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Alginamide Synthesis and Emulsion Preparation

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By

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Abstract

The objective of this project was to optimize the synthesis of the alginamide polymer and investigate its use as a stabilizer in oil/water emulsions and calcium hydrogels. Two modifications were made to the synthesis in attempts to overcome the undesirable secondary cross-linking reaction. The first modification tested was a reduction of reaction time from 24 hours to 1 hour. The second modification tested was the addition of a hydrolysis step with NaOH. Elemental analysis showed that when reducing the reaction time the percent alkylation was also affected. Rheological results confirmed that the added hydrolysis step did reduce the amount of cross-linking present. Thirty-six emulsions were tested for initial particle size and stability. Of these 36 emulsions only 17 contained the desired submicronic particles. The smallest and most stable emulsion particles resulted from the alginamide with a 24 hour reaction time and added hydrolysis step. It was also determined that emulsifying a solution with 50% oil leads to quick visual signs of oil separation. This conclusion encourages further investigation of alginamide for use in applications requiring biocompatible polymers.

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1.0 Introduction

Polymers, such as plastics and rubbers, have proved to be something that we rely on in our everyday lives. Due to a growing number of innovative applications, there is a growing need to polymers which are biocompatible. These biocompatible polymers are needed to replace functional parts of the body or to function in intimate contact with living tissue. Biocompatible polymers have already been developed for uses such as artificial joints, engineered tissues and even contact lenses.

Currently, biocompatible polymers, suitable for implantation in the brain, have proved to be successful in creating scaffolds for tissue growth. Such polymers, when chemically cross-linked, create a hydrophilic insoluble hydrogel network. Recent studies have shown that hydrogels, due to their resemblance to a biological tissue, are a promising research path for implementation in the human body. Specific hydrogels, polyhydroxyethyl- methacrylate (pHEMA) and poly – N (2-hydroxypropyl) – methacrylamide (pHPMA) have been successful in implantation experimentation (Lesny, 2002).

Researchers at the Ecole Nationale Superieure Des Industries Chimiques (ENSIC) are interested in incorporating the direct administration of medication by way of the polymer network or "implanted tissue". Their current challenge is to develop a biocompatible polymer network in which essential drugs can be encapsulated and directly delivered. The theory being that the direct administration and controlled release will hopefully increase the success rate of tissue implantation (Martin, 2006).

ENSIC has begun investigating the networking, stabilizing and encapsulation properties of the naturally occurring alginate polymer. Alginate, which has been widely used in the food and pharmaceutical industries, looks very promising due to its biocompatibility, biodegradability and its ability to form cross-linked polymer networks. In initial work with alginate, ENSIC has encountered an undesired cross-linking reaction which occurs when modifying the alginate polymer. The goal of our research was to optimize the synthesis of the alginamide polymer and investigate the use of the consequent products in oil-in-water emulsions and calcium hydrogels.

2.0 Background

For the past 18 years, ENSIC has been investigating drug delivery methods involving the use of hydrophobically modified polysaccharides (Durand, 2004). The majority of these efforts have involved the use of dextran polymers. Our research, completed in collaboration with ENSIC, involved the use hydrophobically modified alginate polymers. Alginate polymers were researched by the previous WPI students, although our research takes a different approach (Brunetti, 2006).

This section provides information that is essential to understanding the need for this research, as well as background information on alginate polymers and the proposed use of alginamide to create stabilized emulsions and hydrogels.

2.1 Alginate

Since it was discovered in 1880 by a British Chemist named E. Stanford, alginate has proved to have many uses. Fifty years after its discovery, it began to be commercially produced as a food additive and in 1934 started being used as a stabilizer in ice cream. Since then alginate has been used in many commercial products such as juices, salad dressings, cosmetics, waterproofing materials and fireproofing fabrics. Due to alginates hydrophilic nature and ability readily absorb large amounts of water it is also useful as an additive in dehydrated products such as slimming aids, manufacturing paper and textiles.

Alginate, which is a naturally occurring nontoxic copolymer, is extracted from seaweed, including the giant kelp *Macrocystis pyrifera*, *Ascophyllum Nodosum* and various types of *Laminaria*. It can also be extracted from some types of bacteria. Due to its nontoxicity and biocompatibility, it is widely used in the pharmaceutical industry for cell demobilization and encapsulation.

Commonly in the form of an acid, alginate is a viscous gum that is abundant in the cell walls of brown algae. Chemically, alginate is a linear copolymer with homopolymeric blocks of (1-4)-linked ß-D-mannuronate (M) and its C-5 epimer α-L-guluronate (G) residues, respectively, covalently linked together in different sequences or blocks. The monomers can appear in homopolymeric blocks of consecutive G-residues

(G-blocks), consecutive M-residues (M-blocks), alternating M and G-residues (MG-blocks) or randomly organized blocks, see Figure 1.

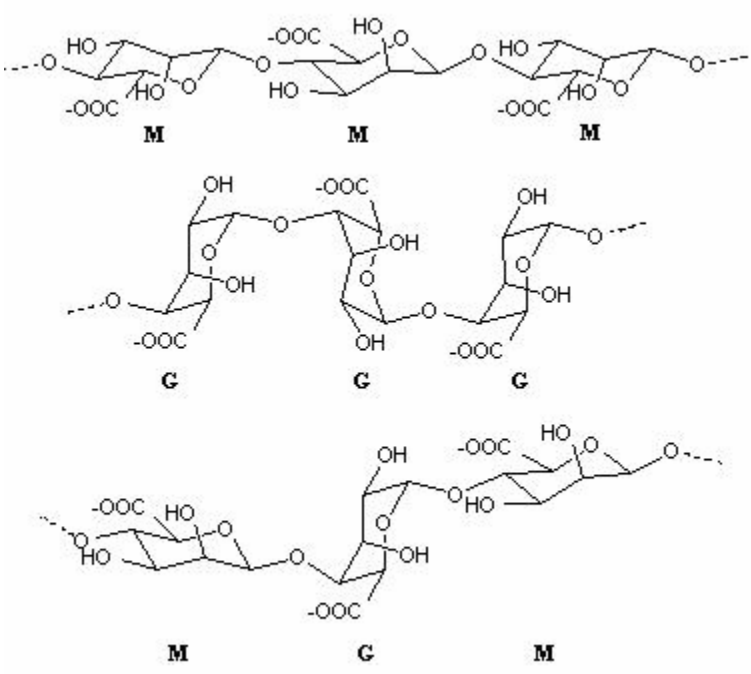


Figure 1. The M and G residues that form alginate polymers
http://www.kjemi.uio.no/Polymerkjemi/Research/alginate.jpg

The chemical compound sodium alginate is the sodium salt of alginic acid with an empirical chemical formula of $NaC_6H_7O_6$.

2.2 Modified Alginate

Alginate is a "water loving" polymer. When placed in water alginate readily absorbs large amounts of water which is ideal for its gelling and thickening applications. Although, when the desired use is as a stabilizer the properties must be altered. In order for alginate to function as a stabilizer in oil-in-water emulsions, it must be modified with hydrophobic chains. In attaching hydrophobic chains, alginate is able to possess hydrophilic and hydrophobic properties. The addition of hydrophobic chains can allow the polymer to hydrophobically associate in an aqueous solution (Pelletier, 2000).

Alginate can be made amphiphilic by the addition of alkyl chains attached with various types of linkages such as ester or amide. Researchers at ENSIC have determined that amide attached alkyl chains are preferred for biological applications. Ester attached alkyl chains would be rapidly hydrolyzed in physiological media. The amide functions allow the alkylated polymer to be stable for long periods of time (Leonard, 2007).

It also has been determined that in alkylating the alginate with amide functions a secondary cross-linking reaction occurs. This cross-linking is believed to be hindering the polymers ability to function as a stabilizer (Martin, 2006). Figure 2 illustrates the attached alkyl chains and secondary cross-linking.

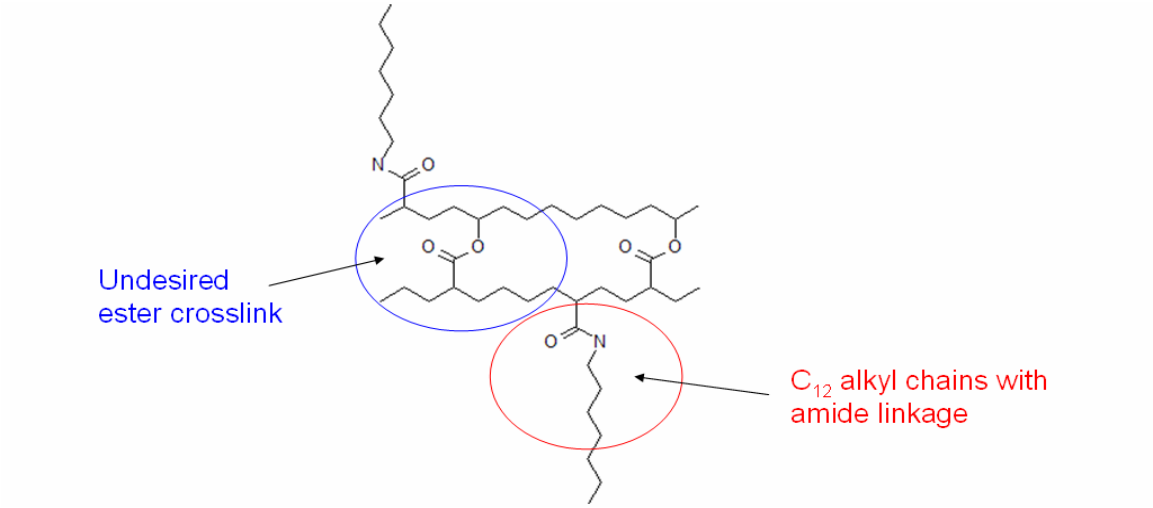


Figure 2. Alkylated alginate polymer with undesired ester crosslink

2.3 Emulsions

Emulsions, which contain two immiscible liquids, are unstable mixtures that do not spontaneously form. The colloidal system, one phase, consists of a dispersed phase and a continuous phase.

The heterogeneous mixture requires the input of energy in order to create stable mixture of the two phases. This process is called emulsification. Two immiscible substances commonly used are oil and water. A prime example of this situation is oil and

vinegar salad dressing. The oil and water will combine when shaken vigorously but when allowed to sit the two immiscible liquids will visibly separate, as seen in Figure 3.

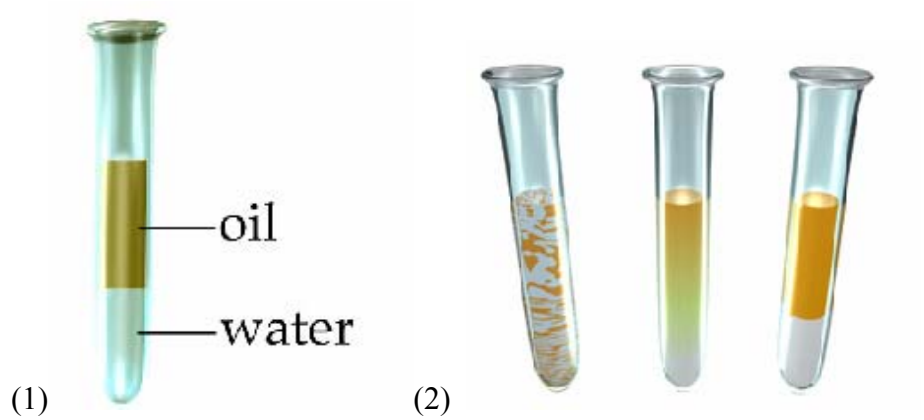


Figure 3. (1). The two phases present in an oil-water emulsion. (2) a. An emulsified oil-water emulsion. b. The beginning separation of an unstable emulsion. c. The separated liquids.
simscience.org/membranes/advanced/page3.html

The undesirable separation seen in many emulsions can be altered by the use of emulsifiers also known as surfactants. Emulsifiers assist in stabilizing emulsions by lowering the surface tension between the two liquids. As seen in Figure 4, the surfactant surrounds select oil droplets hence stabilizing the interface between the oil and water.

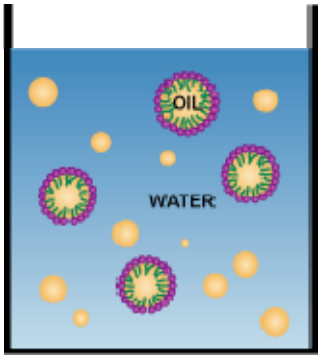


Figure 4. Encapsulated oil particles in a oil in water emulsion
http://www.specialchem4coatings.com/tc/wax/index.aspx?id=emulsions

The liquid-liquid interface of emulsions requires the input of energy to form stable or even unstable emulsions. Due to the immiscibility of the two liquids, they will not combine spontaneously. The most common methods of preparation include vortexing and sonciation.

Once prepared, emulsions can be characterized in many ways such as by particle sizing, rheology, stability, viscosity, density and separation. There are many characteristics which can influence the properties of an emulsion. Important characteristics include droplet size, surfactant concentration, oil-water ratio, time, temperature, and pH.

The changes that are observed after emulsions are prepared can be referred to as emulsions aging. Two important changes that can affect emulsion properties are flocculation and coalescence. Flocculation refers the aggregation of the formed droplets. Flocculation is then followed by the coalescence of particles. Coalescence is joining of droplets which results in an increase in particle size.

2.4 Hydrogels

Hydrogels are hydrophilic three-dimensional networks which have a soft consistency and highly resemble biological tissues. These amphiphilic networks are able to absorb large amounts of water. The swelling ratio of hydrogels can be affected with alterations in pH, temperature, ionic strength and electromagnetic radiation.

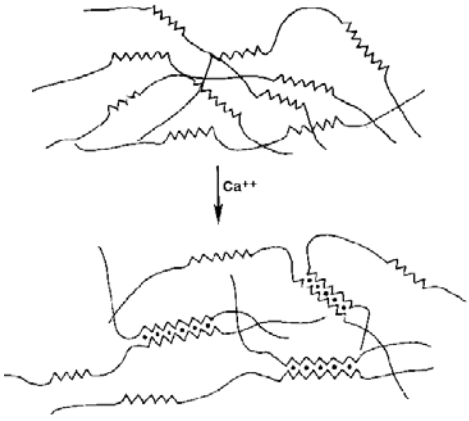


Figure 5. Formation of hydrogels through the addition of Ca+2
www.fao.org/docrep/X5822E/x5822e04.htm

Another important property of hydrogels is their insolubility when cross-linked. The addition of divalent cations, such as Ca^{2+}, Cu^{2+}, Zn^{2+}, Mn^{2+}, to solutions of sodium alginate cause cross-linking to occur throughout the polymer chains (Rastello De

Boisseson, 2004). The cations exchange with the Na+ ions causing the formation of the cross-linked polymer and the resulting "egg-box" which can be seen in Fig 5.

Another important aspect of alginate hydrogels is there mechanical strength. By altering the molecular weight or ratios of M/G residues the strength and viscosity of the gel can be altered (Rastello De Boisseson, 2004).

2.5 Biomaterials

Biomaterials function in intimate contact with living tissues. There are a range of materials which are considered to be biomaterials, such as metals, alloys, ceramics, and synthetic or naturally occurring polymers. It regards to this work it is important to examine the potential uses of biocompatible polymers. Popular fields where biopolymers are currently being used are tissue engineering and drug delivery systems.

Tissue Engineering

Tissue engineering is a promising field which combines biopolymer engineering and surface chemistry (Woerly, 1996). Engineers in this field aim to create synthetic biomaterials that can emulate the functions of damaged human tissues. Hydrogels have proved to be successful scaffolds on which tissue growth can be promoted. Two synthetic hydrogels, polyhydroxyethyl-methacrylate (pHEMA) and poly *N*-(2-hydroxypropyl)-methacrylamide (pHPMA), have been successful *in vitro* (Lesny, 2002). Other polymers such as polylactic acid and polyglycolic acid have been researched due to their being biodegradable (Kuo, 2001) Biocompatible polymers, which are also biodegradable, have shown positive results in various tissue engineering applications and will continue to be a very well researched area.

Drug Delivery

Biomaterials are being widely used in the field of drug delivery. Particularly biocompatible polymers are being designed to function as inert, triggered response, and controlled release delivery vehicles. Alginate when modified can be used in the formation of emulsions and hydrogels. Hydrogels have proved to be popular vehicles for drug delivery because drugs can be trapped during hydrogel polymerization.

3.0 Methodology

Characterization of Starting Material

The sodium alginate which was used in all synthesis was obtained from Sigma, France. The alginate was characterized by SEC-MALLS: molecular weight ($M_{w)}$ = 273,000 g/mol, molecular number ($M_{n)}$ = 159,000 g/mol, and polydispersity index ($I_{p)}$ = 1.7. This particular alginate was chosen due to its high molecular weight. Rheological flow testing was also completed to obtain the viscosity behavior as a function of shear rate. Rheology was chosen as a method of characterization because it shows the polymers shear thinning region and is helpful in determining relative viscosities. This method of characterization was used throughout the remainder of the project to determine the relative success of modification of the alginate's mechanical behavior. The rheological flow testing was completed on an AR2000 Rheometer from TA Instruments. The geometry used for the majority of testing was the concentric cylinder. The following conditions were manually set in the "run parameters" for the flow testing.

Pre-shear	1 Pa; 10 min
Equilibration	10 min
Pressure Range	0.1 – 50 Pa
Tolerance	10%

Table 1. Parameters to be changed for flow rheology testing.

All of the other parameters were pre-set in the software and were not altered. All further rheological testing followed this method unless otherwise stated.

3.1 Optimization of Alginamide Synthesis

Our goal was to optimize the synthesis in order to obtain an alginamide with the best encapsulation properties. Researchers in the Laboratoire de Chimie Physique Macromoleculaire (LCPM) at ENSIC recently discovered an undesired side reaction occurring during the alkylation of the alginate. The reaction resulted in cross-linking

between the alginate chains that would in turn hinder the encapsulation capabilities of the polymer. This work contains the attempts made to prevent or decrease such cross-linking.

3.1.1 Synthesis of Alginate TBA Salt

The alginate tetrabutylammonium (TBA) salt used in all experiments was prepared by first acidifying a sodium alginate solution followed by neutralization with TBAOH. A solution of 5.0 g sodium alginate (M_w = 273,000 g/mol) in 100mL of ethanol (70%) with 5mL HCl (12N) was stirred in an ice bath at 4°C for 30 minutes. The solution was then filtered and washed with 0.5L of ethanol (70%) and then with 300mL of acetone. The product was dried in a vacuum for 2 hours. A sample of the dried product was titrated with NaOH to determine the percent acidification. It was determined that 62% of the sodium ions were acidified and 38% remained untouched.

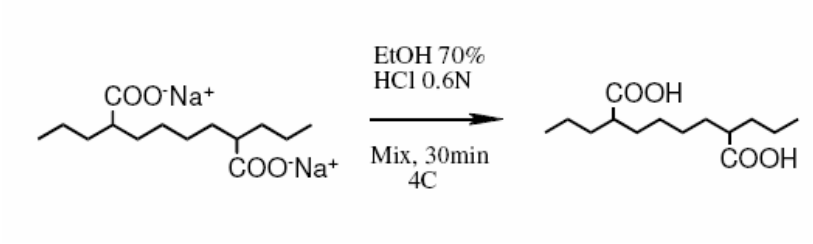


Figure 6. Synthesis of acidified alginate

The acidified alginate (4.14g) was then dissolved in 450mL of H_2O and allowed to stir for 1 hour. TBAOH (0.15M) was then titrated into the alginate solution until a pH of 7 was obtained. The neutralized solution was then divided into 2 flasks, frozen and lyophilized for 2 days. The product was collected and massed at 7.35g, a 70% yield. This synthesis was also completed a second time to obtain 7.64g, a 73% yield. This second round of product was also 62% acidified. The alginate TBA was characterized by SEC-MALLS: M_w= 173,000 g/mol, M_n = 90,000 g/mol, and I_p = 1.9. The drop in molecular weight can be attributed to partial degradation of the alginate by TBAOH.

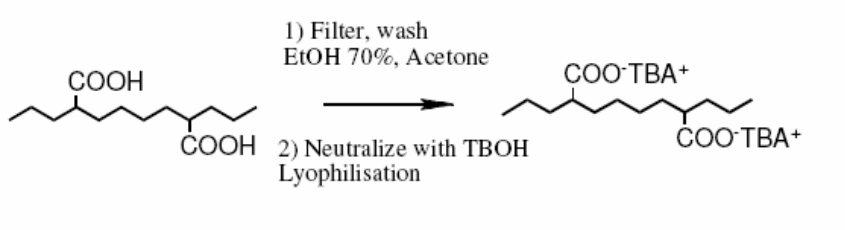


Figure 7. Synthesis of Alginate TBA

3.1.2 Synthesis of Alginamide

In attempts to examine the alginamide and undesired crosslinking, two different reactions were carried out. The first synthesis was performed in order to obtain the alkylated alginate and the cross-linked alginate side product, while the second reaction was carried out to obtain solely the cross-linked alginate. The same reaction conditions were used for each synthesis.

3.1.3 Synthesis of Alginamide under 45min/ 24hr Conditions

The alginate TBA salt (2g), dodecylamine (1.11g), triethylamine (0.607g), and 2-chloro-1-methylpyridinium iodide (CMPI, 0.535g) were dissolved in 350mL of dimethylformamide (DMF). The solutions were pre-cooled for 1 hour and added simultaneously. The solution was then allowed to react in an ice bath for 45 min followed by ambient temperature for 24 hours. The reaction was stopped after the 24 hours by the addition of 60ml of NaCl (2.5M). The product was then precipitated with approximately 300 ml of ethanol (96%), filtered, washed with 0.5L ethanol (70%), and placed in a vacuum overnight.

The alkylated alginate recovered (0.623 g) was divided into three samples of 0.206 g, 0.206 g, and 0.209 g. The samples were dissolved in 50 mL H_2O, 50 mL NaOH (10^{-3} M, pH 11.0), and 50 mL NaOH (10^{-4} M, pH 10.0) respectively. The pH of the NaOH samples was measured after 24 hours of dissolution and was adjusted back to 10.98 and 10.13 by the addition of NaOH (1M). The samples were then dialyzed against de-ionized water in membrane tubing for 6 hours with the water being changed every 2hours. Following dialysis the samples were frozen with liquid nitrogen and lyophilized.

The samples were then dissolved in water, stirred overnight and analyzed on the rheometer in 0.5% solutions. A 0.5% sample of the starting sodium alginate was also analyzed for relative comparison purposes. The conditions mentioned earlier for flow testing with the concentric cylinder were used. In addition, observations were made on each sample's solubility based on visual inspection.

3.1.4 Synthesis of Crosslinked Alginate under 45min/ 24hr Conditions

The alginate TBA salt (2g) and CMPI (0.536g) were dissolved in 350mL of DMF. The solution was allowed to react in an ice bath for 45 min and then at ambient temperature for 24 hours. The reaction was stopped after 24 hours by the addition of 60mL of NaCl (2.5M). The product was then precipitated with approximately 300 ml of ethanol (96%), filtered, washed with 0.5L ethanol (70%), and placed in a vacuum overnight. The recovered reference product (3.86g) was divided into three samples of 1.27g, 1.27g and 1.26g. The samples were dissolved in 50 mL H_2O, 50 mL NaOH (10^{-3} M), and 50 mL NaOH (10^{-4} M) respectively. The pH of the alginate in NaOH solutions were measured after a 24 hour dissolution period and additional NaOH (1M) was added to bring the pH to 11.10, and 9.95. The samples were then dialyzed against de-ionized water for 6 hours, frozen, and lyophilized. In order to determine the molecular weights and solubilities in water, the samples were prepared for size-exclusion chromatography (SEC). The three products were dissolved in 5mg/mL $NaNO_3$ solutions.

3.1.5 Synthesis of Alginamide under 1hr Conditions

After examining the 45min/24hr product, a decision was made to examine the effects of carrying out the reaction at a shorter length. We were interested to see if shortening the length of the reaction would effect the % alkyl substitution. We also wanted to see if the cross-linking was also conducted within the first hour of combining the solutions. The only condition of the alginamide reaction (seen in section 3.1.3) that was altered was the length. The solutions were mixed and allowed to react in ice for 1 hour. After the hour, the reaction was immediately stopped by the addition of NaCl. The remaining steps and characterization described in Section 3.1.3 were then carried out.

3.1.6 Synthesis of Crosslinked Alginate under 1hr Conditions

It was also decided to synthesize the cross-linked alginate under 1 hour conditions. We wanted to see a reduction in time affected the amount of cross-links formed. The synthesis of the cross-linked alginate was also carried out under the 1 hour condition. The synthesis was then completed as described in Section 3.1.4.

3.2 Synthesis of Alginamide for Emulsions

The next phase of research involved using the synthesized alginamide as a surfactant in oil-in-water emulsions. In the attempts to synthesize an alginamide with less cross-linking, four different products were made. Hence, all alkylated alginates, the 45min/ 24hr hydrolyzed and unhydrolyzed as well as the 1hr hydrolyzed and unhydrolyzed, obtained up until this point were tested as surfactants in oil-in-water emulsions.. Due to low remaining amounts of product, each was resynthesized for use as a surfactant. Each was synthesized under the same conditions but with the addition of the hydrolysis step.

3.2.1 Second Synthesis of 45min/24hr Alginamide, Hydrolyzed and Unhydrolyzed

The 45min/24hr alginamide was synthesized under the same conditions described in Section 3.1.3. Next, it was decided that NaOH would be used in an attempt to hydrolyze the ester cross-links. It was desired that the base would hydrolyze the cross-links while not altering the attached alkyl chains. In order to qualitatively complete this, the product was then split into 2 samples, one of which was set aside for use in emulsion preparation and the other to be hydrolyzed with NaOH. The latter sample (1.00g) was dissolved in 100mL NaOH 10^{-3} M (pH 11.17). The solution was allowed to stir for approximately 15 min. After stirring, the pH was found to have dropped below 11. Additional NaOH 10^{-3} M was added until the pH reached 11 again. The solutions were then stirred overnight.

After 24 hrs of stirring the solution was dialyzed for 24hrs. The water was changed every 2 hrs for the first 6 hrs. Next, the sample was frozen and freeze dried for 48hrs. The final hydrolyzed product was massed at 0.97g.

3.2.2 Second Synthesis of 1hr Alginamide, Hydrolyzed and Unydrolyzed

The 1 hr alginamide was synthesized under the same conditions described in Section 3.1.4. As with the 24hr product, an attempt was made to hydrolyze the ester cross-links. The hydrolysis, dialysis, and drying procedure were carried out under the same conditions described in Section 3.2.1.

3.2.3 Characterization of Products to be used in Emulsion Preparation

The four products seen in Table 1 were characterized by flow and oscillatory rheological testing as well as elemental analysis.

4 Sets of Conditions to be Characterized and Examined with Emulsions
45min/24hr synthesis, unhydrolyzed
45min/24hr synthesis, hydrolyzed
1hr synthesis, unhydrolyzed
1hr synthesis, hydrolyzed

Table 2. Four products characterized and used in oil-in-water emulsions.

Characterization of these products was important to determine what differences, if any, exist between the 24 and 1 hour products and the hydrolyzed and unhydrolyzed products. The flow testing was conducted using the parallel plate geometry and 2% solutions in water. The concentric cylinder was not used in this case due to the high viscosities of the products. After the flow test run was completed, the sample was left in place and allowed to equilibrate for 20 min in preparation for the oscillatory test.

In addition to rheological testing, approximately 20mg of each product was sent out for elemental analysis. Elemental analysis was chosen as the method to be used in determining the number of alkyl chains attached to each alginate molecule. The results would give the % Carbon and % Nitrogen found in the sample. These numbers would

then help in identifying each alkyl chain due to the presence of Nitrogen in each amide linkage.

3.3 Emulsions and Hydrogel Preparation

Although alginate emulsions were examined by the previous WPI students, little was known about alginamide emulsions and their hydrolyzed products (Brunetti, 2006). Various alginate concentrations and oil percentages were examined to gain some knowledge on particle sizes, emulsions stability and oil separation.

The oil used for emulsion preparation was Miglyol 810 (Sasol-Condea, Belgium). This oil was chosen due to its low toxicity and biocompatibility. Different ratios of oil to water were examined. The four products seen in Figure XX (Section 3.2.3) were prepared in water concentrations of 0.5%,1% and 2%.

In examining these 4 products at 3 different concentration and 3 oil percentages, a matrix of 36 emulsions was created. This matrix can be seen in Table 2.

Emulsion #	Concentration of Product	% Oil		Emulsion #	Concentration of Product	% Oil
A - Unhydrolyzed				A - Hydrolyzed		
1	0.5%	10%		19	0.5%	10%
2	0.5%	20%		20	0.5%	20%
3	0.5%	50%		21	0.5%	50%
4	1%	10%		22	1%	10%
5	1%	20%		23	1%	20%
6	1%	50%		24	1%	50%
7	2%	10%		25	2%	10%
8	2%	20%		26	2%	20%
9	2%	50%		27	2%	50%
B - Unhydrolyzed				B - Hydrolyzed		
10	0.5%	10%		28	0.5%	10%
11	0.5%	20%		29	0.5%	20%
12	0.5%	50%		30	0.5%	50%
13	1%	10%		31	1%	10%
14	1%	20%		32	1%	20%
15	1%	50%		33	1%	50%
16	2%	10%		34	2%	10%
17	2%	20%		35	2%	20%
18	2%	50%		36	2%	50%

Table 3. The 36 emulsions prepared and particle sized.

Each 0.5% and 1% emulsion was vortexed for 1 min on a Yellow Line vortexer at a speed of 2500 rpm. The oil/water solutions were then sonicated to further stabilize each solution. A Bioblock Scientific Vibra- Cell emulsifier was used to agitate each solution with high frequency sound waves. The sonicator probe was inserted into each conical tube to the same distance for each emulsion. The probe was also carefully placed in the center of the tube as to not touch any of the sides. A beaker of cold water was placed around the vial to absorb any produced heat. Each oil/water solution was sonicated for 3 minutes at a power level of 3 and a 50% active cycle. These conditions were held constant for the 0.5% and 1% emulsions and altered for the 2% emulsions. After each emulsion (10mL) was prepared, particle size and daily oil separation data was obtained.

Some difficulties were encountered when preparing the 2% alginate emulsions. When preparing the first 2% emulsion under the conditions used for the 0.5 and 1%, it was noticed that the emulsion was very unstable; there was visible amounts of oil not encapsulated. It was then decided that it would be necessary to re-vortex and re-sonicate each 2% emulsion. Therefore, each 2% emulsion was vortexed (1min), sonicated (3min), vortexed (1min), and sonicated (3min).

3.3.1 Particle Size Data

After preparing each emulsion, it was necessary to gain important particle size data as soon as possible. Our goal was to obtain initial particle size data as well as to re-particle size each emulsion 5 days later. These measurements would be a direct relation to each particle's stability over a 5 day period. A Malvern Instrument High Performance Particle Sizer (HPPS) was used for all particle size measurements. Each analyzed sample was made using 15 µl of emulsion and 3 ml of NaCl (10^{-3} M). Once combined, the sample was mixed with a pipette and any signs of bubbles were removed.

At the conclusion of each run, the HPPS provides a polydispersion index (PDI) and count rate for each run. For reliable HPPS results, it is important to monitor the PDI. The PDI should be under 1 and preferably around 0.1 to 0.2. Large PDI's such as 1 show that the sample has a broad size distribution. All particle size data can be seen in Appendix 7.1 and 7.2.

Due to the lack of previous data with alginamide emulsions, there were limited expectations for the particle sizes. The previous MQP was consulted to obtain a range of particle sizes that might be observed although they utilized a different modified alginate. In meeting with researchers at ENSIC, it was determined that submicronic particle sizes, particles under 1 micron, would be the most desirable.

3.3.2 Emulsion Oil Separation

After each emulsion was formed, a 4ml sample was set aside for oil separation observations. The 4ml sample was placed in a 5ml graduated plastic test tube. Observations were made daily for any signs of oil separation. Daily observations can be found in Appendix 7.3.

3.3.3 Hydrogel Oil Separation

In addition to observing emulsion oil separation, a hydrogel was formed and also monitored for oil separation. A 5 mL sample of each emulsion was used to prepare a Calcium alginate hydrogel network. The hydrogel was prepared by adding 10 mL of $CaCl_2$ (0.1M) to the 5 mL of emulsion. The mixture was then shaken and transferred to a glass test tube. Oil separation observations were then taken an hour after the hydrogel was placed in the test tube and also taken daily for the next 5 days. Daily observations can be found in Appendix 7.4.

4.0 Results and Discussion

4.1 Optimization of Alginamide Synthesis

4.1.1 Rheology Flow Testing

The goal of the optimization of the alginamide synthesis was to reduce or avoid the secondary cross-linking reaction. Rheological testing was chosen as a method of characterizing the products and determining the success of altering the amount of crosslinks. The exact viscosity of each sample was not important. Instead, knowing the viscosities relative to each other was most important. For example, if the viscosity of a hydrolyzed sample was found to be lower than the unhydrolyzed sample, this would be considered a way of stating that amount of cross-linking was affected with hydrolyzation.

Alginamide vs. Hydrolyzed Alginamide (NaOH 10^{-3}M)

The flow plot of the unhydrolyzed alginamide was compared to the flow plot of the alginamide hydrolyzed with NaOH 10^{-3} M. This comparison was made to see if the hydrolysis of the alginamide caused any decrease in viscosity. A decrease in viscosity could then be attributed to hydrolysis of the secondary cross-links. The plot seen in Figure 8 shows the unhydrolyzed alginamide having a viscosity between 1 and 0.1 Pa/s. The alginamide hydrolyzed with NaOH, seen in Figure 9, is shown having a viscosity of less than 0.1 Pa/s. The two products compared were both synthesized under the same reaction conditions. The only difference existing between the two was the hydrolysis of the second with NaOH 10^{-3} M. The decrease in viscosity was a direct result of the hydrolysis. This shows hydrolysis does affect the secondary cross-links which exist in the alginamide.

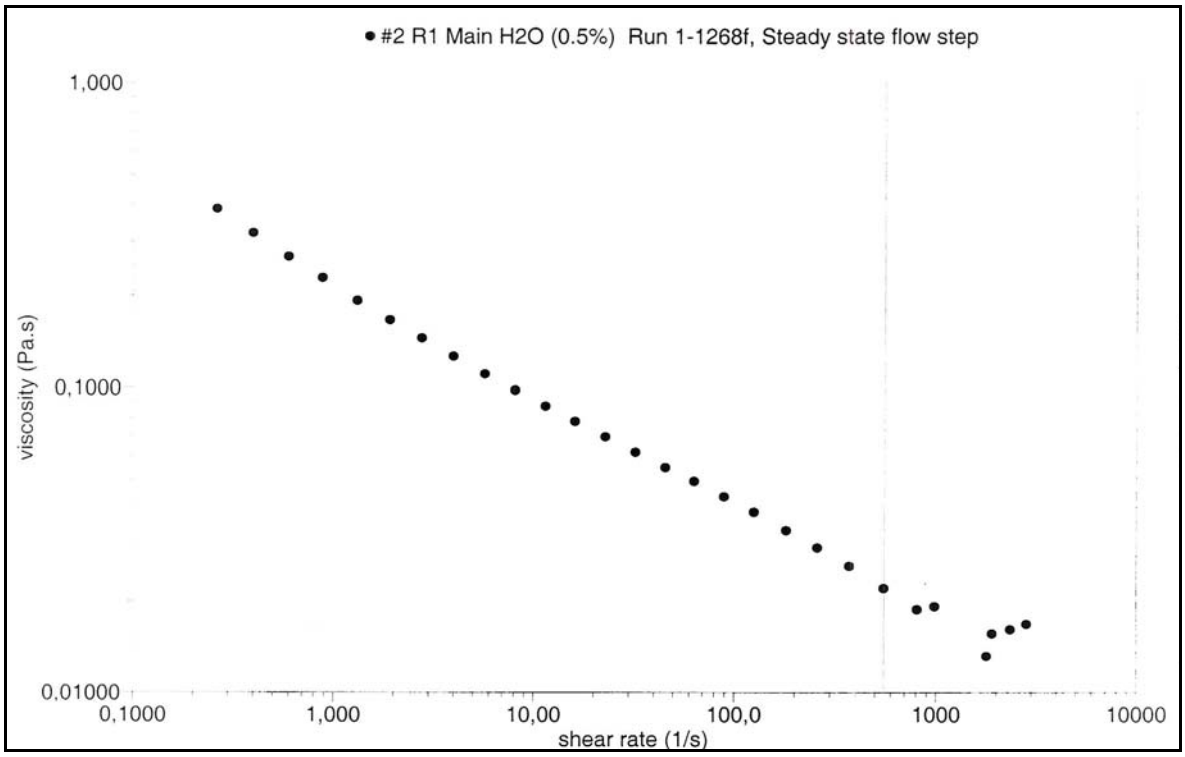


Figure 8. Rheological flow plot of alginamide at 0.5%

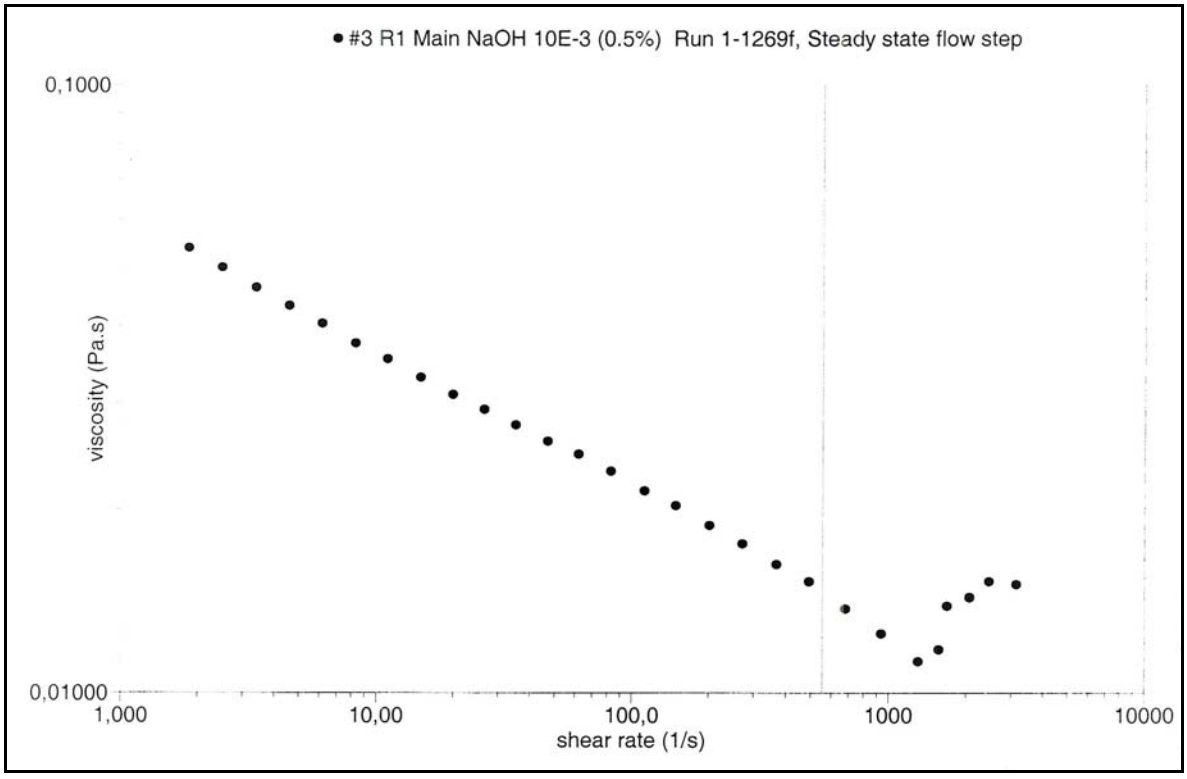


Figure 9. Rheological flow plot of alginamide hydrolyzed with NaOH

4.1.2 Elemental Analysis

Elemental analysis was used to determine the number of C_{12} chains attached in the alkylation reaction. Due to the amide linkage of each alkyl chain, the ratio of carbon to nitrogen led to the determination of the number of successfully attached C_{12} chains. The absolute values of C and N were not used due to suspected amounts of water trapped in the samples. In order to determine the substitution ratio of the alginamide the following equation was used.

$$\frac{C}{N} = \left[\frac{(6)((100-T)+(18(T)))(12)}{14(T)} \right]$$

Equation 1. Substitution ratio of carbon to nitrogen

In this equation, *C* and *N* are the percent of carbon and nitrogen present and *T* is the percentage of modified units per 100 units of alginate. A ratio of *C/N* was used rather than the absolute value of *C* and *N* because it was probable that water was in trapped in the sample which would alter the values of *C* and *N*. The calculated substitution ratios can be seen in Table 4.

Sample	C %	N %	% substitution
24 hr in H_2O	36.0	1.5	32.0
24 hr in NaOH (10^{-3} M)	39.1	1.5	31.5
24 hr NaOH (10^{-4} M)	36.7	1.4	32.0
1 hr H_2O	34.0	0.9	17.0

Table 4. Substitution ratios for the four alginamide samples

The elemental analysis data was very helpful in determining if hydrolysis affects the attached alkyl chains as well as if reducing the reaction time to 1 hour effects the amount of alkyl chains substituted. It can be seen that the alkylated alginate which was synthesized under the 24 hour conditions and was not hydrolyzed had a percent

substitution of 32. Next, it can be seen that when hydrolyzing that sample with NaOH 10^{-3} M or 10^{-4} M, the percent substitution was not effected. This means that hydrolysis with either molarity of NaOH does not effect the alkyl chains attached with amide linkages.

The next important result can be drawn from comparing the 24 hr product to the 1 hr product. Here you can see that the percent substitution dropped from 32 to 17 with the reduction in reaction time. This means that in reducing the reaction length the number of attached alkyl chains will be affected.

4.2 Characterization of Alginamide Products

4.2.1 Rheological Flow Testing

As with the first round of rheological flow testing, the rheometer was used to determine any relative changes in viscosity. It was hoped that the each hydrolyzed product would have a reduced amount of cross-linking and hence a lower viscosity. Unfortunately, the results did not show any differences in viscosity. The plots were all similar to the one seen in Figure 10. It was determined that no statement on the successfulness of hydrolyzation in reducing cross-linking could be made based on these results. This may have been attributed to the increase in polymer concentration to 2%.

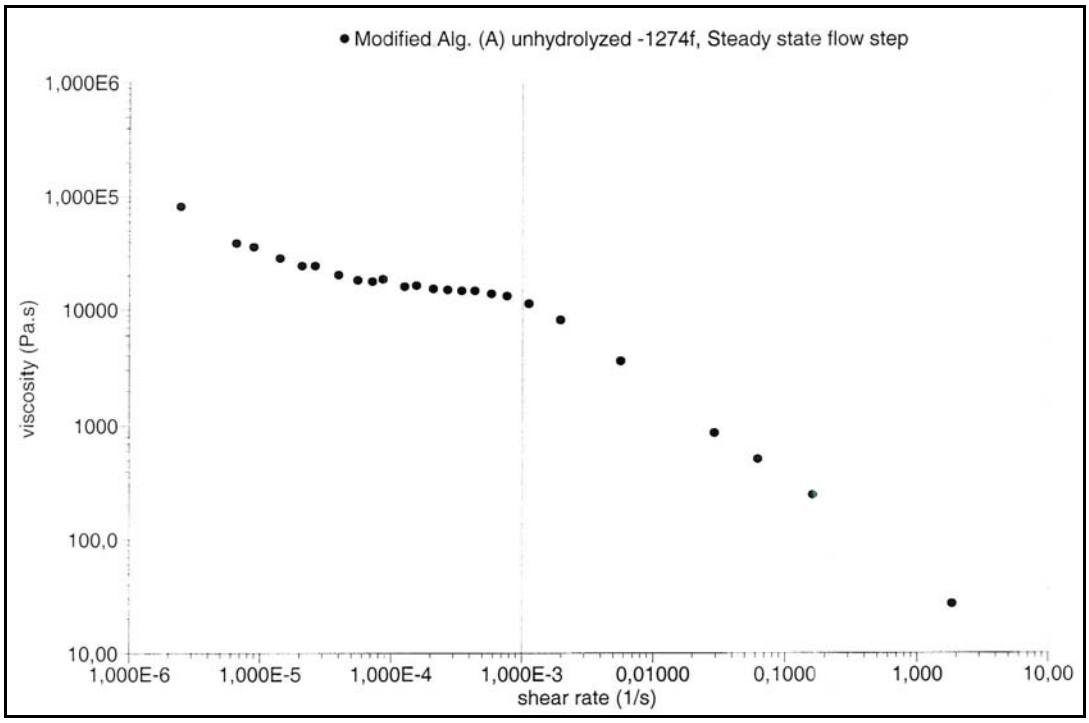


Figure 10. Flow rheology plot for 24 hr unhydrolyzed

4.2.2 Viscosities from Visual Observations

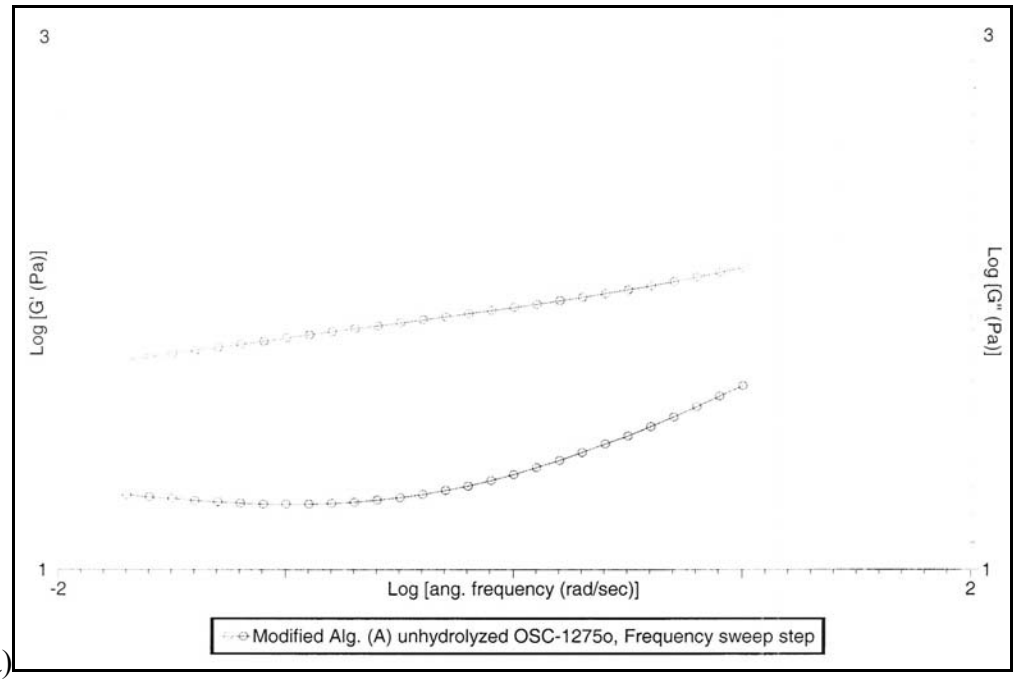
Although rheology was unable to determine any differences in the viscosities of the products, some visual differences were observed. In examining the visual movement and flow of each 2% solution, it was observed that differences did exist. The 24 hr unhydrolyzed product appeared to be the most viscous of the three solutions. The 24 hr hydrolyzed product appeared to be less viscous than its corresponding unhydrolyzed product. The 1 hr products appeared to be less viscous than the 24 hr products with the 1 hr hydrolyzed product being the least viscous of the four. These visual observations led us to think that rheological flow testing might not be the best way to measure differences in cross-linking for these highly viscous solutions. Possibly aqueous solutions of 0.5% or 1% may have been more successful at highlighting the differences between the samples.

4.2.3 Rheological Oscillatory Testing

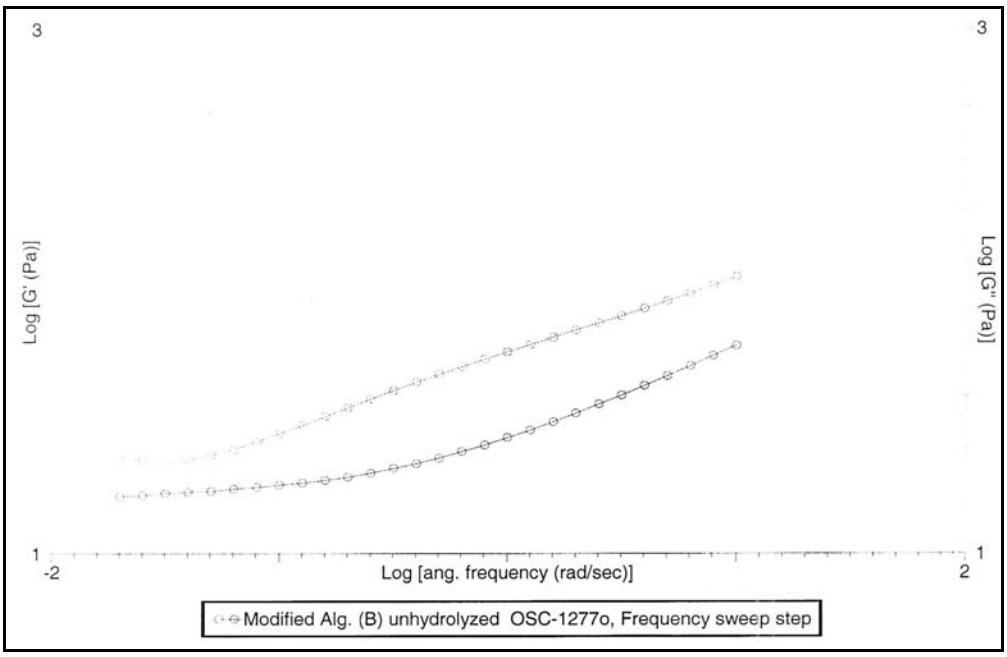
Unlike rheological flow testing, rheological oscillatory testing was able to highlight difference between the samples. Oscillatory testing was able to distinguish if a sample had a more pronounced viscous behavior or elastic behavior. The plots obtained were used to qualitatively compare the differences in these behaviors or the two samples.

The 24 hr unhydrolyzed product (Figure 11 a) showed strong gel-like behavior. The elastic modulus (G') portion of the product was independent of frequency. This behavior is a characteristic of high viscosity. The 1 hr unhydrolyzed product (Figure 11 b) showed weaker gel-like behavior due to the proximity of elastic modulus and viscosity line (G"). The 24 hr hydrolyzed product (Figure 11. c) also had a G' that was independent of frequency. Although, when compared to the 24 hr unhydrolyzed product there was a shift to less gel-like behavior. The 1 hr hydrolyzed product (Figure 11 d) also showed a down shift compared to the unhydrolyzed product.

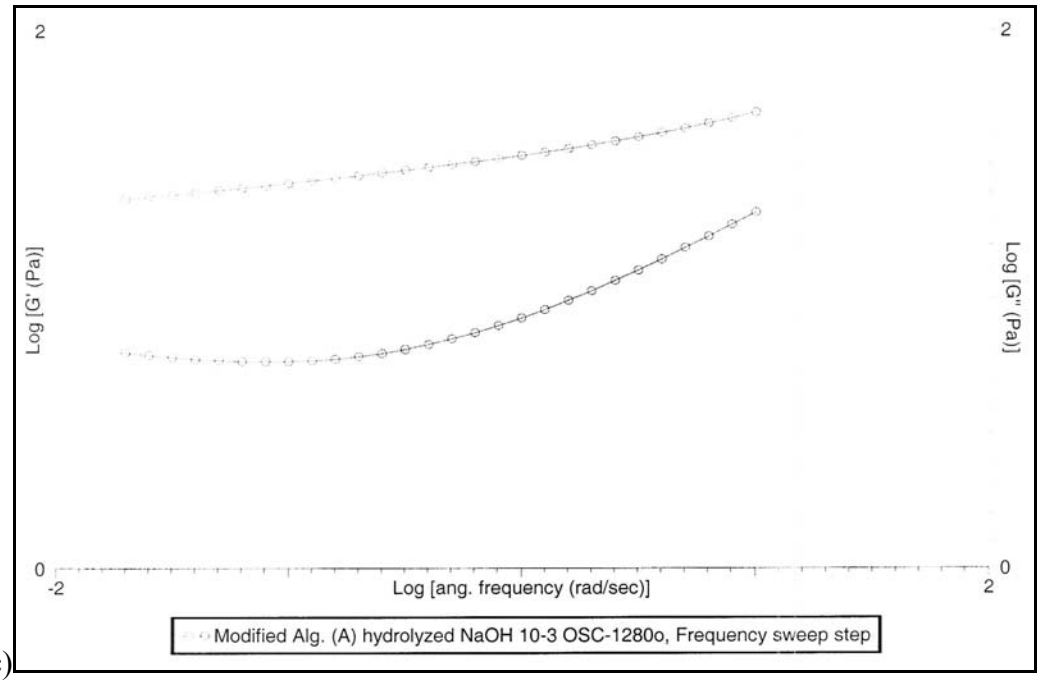
(a)


3
3
Log [G' (Pa)]
Log [G" (Pa)]
1
-2
Log [ang. frequency (rad/sec)]
2
1
Modified Alg. (A) unhydrolyzed OSC-1275o, Frequency sweep step

(b)


3
3
Log [G' (Pa)]
Log [G" (Pa)]
1
-2
Log [ang. frequency (rad/sec)]
2
1
Modified Alg. (B) unhydrolyzed OSC-1277o, Frequency sweep step

(c)



(d)

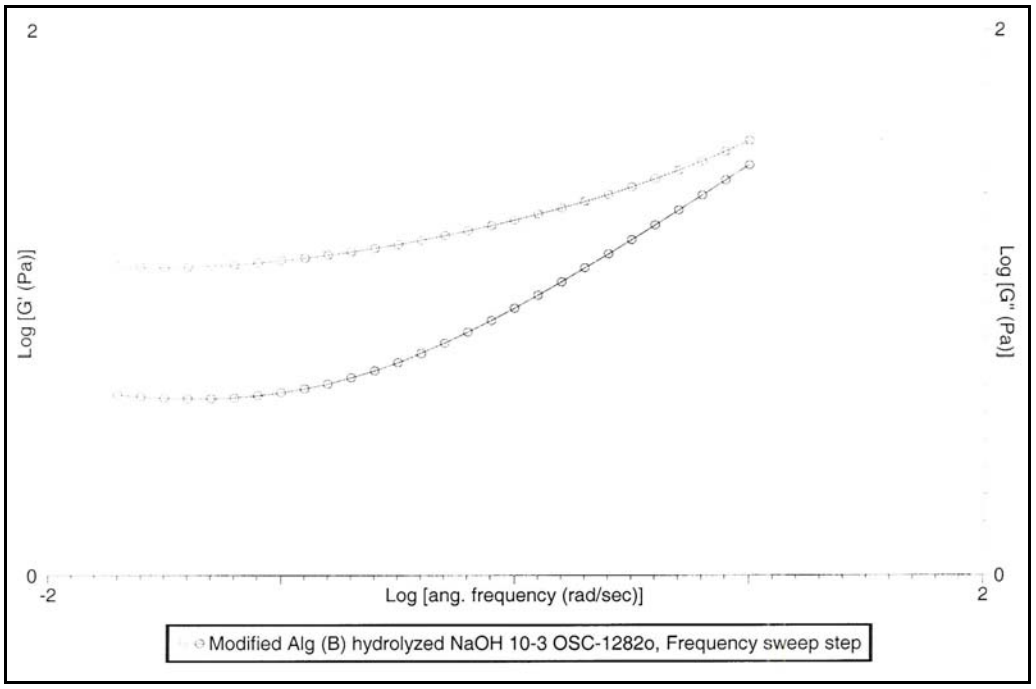


Figure 11. Oscillation rheology plots (a) 24 hr unhydrolyzed (b) 1 hr unhydrolyzed (c) 24 hr hydrolyzed (d) 1 hr hydrolyzed

4.2.4 Elemental Analysis

Equation 1 was used to calculate the substitution ratios for the 24 hr and 1 hr hydrolyzed and unhydrolyzed products. The substitution ratios are found in Table 5.

Sample	C %	N %	% Substitution
24 hr unhydrolyzed	37.5	1.1	20.7
1 hr unhydrolyzed	37.7	0.6	10.6
24 hr hydrolyzed	37.3	1	18.0
1 hr hydrolyzed	34	0.6	9.6

Table 5. Substitution ratios for the four alginamide samples

This round of elemental analysis confirmed results found in the previous elemental analysis results. In comparing the 24 hr unhydrolyzed and hydrolyzed products, the percent substitution was not affected. Again confirming that hydrolysis has no effect on the amide linkage of each alkyl chain. Also in comparing the 24 hr unhydrolyzed to the 1 hr unhydrolyzed, it is important to notice that the percent substitution for the 1 hr product was less. This also confirms that the reduction in reaction time does affect the number of alkyl chains which get substituted on the alginate.

4.3 Particle Size Analysis of Emulsions

4.3.1 Initial Size: Day of Formation

It was important to obtain particle size data immediately following emulsion preparation. Over time emulsion particles can begin to show signs of coalescence, therefore increasing the observed particle size. The particle size data obtained for each emulsion one hour after preparation showed sizes ranging from 469 to 2988 nm.

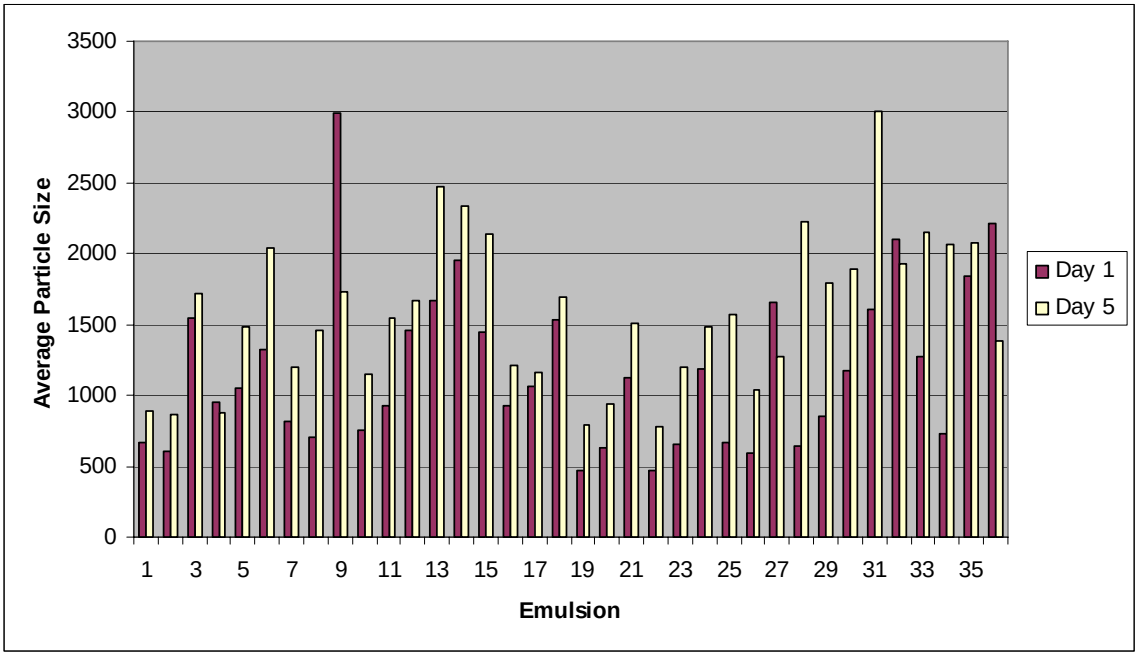


Figure 12. Average particle size data for all 36 emulsions

Due to the lack of previous data on alginate emulsions, there were no real expectations going into analysis. Although due to potential uses in biological applications, it was determined that submicronic particles (less than 1 micron) would be desirable. From the data obtained, only 17 out of the 36 emulsions had submicronic particle sizes. The mean count rate of each run was monitored. If the mean count rate fell between 100 and 500 kcps the run was considered accurate. The mean count rates of these 17 emulsions fell in between these parameters.

It was decided to focus on the submicronic emulsions and examine there compositions. Figure 13 shows the four products which were used in preparing emulsions and the percentage of submicronic emulsions that correspond to each.

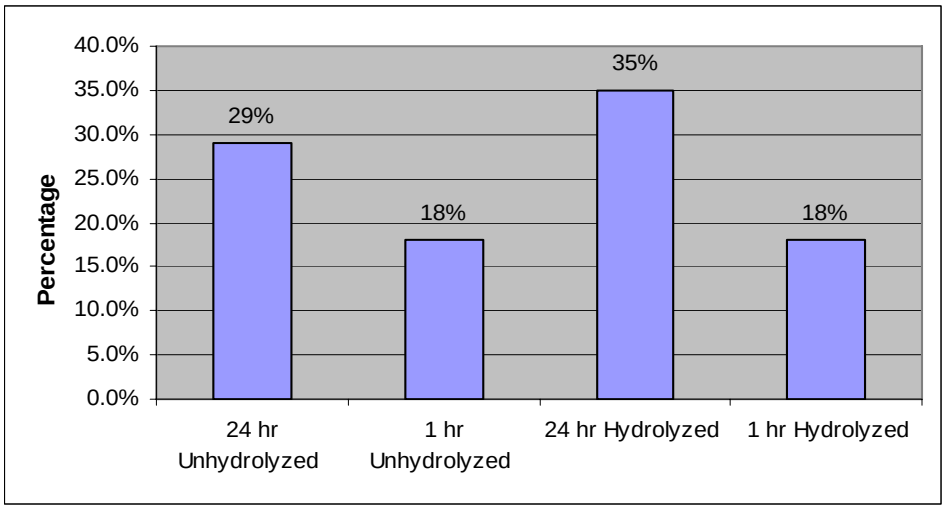


Figure 13. Percentage of the 17 submicronic emulsions from the alginamide products

In examining Figure 13, it can be seen that the 24 hr products make up the majority of the submicronic emulsions. The 24 hr hydrolyzed product had the most submicronic emulsions. The average particle size of each products submicronic emulsions was also analyzed in Figure 14.

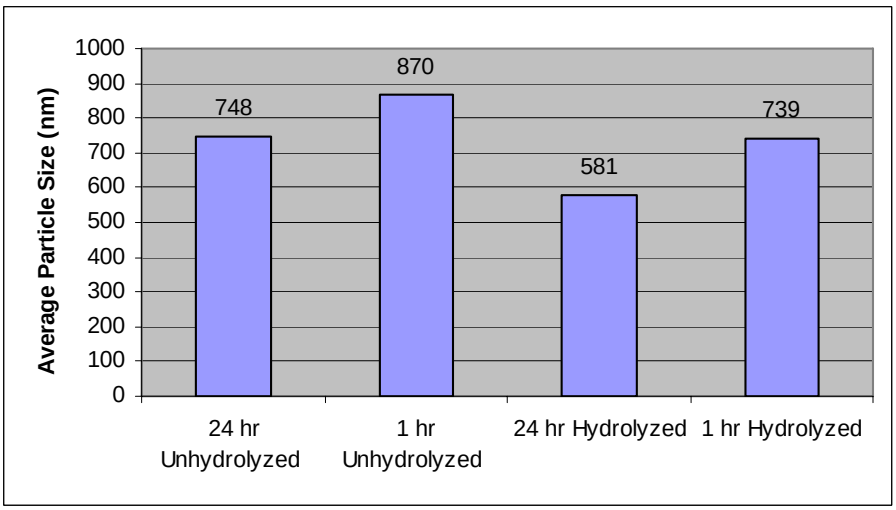


Figure 14. Average particle sizes of the 17 submicronic emulsions

It was determined that the 24 hr hydrolyzed product had the most submicronic emulsions as well as the smallest average particle size.

4.3.2 Stability after Five Days

The particle sizes of all but 4 emulsions increased in size over the 5 day period which can be seen in Table 6 & 7. Out of the 17 submicronic emulsions, only 6 of them remained under 1 micron all of which showed an increase in particle size.

24 hr Unhydrolyzed				
Aqueous Polymer	Oil	Day 1 (nm)	Day 5 (nm)	% Change
0.5%	10%	672	895	33.2
0.5%	20%	602	864	43.5
0.5%	50%	1546	1718	11.1
1.0%	10%	954	880	-7.7
1.0%	20%	1054	1482	40.6
1.0%	50%	1323	2035	53.9
2.0%	10%	811	1199	47.9
2.0%	20%	705	1465	107.7
2.0%	50%	2988	1736	-41.9
24 hr Hydrolyzed				
0.5%	10%	469	788	68.1
0.5%	20%	629	942	49.7
0.5%	50%	1128	1504	33.4
1.0%	10%	471	782	66.1
1.0%	20%	656	1197	82.5
1.0%	50%	1183	1484	25.5
2.0%	10%	674	1565	132.4
2.0%	20%	588	1039	76.6
2.0%	50%	1654	1274	-23.0

Table 6. The calculated % change in particle size for 24 hr product emulsions.

1 hr Unhydrolyzed				
Aqueous Polymer	Oil	Day 1 (nm)	Day 5 (nm)	% Change
0.5%	10%	756	1147	51.8
0.5%	20%	924	1547	67.4
0.5%	50%	1454	1675	15.2
1.0%	10%	1667	2479	48.7
1.0%	20%	1959	2335	19.2
1.0%	50%	1452	2144	47.7
2.0%	10%	930	1206	29.7

2.0%	20%	1058	1164	10.0
2.0%	50%	1531	1697	10.8
1 hr Hydrolyzed				
0.5%	10%	640	2223	247.2
0.5%	20%	854	1794	110.1
0.5%	50%	1176	1893	61.0
1.0%	10%	1607	5705	255.1
1.0%	20%	2101	1935	-7.9
1.0%	50%	1273	2158	69.5
2.0%	10%	724	2068	185.7
2.0%	20%	1841	2079	12.9
2.0%	50%	2215	1388	-37.3

Table 7. The calculated % change in particle size for 1 hr product emulsions.

In examining the changes in particle size for each emulsion, it was determined that only 6 of the emulsion had particle sizes which were below 1 micron.

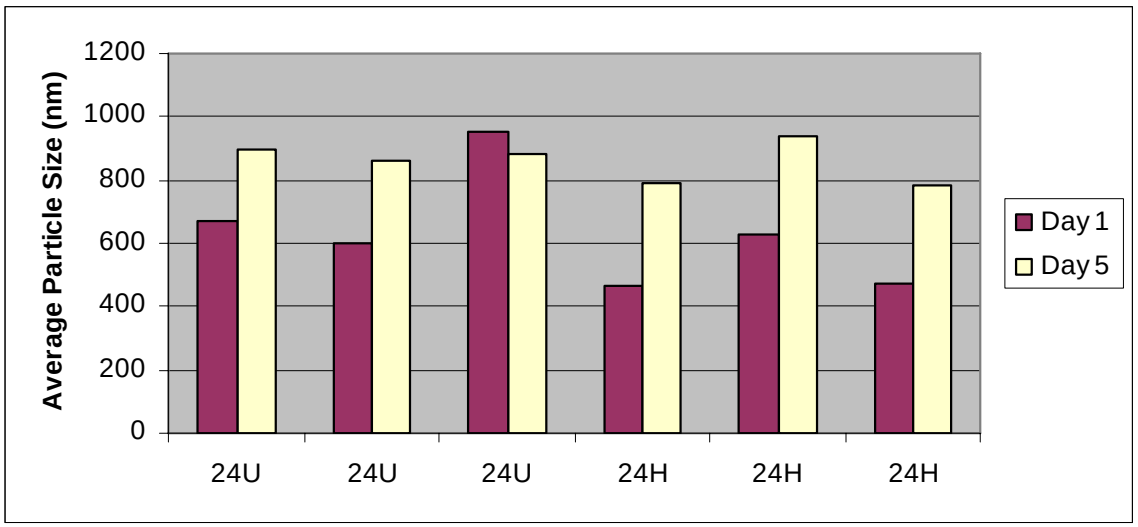


Figure 15. Stability of submicronic particles which were found on Day 5

The 24 hour unhydrolyzed and hydrolyzed products composed the 6 emulsions which retained submicronic particle sizes. The few 1 hr product emulsions which had initial submicronic particles did not retain them over the 5 day period.

In analyzing all the particle size results it can be stated that the 24 hr products out performed the 1 hr products in smallest particle size and in emulsion stability. In recalling the elemental analysis results, the 24 hr products had a high percentage of alkyl chains attached. It is apparent through these results that this difference in % substitution does affect the stabilizing abilities of the alginate polymer. The role of the secondary cross-linking in effecting the encapsulation abilities of the alginamide is not apparent

when analyzing the results. The 24 hr unhydrolyzed product, which contains the secondary cross-links, did produce 29% of emulsions with submicronic particles and 3 of the stable submicronic emulsions.

4.4 Oil Separation

4.4.1 Emulsion

No visible oil separation was observed for the samples set aside in the graduated 5ml plastic test tubes. This was seen even for emulsions which showed signs of oil separation upon formation. The oil separation data that was obtained was determined to be inconclusive.

4.4.2 Hydrogel

After each hydrogel was prepared it was set side so observations for oil separation could be made. Any visible layer of oil or oil bubbles was noted as oil separation. Estimates were made as to the quantitative amount of oil observed. These observations can be seen in Appendix 7.4.1.

Within the first hour of preparation, the hydrogels containing 50% oil were very unstable. Only samples containing 50% oil showed a visible layer of oil which had formed on top of the hydrogel.

Hydrogels with visible Oil Separation		
Hydrogel	% Oil	Initial Particle Size (nm)
6	50%	1323
9	50%	2988
15	50%	1452
18	50%	1531
27	50%	1654
33	50%	1273
36	50%	2215

Table 8. **The oil percentages and particle sizes of the 7 hydrogels with visible oil separation**

All 7 of the hydrogels containing oil separation did not have submicronic particle sizes.

5.0 Conclusions and Recommendations

The main objective of our research was to synthesize an alginamide product that had the best encapsulation properties for emulsion formation, that is, with the highest percent of alkyl chain substitution and the least amount of ester cross-linking. It was advantageous to obtain emulsions with the smallest average particle sizes, preferably under one micron. Additionally, we wanted the emulsions with submicronic particles to retain their average sizes over time.

It was found that reducing the reaction time of the synthesis from 24 hours to 1 hour reduced the percent of alkyl chain substitution by approximately 50%. Such a drastic drop in percent alkylation suggests that 1 hour does not leave sufficient time for the alkyl chains to attach. We were unable to state whether the decrease in reaction time had any effects on the level of cross-linking. This was due to the difference in percent alkylation seen between the two products. Other analytical tests such as mass spectroscopy or nuclear magnetic resonance (NMR) should be investigated to help quantify the degree of cross-linking.

Hydrolysis of the alginamide products with sodium hydroxide (NaOH) at low concentrations was able to reduce the amount of cross-linking but the degree of which could not be quantified. Furthermore, the addition of the hydrolysis step did not disrupt the alky chain substitution. Hydrolysis experiments with higher concentrations of NaOH should be carried out to test the extent of which the cross-linking can be reduced without jeopardizing the alkyl chain attachment.

The alginamide product that underwent a 24 hour reaction time and was hydrolyzed yielded the most promising results in emulsion formation and particle size stability. This was a direct result from the higher percent alkylation and reduced cross-linking when compared to the other three products. The 24 hour hydrolyzed product yielded the highest percent of emulsions with submicronic particle on day 1 at 35% and yielded 50 % of the emulsions after the 5 day period. It is unknown whether the percent alkylation or the reduced cross-linking is more closely tied to the better encapsulation results. Future research in this area can potentially uncover the more dominant factor and can possibly lead to even more promising results.

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7.0 Appendix

7.1 Particle Size Data: Day of Preparation

Day of Preparation: HPPS Emulsion Data

Record Number	Sample Name	Mean Count Rate (kcps)	Z-Ave.(nm)	PDI	Average Z-ave
1	#1 - AU 0.5	418	699	0.353	672
2	#1 - AU 0.5	402	659.7	0.374	
3	#1 - AU 0.5	410	656.6	0.216	
5	#2 - AU 1%	240	597.2	0.074	602
6	#2 - AU 1%	243	607	0.105	
7	#3 - AU 0.5	300	1499	0.081	1543
8	#3 - AU 0.5	311	1593	0.379	
9	#3 - AU 0.5	307	1538	0.154	
10	#4 - AU 1%	256	913.2	0.673	935
11	#4 - AU 1%	267	994.2	0.43	
12	#4 - AU 1%	258	897.7	0.383	
13	#5 - AU 1%	423	942.3	0.349	1064
14	#5 - AU 1%	428	1166	0.231	
15	#5 - AU 1%	429	1084	0.282	
17	#6 - AU 1%	151	1188	0.311	1384
18	#6 - AU 1%	148	1457	1	
19	#6 - AU 1%	147	1508	1	
20	#6 - AU 1%	413	1420	0.268	1396
21	#6 - AU 1%	402	1299	0.03	
22	#6 - AU 1%	405	1469	0.783	
23	#10 - BU 0.5%	387	768	0.169	756
24	#10 - BU 0.5%	393	749.5	0.036	
25	#10 - BU 0.5%	395	749.6	0.275	
26	#11 - BU .5%	229	937.9	0.251	924
27	#11 - BU .5%	241	919.5	0.228	
28	#11 - BU .5%	232	915.7	0.033	
29	#12 - BU 0.5%	342	1160	1	1454
30	#12 - BU 0.5%	341	1552	1	
31	#12 - BU 0.5%	341	1649	0.979	
32	#13 - BU 1%	237	1161	0.201	1667
33	#13 - BU 1%	233	1856	1	
34	#13 - BU 1%	235	1983	1	
35	#13 - BU 1%	230	1995	1	1976
36	#13 - BU 1%	220	1988	1	
37	#13 - BU 1%	227	1946	0.775	
38	#14 - BU 1%	216	1949	1	1959

39	#14 - BU 1%	214	1607	0.354	
40	#14 - BU 1%	213	2320	1	
41	#15 - BU 1%	273	1241	0.157	1452
42	#15 - BU 1%	274	1555	0.939	
43	#15 - BU 1%	269	1560	0.306	
44	#19 - AH 0.5%	296	470.1	0.392	469
45	#19 - AH 0.5%	289	472.5	0.276	
46	#19 - AH 0.5%	295	463.5	0.273	
47	#20 - AH 0.5%	279	632.5	0.145	629
48	#20 - AH 0.5%	276	634.5	0.043	
49	#20 - AH 0.5%	277	620.3	0.124	
50	#21 - AH 0.5%	224	1119	0.182	1128
51	#21 - AH 0.5%	217	1107	0.034	
52	#21 - AH 0.5%	215	1157	0.131	
53	#22 - AH 1.0%	200	473.3	0.17	471
54	#22 - AH 1.0%	196	468.7	0.215	
55	#22 - AH 1.0%	201	470.4	0.267	
56	#23 - AH 1.0%	416	651.8	0.3	656
57	#23 - AH 1.0%	422	659.7	0.269	
58	#23 - AH 1.0%	416	656.3	0.087	
59	#24 - AH 1.0%	154	1258	0.509	1183
60	#24 - AH 1.0%	157	1130	0.109	
61	#24 - AH 1.0%	157	1160	0.209	
62	#28 - BH 0.5%	460	642.8	0.164	640
63	#28 - BH 0.5%	455	647	0.02	
64	#28 - BH 0.5%	469	630.8	0.156	
65	#29 - BH 0.5%	348	855.7	1	854
66	#29 - BH 0.5%	350	856	1	
67	#29 - BH 0.5%	348	849.4	0.465	
68	#29 - BH 0.5%	347	841.1	0.374	838
69	#29 - BH 0.5%	347	854.4	0.223	
70	#29 - BH 0.5%	346	818.4	0.201	
71	#30 - BH 0.5%	211	1204	0.185	1176
72	#30 - BH 0.5%	212	1171	0.134	
73	#30 - BH 0.5%	206	1152	0.206	
74	#31 - BH 1.0%	276	2124	0.787	1607
75	#31 - BH 1.0%	270	1300	0.455	
76	#31 - BH 1.0%	261	1396	0.319	
77	#32 - BH 1.0%	356	2264	0.576	2815
78	#32 - BH 1.0%	358	3822	1	
79	#32 - BH 1.0%	344	2359	0.907	
80	#32 - BH 1.0%	346	2435	1	2101
81	#32 - BH 1.0%	352	1931	0.519	
82	#32 - BH 1.0%	345	1938	0.52	
83	#33 - BH 1.0%	257	1127	0.155	1273
84	#33 - BH 1.0%	275	1334	0.037	
85	#33 - BH 1.0%	264	1358	0.39	
86	#7 - AU 2.0%	399	788.3	0.031	811
87	#7 - AU 2.0%	394	765.6	0.178	
88	#7 - AU 2.0%	382	878.4	0.549	

89	#8 - AU 2.0%	212	721	0.275	705
90	#8 - AU 2.0%	211	705.4	0.288	
91	#8 - AU 2.0%	208	689.6	0.334	
92	#9 - AU 2.0%	357	5140	0.765	4466
93	#9 - AU 2.0%	352	3881	1	
94	#9 - AU 2.0%	364	4377	1	
95	#9 - AU 2.0%	348	2984	1	2988
96	#9 - AU 2.0%	331	3007	0.848	
97	#9 - AU 2.0%	344	2972	0.945	
98	#16 - BU 2.0%	297	1009	0.36	930
99	#16 - BU 2.0%	287	913.4	0.343	
100	#16 - BU 2.0%	288	867.1	0.311	
101	#17 - BU 2.0%	198	994.3	0.156	1058
102	#17 - BU 2.0%	195	991	0.124	
103	#17 - BU 2.0%	189	1190	0.369	
104	#18 - BU 2.0%	314	1398	0.378	1531
105	#18 - BU 2.0%	325	1573	0.187	
106	#18 - BU 2.0%	315	1623	0.736	
107	#25 - AH 2.0%	200	677.1	0.19	673
108	#25 - AH 2.0%	195	668.9	0.109	
109	#25 - AH 2.0%	190	674.4	0.179	
110	#26 - AH 2.0%	246	600.5	0.367	588
111	#26 - AH 2.0%	239	588.3	0.203	
112	#26 - AH 2.0%	242	576.5	0.244	
113	#27 - AH 2.0%	239	1523	0.125	1654
114	#27 - AH 2.0%	235	1658	0.272	
115	#27 - AH 2.0%	239	1781	0.577	
116	#34 - BH 2.0%	176	742.5	0.334	724
117	#34 - BH 2.0%	176	701	0.184	
118	#34 - BH 2.0%	178	728.2	0.185	
119	#35 - BH 2.0%	403	1959	0.223	1841
120	#35 - BH 2.0%	391	1866	0.463	
121	#35 - BH 2.0%	386	1698	1	
122	#36 - BH 2.0%	363	2309	1	2215
123	#36 - BH 2.0%	364	1877	0.3	
124	#36 - BH 2.0%	368	2458	1	

7.2 Particle Size Data: Stability after Five Days

Stability after 5 Days: HPPS Emulsion Data

Record Number	Sample Name	Mean Count Rate (kcps)	Z-Ave.(nm)	PDI	Average Z-ave
125	#1 - AU 0.5% D5	431	895	0.523	895
126	#1 - AU 0.5% D5	419	880	0.1	
127	#1 - AU 0.5% D5	420	911	0.158	
128	#2 - AU 0.5% D5	282	825	0.193	864
129	#2 - AU 0.5% D5	270	874	0.125	
130	#2 - AU 0.5% D5	277	894	0.121	
131	#3 - AU 0.5% D5	425	1571	1	1718
132	#3 - AU 0.5% D5	422	1828	1	
133	#3 - AU 0.5% D5	421	1755	0.328	
134	#3 - AU 0.5% D5	443	1886	0.161	1948
135	#3 - AU 0.5% D5	454	1953	0.229	
136	#3 - AU 0.5% D5	456	2004	0.383	
137	#4 - AU 1.0% D5	433	792	1	880
138	#4 - AU 1.0% D5	428	911	0.568	
139	#4 - AU 1.0% D5	428	937	0.166	
158	#4 - AU 1.0% D5	411	612	0.214	783
159	#4 - AU 1.0% D5	391	816	0.684	
160	#4 - AU 1.0% D5	401	922	0.521	
140	#5 - AU 1.0% D5	186	1628	1	1482
141	#5 - AU 1.0% D5	187	1474	1	
142	#5 - AU 1.0% D5	190	1343	0.224	
143	#5 - AU 1.0% D5	434	1682	0.02	1691
144	#5 - AU 1.0% D5	441	1732	0.092	
145	#5 - AU 1.0% D5	439	1660	0.112	
146	#6 - AU 1.0% D5	170	1832	1	2035
147	#6 - AU 1.0% D5	169	2270	0.363	
148	#6 - AU 1.0% D5	169	2004	0.213	
149	#10 - BU 0.5% D5	199	805	0.061	1147
150	#10 - BU 0.5% D5	201	1038	0.268	
151	#10 - BU 0.5% D5	199	1599	0.214	
152	#11 - BU 0.5% D5	275	1509	1	1547
153	#11 - BU 0.5% D5	278	1594	1	
154	#11 - BU 0.5% D5	276	1538	1	
155	#11 - BU 0.5% D5	231	1797	0.149	1730
156	#11 - BU 0.5% D5	224	1689	0.022	
157	#11 - BU 0.5% D5	223	1704	0.034	
161	#12 - BU 0.5% D5	437	1250	0.159	1675
162	#12 - BU 0.5% D5	434	1702	0.078	
163	#12 - BU 0.5% D5	440	2074	0.223	
164	#13 - BU 1.0% D5	339	2867	0.315	2479
165	#13 - BU 1.0% D5	345	2199	0.092	

166	#13 - BU 1.0% D5	334	2371	1	
167	#14 - BU 1.0% D5	230	3047	1	2335
168	#14 - BU 1.0% D5	228	1961	0.088	
169	#14 - BU 1.0% D5	220	1996	0.048	
170	#14 - BU 1.0% D5	223	3511	1	2618
171	#14 - BU 1.0% D5	226	1951	0.445	
172	#14 - BU 1.0% D5	223	2391	1	
173	#15 - BU 1.0% D5	155	1675	1	2144
174	#15 - BU 1.0% D5	149	2328	1	
175	#15 - BU 1.0% D5	155	2430	1	
176	#19 - AH 0.5% D5	450	693	0.032	788
177	#19 - AH 0.5% D5	455	738	0.233	
178	#19 - AH 0.5% D5	454	932	1	
179	#20 - AH 0.5% D5	275	921	0.648	942
180	#20 - AH 0.5% D5	279	956	0.581	
181	#20 - AH 0.5% D5	275	949	0.309	
182	#21 - AH 0.5% D5	411	1482	0.036	1504
183	#21 - AH 0.5% D5	407	1482	0.145	
184	#21 - AH 0.5% D5	407	1549	0.254	
185	#22 - AH 1.0% D5	184	797	0.043	782
186	#22 - AH 1.0% D5	184	753	0.19	
187	#22 - AH 1.0% D5	182	797	0.099	
188	#23 - AH 1.0% D5	467	1287	0.14	1197
189	#23 - AH 1.0% D5	463	1282	1	
190	#23 - AH 1.0% D5	464	1022	0.232	
191	#24 - AH 1.0% D5	172	1434	1	1484
192	#24 - AH 1.0% D5	174	1510	0.936	
193	#24 - AH 1.0% D5	172	1508	0.496	
194	#28 - BH 0.5% D5	274	1443	0.447	2223
195	#28 - BH 0.5% D5	259	2665	1	
196	#28 - BH 0.5% D5	277	2561	1	
197	#29 - BH 0.5% D5	352	1877	1	1794
198	#29 - BH 0.5% D5	353	1825	1	
199	#29 - BH 0.5% D5	345	1680	1	
200	#30 - BH 0.5% D5	202	1682	0.374	1893
201	#30 - BH 0.5% D5	235	1952	0.16	
202	#30 - BH 0.5% D5	282	2044	0.176	
203	#31 - BH 1.0% D5	246	2143	0.268	5705
204	#31 - BH 1.0% D5	260	5598	1	
205	#31 - BH 1.0% D5	247	9375	1	
206	#32 - BH 1.0% D5	448	1747	0.436	1935
207	#32 - BH 1.0% D5	433	1961	0.434	
208	#32 - BH 1.0% D5	440	2097	0.498	
209	#33 - BH 1.0% D5	171	2094	1	2158
210	#33 - BH 1.0% D5	177	2060	1	
211	#33 - BH 1.0% D5	177	2319	1	
212	#7 - AU 2.0% D5	200	1265	0.345	1199
213	#7 - AU 2.0% D5	180	1079	0.175	
214	#7 - AU 2.0% D5	177	1252	0.442	
215	#8 - AU 2.0% D5	197	1510	0.337	1465

216	#8 - AU 2.0% D5	194	1484	0.253	
217	#8 - AU 2.0% D5	187	1401	0.161	
218	#9 - AU 2.0% D5	274	1857	0.405	1736
219	#9 - AU 2.0% D5	266	1663	0.059	
220	#9 - AU 2.0% D5	269	1689	0.362	
221	#16 - BU 2.0% D5	408	1147	0.045	1206
222	#16 - BU 2.0% D5	404	1253	1	
223	#16 - BU 2.0% D5	403	1219	0.366	
224	#17 - BU 2.0% D5	192	967	0.38	1164
225	#17 - BU 2.0% D5	196	1081	0.359	
226	#17 - BU 2.0% D5	192	1444	1	
227	#18 - BU 2.0% D5	267	1646	0.782	1697
228	#18 - BU 2.0% D5	267	1766	0.778	
229	#18 - BU 2.0% D5	273	1680	0.509	
230	#25 - AH 2.0% D5	342	1208	0.376	1565
231	#25 - AH 2.0% D5	359	1769	0.9	
232	#25 - AH 2.0% D5	340	1717	0.678	
233	#26 - AH 2.0% D5	429	925	0.242	1039
234	#26 - AH 2.0% D5	425	1017	0.299	
235	#26 - AH 2.0% D5	421	1175	0.401	
236	#27 - AH 2.0% D5	196	1263	1	1274
237	#27 - AH 2.0% D5	205	1278	0.787	
238	#27 - AH 2.0% D5	202	1282	0.868	
239	#34 - BH 2.0% D5	190	2169	1	2068
240	#34 - BH 2.0% D5	191	1540	0.229	
241	#34 - BH 2.0% D5	192	2496	1	
242	#35 - BH 2.0% D5	205	1427	0.269	2079
243	#35 - BH 2.0% D5	198	2506	1	
244	#35 - BH 2.0% D5	200	2305	1	
245	#36 - BH 2.0% D5	325	1322	0.079	1388
246	#36 - BH 2.0% D5	327	1393	0.022	
247	#36 - BH 2.0% D5	327	1448	0.082	

7.3 Hydrogel Oil Separation Observations

Hydrogel Oil Separation Observations						
	Day 0	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
24 hr Unhydrolyzed						
Emul #						
1						
2						
3						
4						
5						
6	.5mm	1mm	1mm			
7						
8						
9	slight					
1 hr Unhydrolyzed						
10						
11						
12						
13						
14						
15	slight	.5mm				
16						
17						
18	slight					
24 hr Hydrolyzed						
19						
20						
21						
22						
23						
24						
25						
26						
27	slight					
1 hr Hydrolyzed						
28						
29						
30						
31						
32						
33		.5mm				
34						
34						
36	slight					