<p>| Table I: Table depicts the different amounts of solvent, co solvent, and aspirin used for all cases considered. |</p>
<table>
<thead>
<tr>
<th>Solvent</th>
<th>Co solvent</th>
<th>Aspirin</th>
<th>Surfactant</th>
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<tbody>
<tr>
<td>Solution 1</td>
<td>100 mL ethanol</td>
<td>–</td>
<td>25 g</td>
</tr>
<tr>
<td>Solution 2</td>
<td>100 mL ethanol</td>
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<td>25 g</td>
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<tr>
<td>Solution 3</td>
<td>100 mL ethanol</td>
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<td>25 g</td>
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<tr>
<td>Solution 4</td>
<td>100 mL ethanol</td>
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<td>25 g</td>
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<tr>
<td>Solution 5</td>
<td>100 mL ethanol</td>
<td>–</td>
<td>25 g</td>
</tr>
<tr>
<td>Solution 6</td>
<td>200 mL hexane</td>
<td>3 mL ethanol</td>
<td>0.5 g</td>
</tr>
<tr>
<td>Solution 7</td>
<td>200 mL hexane</td>
<td>20 mL ethanol</td>
<td>5 g</td>
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<tr>
<td>Solution 8</td>
<td>200 mL hexane</td>
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<td>5 g</td>
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<tr>
<td>Solution 9</td>
<td>200 mL hexane</td>
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<tr>
<td>Solution 10</td>
<td>200 mL hexane</td>
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</tr>
<tr>
<td>Solution 11</td>
<td>100 mL ethanol</td>
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<tr>
<td>Solution 12</td>
<td>100 mL ethanol</td>
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<tr>
<td>Solution 13</td>
<td>100 mL ethanol</td>
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4. Observations and Results

The research done can be divided into several independent studies. One of these studies examined the effect different poloxamers have on the crystallization of aspirin when added to aspirin dissolved in ethanol. It was observed that when Aspirin crystals are dissolved in ethanol and later come out of a super-saturated solution, they are almost perfectly square in shape, macroscopically, and form fairly large, indistinct rectangular crystal plates and fragments when looked at under the microscope. This solution of pure ethanol and aspirin was the control for the first set of deviations (Figure 1).

The second solution made differed from the first in that it contained Poloxamer 188. It was observed that compared to the control, the crystals that formed were smaller in size, less distinct and had sharper, fractured ends on the microscopic level. Macroscopically, this particular solution had fewer large sized crystals and more fragments, however, the crystals were still almost perfectly square in shape. The third solution contained less Poloxamer 188 by mass, more specifically - one fourth of that used in the second solution - and some needle formation was observed microscopically. Macroscopically, the plates were observed to be smaller in size than the control, and again, were almost perfectly square in shape. The plates were, however, larger than those of solution two.

From this set of results, it can be concluded that the addition of Poloxamer 188 indeed affects the size of aspirin in ethanol. The needle-like structures present in the third solution cannot be explained fully with this experiment’s findings.

The fourth solution contained Poloxamer 338 and did not differ significantly from the second solution. Macroscopically, it exhibited large plates and many crystals that were broken into smaller parts. Macroscopically, the same effect was observed. Therefore, the exact effect of Poloxamer 338 on the crystallization of aspirin in a solution of ethanol cannot
be determined from the results and observations acquired in this study.

The fifth solution exhibited a very different effect because it contained Poloxamer 407. Under the microscope, there was a significant reduction in the number of crystals. Also, the crystals themselves showed a more distinct nature and were much smaller in size as can be seen in Figure 2. The crystallization pattern was also not as jumbled and cluttered as observed in the slides of previous solutions.

The flask containing the solution, when observed with the naked eye, exhibited much smaller, perfectly square crystals out of solution and there were fewer of them than in previous samples. From these observations, it can be determined that Poloxamer 407 inhibits crystal growth. Because the shape of the crystal was not changed, this surfactant can also be characterized as non-surface-specific which means that it inhibits growth on all surfaces of the crystal and not only on specific faces.

The next sub-study of this research project dealt with aspirin dissolved in hexane. In this case, the control was a flask that contained only hexane, ethanol and aspirin. Aspirin is only slightly soluble in hexane solutions and therefore a small amount of ethanol had to be added in order to dissolve aspirin to form enough crystals for comparison purposes. The crystals that formed were needle-like in shape, macroscopically as well as microscopically, as seen in Figure 3.

The bottom of the flask was also observed to contain a few small, thin, rectangular plates as well as a cover of very fine needles. For the seventh solution, which was the control for the later deviations, more ethanol and aspirin were added to the same amount of hexane. Microscopically, the needles that were found on the slide containing the crystals from this solution were really wide and also present were broken clusters of rectangular shapes. Macroscopically, however, the bottom of the flask exhibited a growth that is not unlike snow in appearance and the needle-like crystals of aspirin were much larger than those in the sixth flask.

The eighth solution differed from the control of this study (the seventh solution) in that it contained Poloxamer 188. Under the microscope the slide of this solution featured crystals in needle form that were smaller and much more defined in shape. Macroscopically, the needles on the bottom of the flask were shorter and wider but still thin in depth. From these observations, the exact effect of Poloxamer 188 on the crystallization of aspirin in hexane cannot be determined and should be a topic for further study.

The ninth solution contained Poloxamer 338 and aspirin dissolved in ethanol and hexane, as before. Microscopically, this particular solution contained rectangular crystals that were very defined and clear in shape, as seen in Figure 4.
Macroscopically, the flask contained crystals that were needles in shape but were significantly longer and more distinct than that of the control. From these observations, it can be inferred that Poloxamer 338 promotes growth in the (0 1 0) plane of the crystal which is responsible for its length.

The tenth solution contained Poloxamer 407 and microscopically, both needles and rectangular shapes were observed on the slide from this flask. Macroscopically, the needle-like crystals were smaller in length and thicker than the control. From these observations it can be determined that this poloxamer inhibits crystal growth in the (0 1 0) plane and promotes it in the (0 0 1) plane of the crystal.

The third sub-study of this project dealt with examining the effect of the addition of different volumes of Tween 80 on the crystallization of aspirin in ethanol. The first solution made in this research project served as the control in this instance. The eleventh solution of this project and the first solution of this sub-study contained 3 mL of Tween 80, the twelfth - 10 mL, and the thirteenth - 20 mL. The eleventh solution exhibited jumbled squares macroscopically, and needles and fractured rectangles microscopically. The twelfth solution had fewer crystals and they were floating in the solution in contrast to all other flasks in which the crystals grew on the bottom of the flask. Microscopically, the crystals were very big and distinct. There were no needles and no huge crystal plate like on the control slide, but rather a multitude of smaller, more distinct plates and their fragments, as seen in Figure 5.

The thirteenth solution exhibited even larger crystals microscopically. Macroscopically, nothing could be observed because the crystals never came out of solution.

From the results of this sub-study it is clear that Tween 80 has not only a definite effect on the crystallization of aspirin in ethanol, but also on its solubility. This surfactant promotes growth on all three of the crystal planes: (1 0 0), (0 1 0), (0 0 1). Thus, the aspirin crystal increased in size in all directions because of the Tween 80. Moreover, the relationship between the amount of surfactant added and the size of the crystals is directly proportional - as one increases, so does the other one. The solubility of aspirin in the solution is also affected by the Tween 80. The solubility greatly increases as the amount of Tween added increases. For example, it took two days for any crystals to come out of the twelfth solution and no crystals came out of the thirteenth solution by the end of this study.

5. Future Work

The results of the experiment have suggested ideas for further research – mainly involving the use of other surfactants, solvents, and crystals besides those used in these experiments. By using different versions of Tween, besides Tween 80, the effects of a larger molecular weight can be seen. Other types of poloxamers with either larger or smaller molecular weights can also be used. Ethanol and hexane were the only solvents used in this research. Thus, other solvents could be used in future experiments to observe the changes they cause in the crystal size and morphology of acetylsalicylic acid.

The surfactant is not the only variable in this experiment that can be changed. Heat control is a variable that can be studied. Experiments can be done in the future using different crystals, both similar to and very different from acetylsalicylic acid, as there is
a large variety of crystalline compounds used in the pharmaceutical industry that could be researched.

In a more general sense, the experiments performed could be more elaborate and thorough, in order to retrieve better results. Only one slide was taken and viewed from each of the samples. If more slides were made of each sample, better statistics can be obtained and the results can be clarified and validated. The conclusions made from the slides would provide a stronger argument if there is a greater number of slides used as evidence.

6. Conclusions

As a result of this study, it was found that there are several surfactants that have a definite effect on the crystallization of aspirin in ethanol and hexane. This study examined the particular effects of Poloxamers 188, 338, and 407 as well as the Tween 80 on crystalline growth of aspirin in two different solvents.

The results of this research indicate that the Poloxamer series is a non-surface-specific surfactant that inhibits crystal growth on all planes in an ethanol solution. Poloxamer 338, on the other hand, was observed to promote crystal growth on the (0 1 0) plane in a hexane solution. Poloxamer 407 behaves differently in ethanol and hexane solutions. This surfactant is non-surface-specific in ethanol and it inhibits growth on all surfaces of the crystal. In hexane however, this poloxamer inhibits growth in the (0 1 0) plane and promotes it in the (0 0 1) plane of the crystal. Tween 80 promotes crystal growth on all planes and also increases the solubility of aspirin in the ethanol solvent.

This research project was very informative for the group and introduced us to chemical engineering and the projects and processes involved in this field. We were able to talk one-on-one with several graduate students in the field and also had access to a professor in the field that helped us with our research.

7. Acknowledgments

We would like to thank Rutgers University for the use of their laboratory, equipment, and chemicals. We would also like to thank those involved in the Governor’s School of Engineering and Technology, for providing us with the opportunity to research this interesting topic. Specifically, Anthony Welch has been a great coordinator for this program and a huge role model for us. There are a few people we would like to thank for their time, energy, and brilliance. Dr. Silvina Tomassone is the professor who began the research on this topic and has been a great resource for us during the research process. We would especially like to thank the graduate students whose commitment to our group has allowed us to learn so much in the field of chemical engineering. They are Frank Romanski, Yangyang Shen, Atul Dubey and Syeda Sharmin Hussain. Lastly, for taking us every day to our laboratory and helping us with our paper, we would like to give a special thanks to our project advisor, Sean DiStefano.

8. References

“Poloxamer.” 20 July 2006 <http://www.fisk.edu/~aburger/Published03_06/Sample_prep/Crystal_growth/crystal_growth.html>.