Synthesis of a biodegradable coating through direct transesterification of starch and sunflower oil.

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Abstract

A fatty acid starch ester was synthesized through direct transesterification of starch and sunflower oil. The resulting product was cross-linked in an attempt to form a biodegradable coating. The transesterification was carried out at 80°C and 115°C, using the catalysts NaOH/CTAB and TBD. The reaction products had a degree of substitution ranging from 4.60-13.5% of all alcohol groups. Products were analysed through ¹H-NMR and IR spectroscopy. Analysis of the products clearly indicate formation of a fatty acid starch ester. The reaction products were used to produce a film, the product was dissolved and subsequently mixed with a crosslinking agent. As the solvent dried out a film was formed, the resulting films were analysed using IR spectroscopy. The resulting films clearly showed a decrease in double bonds, which indicates that crosslinking was successful. Overall a direct transesterification of sunflower oil and starch does yield a fatty acid starch ester, but the degree of substitution of the products were too low to have any real application as a coating.

Introduction

Biodegradable coatings are becoming increasingly relevant for the agricultural sector as the focus on sustainability and the environment is ever increasing. Over fertilizing has a significant impact on the fertility of the land and on the climate. Biodegradable polymer coated controlled release fertilizers reduce these harmful effects by slowly releasing the fertilizer packed in the coating. However, these slow release fertilizers are still high in cost, which limits the use of these products. Polysaccharides such as starch are attractive as a feedstock because of its renewability and

availability. As starch can be found crops that are grown on a large scale such as potatoes, wheat and corn. Plant oils are also readily available as a feedstock from various sources such as sunflowers, palm seed and linseed. These oils are composed of various fatty acid compositions and are a renewable feedstock.

Polysaccharides are found widespread in forms such as starch, glycogen and amylose just as fatty acids are found abundantly in various plant oils. An ester product of these polysaccharides and fatty acids would be biodegradable as an ester can be hydrolysed and would provide grafted polymer structure. However, a coating requires some interconnected network within its structure. Luckily many fatty acids are mono- or polyunsaturated fats. These unsaturated fatty acids can be cross-linked, which allows for the formation of a three-dimensional structure and should provide the needed properties for a coating.

Various research papers document the synthesis of such fatty acid starch esters.

A research group from Argentina describes two methods of esterification of corn starch. Corn starch was esterified using tartaric acid and acetic anhydride or butyric acid and tartaric acid. Acetic anhydride and butyric acid function as solvents. A reaction temperature of 120°C yielded a degree of substitution ranging from 0.06 to 1.54. [1]

Amylomaize starch was used in a transesterification reaction using lauric acid methyl ester using a potassium laurate as catalyst. The reagents were dispersed in water. The transesterification occurred at 190°C and a maximum degree of substitution of 0.45 was obtained. [2]

Similarly a research group from Germany used fatty acid vinyl esters for a transesterification of maize starch using a solid carbonate catalyst and a lauroyl chloride reacted with starch in pyridine. DMSO and pyridine were used as solvents respectively. Both reactions were performed at a temperature of 110°C. [3]

These papers describe a synthesis route towards a fatty acid starch ester, using reagents that are not extremely toxic. All these research papers use derivatives of triglycerides which are found abundantly in plant oils. However, using these triglyceride derivatives would require chemical alterations to naturally obtained plant oils, which increases costs of the process and waste products produced. One research paper describes a direct transesterification of maize starch using olive oil and sunflower oil directly. 1,5,7-Triazabicyclo[4.4.0]dec-5-ene (TBD) was used as catalyst and appears to be the most efficient catalyst for a direct transesterification of a fatty acid. DMSO is used as solvent and the reaction occurs at a temperature of 115°C, which yielded a degree of substitution of 1.30. [4]

A direct transesterification of plant oils with starch as described above would be the most efficient route, because plant oils can be used directly instead of first modifying the triglycerides in plant oils. Thus the reaction only requires a polysaccharide and plant oils, which are readily available as a renewable feedstock. Therefore, a direct transesterification of plant oils with polysaccharides was chosen, using TBD as catalyst and DMSO as solvent. Potato starch was chosen as polysaccharide because of its availability and the chosen plant oil was sunflower oil as it has a high content of unsaturated fatty acids.

Additionally, a direct transesterification of sunflower and starch would provide a grafted polymer structure. The unsaturated fatty acids have double bond that can be cross-linked using a crosslinking agent that produces free radicals. The product would be a fatty acid starch ester, that has a three dimensional structure due to the interlinking of unsaturated fatty acids. Both starch and fatty acid starch ester can be hydrolysed, which makes the product biodegradable.

In this research project the possibility of synthesizing a grafted polymer from sunflower oil and potato starch that can be cross-linked will be investigated.



Figure 1:Reaction mechanism TBD catalysed transesterification of starch and sunflower oil.



Figure 2: Reaction mechanism of crosslinking fatty acid chains.

Materials and Methods

Materials

Potato starch,1,5,7-triazabicyclo[4.4.0]dec-5-ene (98%), cetrimonium bromide (≥99%), DMSO-d₆ (95.5 atom% D) and absolute ethanol were obtained from Sigma-Aldrich, Zwijndrecht, Netherlands. Starch was dried for 24 hours at 90°C.THF was obtained from Boom Laboratories, Meppel. Netherlands. DMSO (Baker analysed) was purchased from Avantor Performance Materials. Diethyl ether was used in technical grade. BorchiOxy Coat 1101 on water and Borchers oxycoat (Fe-Drier) were obtained from Borchers. Sunflower oil was purchased at the local supermarket (Brand: Gwoon).

Method

¹H-NMR and IR spectra were collected for starch and sunflower oil. Additionally a ¹H-NMR spectrum was taken of glycerol.

Procedure for synthesis of fatty acid starch ester

Starch (1.0080 gram, 5.60 mmol) was dissolved in DMSO (24 ml) by heating the mixture to 90°C for 30 minutes. After fully dissolving the starch a clear solution is obtained. To this solution sunflower oil (4.83 gram, 5.51 mmol) and TBD (0.2610 gram, 1.88 mmol) were added. The liquid was poured into a 100 ml three-necked flask. The three-necked flask was equipped with a reflux condenser and the reaction mixture was flushed with nitrogen and left under a nitrogen atmosphere and the reaction mixture was stirred for 18 hours at 115°C. Subsequently the reaction mixture was poured into cold ethanol (150 ml), a white powder precipitated. The crude product was collected through filtration and dissolved in THF (20 ml) by heating the mixture to 60°C. Cold ethanol (100 ml) was again used to precipitate the product. The product was washed two times to remove traces of sunflower oil and DMSO. The final product was white and flaky. All ethanol used for the precipitation was collected. Ethanol was evaporated out, the remaining liquid was weighed. ¹H-NMR samples were taken of the starting materials and leftover liquid from each precipitation step. The final product was washed with methanol a ¹H-NMR was taken before and after washing.

Separate reagents under reaction conditions

Each reagent individually was put under reaction conditions to see whether any chemical alterations would occur the separate reagents under these conditions. This was done to rule out any chemical alterations of the starting materials caused by side reactions.

Starch (1.0015 gram, 5.56 mmol) was dissolved in DMSO (24 ml) by stirring the mixture at 90°C for 30 minutes. A clear starch solution was obtained. TBD (0.2627 gram, 1.89 mmol) was added to the solution. The liquid was poured into a 100 ml three-necked flask which was equipped with a reflux condenser and the reaction mixture was put under a nitrogen atmosphere. The reaction mixture was stirred for 18 hours at 115°C. ¹H-NMR samples were taken directly from the reaction mixture before and after the reaction. About 5 ml of the reaction mixture was poured into cold ethanol (50 ml). A white solid precipitated. This white solid was mixed with THF (10 ml) and heated to 60°C. The white solid did not dissolve and remained at the bottom of the glass.

Sunflower oil (4.9010 gram, 5.59 mmol) and TBD (0.2557 gram, 1.84 mmol) were dissolved in DMSO (24 ml). The solution was poured into a 100 ml three-necked flask, equipped with a reflux condenser. The system was flushed with nitrogen and the reaction mixture was put under a nitrogen atmosphere. The reaction mixture was stirred for 18 hours at 115°C.¹H-NMR and IR spectrum were taken directly from the reaction mixture before and after the reaction.

Scale up reactions

Two scale up reactions were performed. In the initial scale up the amount of reagents used was increased 2.5 times compared to the general procedure, the second scale up used double the amount of reagents respectively. The reaction mixture of the second scale up was bubbled with nitrogen for 30 minutes before being put under reaction conditions under a nitrogen atmosphere. For both reaction products an ¹H-NMR and IR spectrum was collected.

Reaction at reduced temperature/Variation of catalyst

The reaction was scaled up to 2.00 grams of starch, CTAB (1.1349, 3.11 mmol) and NaOH (0.1245 gram, 3.11 mmol) were used as catalytic system. The reaction mixture was bubbled with nitrogen for 30 minutes and the reaction temperature was lowered to 80°C while the other reaction conditions were kept the same. Additional to the ¹H-NMR spectra an IR spectrum of the purified product was collected. Similarly a reaction was scaled up to 2.00 grams while using TBD as catalyst. The reaction mixture was bubbled with nitrogen for 30 minutes and subsequently put under a nitrogen atmosphere. The reaction temperature was set at 80°C, other conditions were kept identical. Additional to the ¹H-NMR spectra an IR spectrum of the purified product was collected.

Bulk reaction

The reaction mixture was prepared by suspending starch (2.0075 gram, 11.14 mmol) in sunflower oil (66.9262 gram, 76.38 mmol) through stirring and heating the mixture to 100°C for 30 minutes. Subsequently TBD (0.5264 gram, 3.78 mmol) was added, the mixture was poured into a 250 ml three-necked flask equipped with a reflux condenser and septum. The reaction mixture was bubbled with nitrogen for 30 minutes before the system was closed off an put under a nitrogen atmosphere. The mixture was stirred for 18 hours at 115°C. Cold ethanol (350 ml) was used to precipitate the crude product, the large amount of remaining sunflower oil did not dissolve which hindered collection of the product through filtration. Diethyl ether (150 ml) was used to dissolve the remaining sunflower oil, the crude product was collected through filtration. An attempt was made to dissolve the product in THF, the product did not dissolve in THF. A ¹H-NMR spectrum was taken of the final product.

Preparation of fatty acid starch ester films

The product of the reactions at reduced temperature and second scale up reaction were used to test the film applications of the product. The products of the reaction at reduced temperature were dissolved in DMSO, 0.7wt% of Borchers Oxycoat on 1,2-propylene glycol base was added to each solution. The solutions were mixed and spread out over a small petri dish. The product of scale up 2 was suspended in water by stirring the mixture for 48 hours, subsequently 0.7wt% of Borchi® OXY-Coat 1101 on water was added and the resulting solution was spread over a petri dish. After drying out the solvent an IR spectrum was recorded for each film.

Solubility tests

The solubility of starch and sunflower oil was tested in THF. Starch (0.5036 gram, 2.80 mmol) was mixed with THF (10 ml) the solution was stirred and heated close to the boiling point of THF. After 30 minutes another 10 ml of THF was added. The mixture was stirred for 3 hours, no starch had dissolved in this time period. The solubility of an equimolar mixture of starch (0.5008 gram, 2.80 mmol) and sunflower oil (2.5461 gram, 2.82 mmol) in THF was tested in the same fashion. Sunflower oil was completely dissolved after 3 hours, starch did not. The purified product's solubility was tested in xylene and water. The xylene mixture was heated to 120°C for 2 hours, no product had dissolved in xylene. After 3 hours of mixing no product had dissolved in water and no stable suspension was formed.

Results and Discussion

Starting materials

The starting materials were analysed through ¹H-NMR and IR spectroscopy to obtain information on characteristic peaks. As the reagents undergo chemical alterations these peaks shift or disappear from the product spectra.

The ¹H-NMR spectrum of sunflower oil (figure 3) gave the following result: ¹H-NMR (300 MHz, DMSOd6) δ 5.13 (s, 11H), 4.00 (d, J = 52.5 Hz, 10H), 2.77 – 2.35 (m, 4H), 1.95 (d, J = 64.2 Hz, 12H), 1.39 (s, 6H), 1.09 (s, 48H), 0.70 (s, 9H). The peaks at 0.70 ppm, 1.09 ppm and 1.39 ppm clearly show the aliphatic protons on the fatty acid chains. Protons in the vicinity of the double bond are slightly more deshielded than the other aliphatic protons thus they produce a doublet around 1.95 ppm. The protons on the fatty acid chain, near the carbonyl carbon (position 2, 21 and 40 in molecule) are close to ester functional groups within the molecule. This causes these proton peaks to be fairly downfield. The peaks caused by the glycerol protons coincide with the protons on positions 2, 21 and 40 to form a doublet at 4.00 ppm in the spectrum. Protons connected to the double bonded carbons are found in a large peak at 5.13 ppm together with the lone proton on position 60. The ¹H-NMR spectrum does not provide the expected characteristic peaks for the compound. This is most likely due to several chemical shifts being relatively close and their peaks coinciding. The obtained infrared spectrum (figure A1) shows characteristic peaks for alkanes (2937 cm⁻¹ and 2847 cm⁻¹), alkenes (3010 cm⁻¹ and 720 cm⁻¹) and esters (1740 cm⁻¹, 1167 cm⁻¹ and 1104 cm⁻¹). These peaks correspond to the structure of the triglycerides in sunflower oil which has double bond and ester groups.



Figure 3: ¹H-NMR of sunflower oil

¹H-NMR spectroscopy of starch gave characteristic peaks at: ¹H-NMR (300 MHz, DMSO-d6) δ 5.43 (d, J = 29.1 Hz, 1H), 5.08 (s, 1H), 4.56 (s, 1H), 3.46 (d, J = 80.4 Hz, 7H) (see figure 4). The proton at position 2 is very deshielded due to its proximity to two oxygen molecules, which causes a doublet at 5.43 ppm. As expected the secondary alcohols are found as two singlets at 5.08 ppm and 4.56 ppm. The signals created by the protons inside the ring and primary alcohol group coincide and form a large doublet at 3.56 ppm. What was also concluded from the ¹H-NMR spectrum was the presence of water even after drying

IR spectroscopy shows a broad peak at 3300 cm⁻¹ and a doublet around 1400 cm⁻¹ which indicate the presence of alcohol groups (see figure A2). Specific characteristic peaks for a primary alcohol (1077 cm⁻¹) and secondary alcohol (1145 cm⁻¹) are also present in the spectrum. These correspond to the structure of starch. A small amount of starch was dissolved in sunflower oil, the resulting solution was also analysed with infrared spectroscopy (Appendix IR spectrum of starch and oil). The mixture was found to have the same characteristic alkane (2937 cm⁻¹ and 2847 cm⁻¹), alkene (3010 cm⁻¹) and ester peaks (1740 cm⁻¹, 1167 cm⁻¹ and 1104 cm⁻¹). However only one alcohol peak is found at 1100 cm⁻¹, which indicates a secondary alcohol. The lack of other characteristic peaks for starch is caused by the low solubility of starch in sunflower oil.



Figure 4: ¹H-NMR of starch

Glycerol was also analysed with ¹H-NMR spectroscopy (figure 5), as it is one of the reaction products if all fatty acid groups on triglyceride successfully undergo a transesterification. Therefore, some glycerol is expected within the reaction mixture. The spectrum yielded the following results: ¹H NMR (300 MHz, DMSO-d6) δ 4.46 (d, J = 4.6 Hz, 1H), 4.38 (t, J = 5.6 Hz, 2H), 3.48 – 3.18 (m, 5H). In the spectra the protons on the alcohol group are clearly present at 4.46 ppm and 4.38 ppm. The doublets caused by protons at position 2 and 5 coincide with the triplet that corresponds to the proton at position 3, the resulting multiplet is found at 3.31 ppm.



Figure 5: ¹H-NMR of glycerol

Reactions in DMSO

The first reaction of starch and sunflower oil yielded a white flaky product. Very little product was collected as most of the product had dried into the filter overnight. The yield was not determined. The ¹H-NMR (300 MHz, DMSO-d6) δ 5.24 (s, 8H), 3.48 (s, 1H), 2.70 (s, 1H), 2.25 (s, 4H), 1.93 (s, 8H), 1.21 (s, 38H), 0.83 (s, 6H). The sample taken for 1 H-NMR spectroscopy was not completely dry of methanol, as seen by the peaks produced at 3.14 ppm and 4.09 ppm. The product clearly shows that even after washing the product with methanol some aliphatic chains are in the product, as the ¹H-NMR spectrum (figure 6) shows peaks around the aliphatic region (0.83 ppm, 1.21 ppm and 1.93 ppm). A small singlet at 3.48 ppm is produced by the proton at positions 7 and 17, which are in the ring structure of starch. A similar singlet at 2.25 ppm indicates an ester bond, originating from the protons at position 23 and 43. All these peaks indicate that an ester of starch and a fatty acid was formed. However, many characteristic peaks are missing, such as a doublet that should be around 4 ppm that originates from the protons at position 11 and 21. Furthermore the characteristic peaks caused by the secondary alcohol groups on the starch molecule are also not seen. The lack of these peaks is most likely caused by the bad solubility of the product in deuterated DMSO or due to peaks coinciding. ¹H-NMR spectroscopy strongly indicates that a fatty acid starch ester has formed.



Figure 6: ¹H-NMR of MeOH washed product of starch and sunflower oil in DMSO.

All ethanol used for precipitation and other purification steps was collected. This was done as an extra method to confirm that the a transesterification has occurred and to track whether the method of purification was appropriate. Product should not also be found in the liquid used for precipitation. Ethanol was evaporated out of the mixture. A ¹H-NMR spectrum was taken of the residual liquid (see figure 7). The residue was expected to exist out of sunflower oil, glycerol, ethanol, TBD and DMSO. The ¹H-NMR spectrum clearly shows that the a large amount of oil is still present, as seen by the aliphatic peaks at 1.17 ppm and 1.47 ppm. A small amount of free fatty acid is also present in the mixture, seen by a singlet at 10.60 ppm. As expected DMSO from the reaction mixture is present in large quantities. Two small triplets at 3.09 ppm and 3.21 ppm are most likely caused by the glycerol protons from triglycerides that have taken part in the reaction and have lost one or two fatty acid groups. This causes a slight shift in the protons attached to the glycerol molecule.



Figure 7:¹H-NMR of ethanol residue obtained after evaporating ethanol used for precipitation.

When stacking the collected ¹H-NMR spectra of the residual liquid, glycerol, sunflower oil and predicted spectra of TBD and ethanol together it becomes more clear what is present in the residual liquid (see figure 8). Glycerol is present in a small amount, as seen by a small singlet at 4.35 ppm. Glycerol together with TBD forms a multiplet around 3.42 ppm as their peaks coincide. The presence of TBD is further confirmed by a small multiplet at 1.90 ppm. The residual liquid was not completely free of ethanol as can be seen by a triplet at 1.03 ppm.



Figure 8: ¹H-NMR of ethanol residue stacked over: glycerol, sunflower oil, TBD and ethanol

Analysing the ethanol residue with ¹H-NMR spectroscopy has clearly pointed out that DMSO, sunflower oil and even ethanol are present in large quantities. In addition small amounts of TBD and glycerol are also present in the ethanol residue. The glycerol peaks shift quite a lot as mentioned before because not all fatty acid groups on the molecule have reacted.

Ethanol residues for the subsequent reactions will not be discussed in detail, as their ¹H-NMR spectra were nearly identical to that of the first reaction.

Separate reagents under reaction conditions in DMSO

The starting materials were individually put under reaction conditions to check whether any chemical alterations could happen to these materials under reaction conditions. A ¹H-NMR spectrum was recorded of the reaction mixture before and after the reaction conditions. The resulting spectra were superimposed and analysed to see if any chemical alteration had happened.

The initial spectrum of starch before reaction conditions, has an inflated peak at 3.47 ppm which are the characteristic peaks merged together (see figure 9). Due to the gelling of the reaction mixture the characteristic peaks from protons on the starch ring have merged together. The spectrum obtained of the reaction mixture after reaction conditions (figure C2) does show some of these characteristic protons on the starch ring around 3.29 ppm. Which indicate that starch did not undergo a reaction. Additionally the characteristic singlets at 2.75 ppm and 2.39 ppm, clearly not shift under reaction conditions. The product also did not dissolve nor form a suspension in THF, which was found to be a characteristic property of starch. It was concluded that starch did not undergo any chemical alterations during reaction conditions.



Figure 9: ¹H-NMR of starch before and after reaction conditions in DMSO. Red = Before, Green = After

Sunflower oil did not seem to have reacted with anything under reaction conditions. The ¹H-NMR spectra of the reaction mixture before and after the reaction conditions seemed to have no large differences (see figure 10). When comparing figure D1 and D2 it is clear that a multiplet at 3.00 ppm

that has split into two small triplets. This is most probably caused by the triglyceride interacting with the catalyst, trace amount of water in sunflower oil react with a triglyceride splitting off a free fatty acid. This causes the protons in the glycerol molecule to be in a slightly different chemical environment. Which fatty acid is removed from the triglyceride (centre or outer) causes two triplets to form. What is important to notice is a decrease in the peaks at 5.17 ppm and 5.30 ppm paired with a small increase of the aliphatic peak at 1.20 ppm. This indicates some change in the double bonds of the fatty acid, which could be caused by interference of oxygen at elevated temperatures. There seem to be no further changes to the triglycerides under reaction conditions. However the presence of oxygen should be eliminated to prevent auto oxidative crosslinking, as this might have caused the slight chemical shift of the singlet at 3.38 ppm.



Figure 10: ¹H-NMR of sunflower oil before and after reaction conditions in DMSO. Red = Before, Green = After

Scale up reactions in DMSO

The reaction was scaled up to more accurately determine the yield. The loss of product was carefully followed over the purification steps. As a result the first scale up yielded 3.86 grams of product. A 61.8% weight increase was observed, which indicated a degree of substitution of 13.5%. The second scale up reaction yielded 2.26 grams of product. A total weight increase of 27.3% was observed, which indicates a degree of substitution of 6.0%. The calculated values for percentage weight increase and degree of substitution take into account the loss of product over purification and of transferring the reaction mixture (see figure E6).

Characterisation of the first scale up product

¹H-NMR spectroscopy seems to confirm that fatty acids have been attached to the starch molecule: ¹H-NMR (300 MHz, DMSO-d6) δ 5.25 (s, 8H), 4.35 (s, 4H), 2.67 (s, 2H), 1.94 (s, 8H), 1.19 (s, 38H), 0.78 (s, 6H) (see figure 11). The aliphatic peaks caused by the chain ends of the fatty acid give a clear signal, as well do the other aliphatic protons. However, the characteristic peaks of starch in the range of 4.3 ppm to 6.8 ppm have almost completely disappeared except for one small signal caused by protons at position 11 and 21. The ¹H-NMR spectrum does not provide proof that a fatty acid starch ester has formed, as a doublet for the protons at position 23 and 43 was expected around 2.30 ppm. IR spectroscopy confirms that an ester is present in the product as a peak is found at 1731 cm⁻¹ (see figure E1). The IR band for the ester peak has shifted slightly, compared to the triglycerides in sunflower oil. The IR spectrum confirms the presence of alcohol functionalities with a broad peak at 3350 cm⁻¹ and aliphatic chains (2922 cm⁻¹, 2848 cm⁻¹ and 1458 cm⁻¹) with alkene functionalities (3020 cm⁻¹, 1645 cm⁻¹ and 708 cm⁻¹). The shift of the ester peak in the IR spectrum and ¹H-NMR spectra combined indicate that a fatty acid starch ester has formed.



Figure 11:¹H-NMR of product of first scale up, after reacting starch and sunflower oil in DMSO.

Characterisation of the second scale up product

¹H-NMR showed the following characteristic peaks: ¹H-NMR (300 MHz, DMSO-d6) δ 5.21 (d, J = 96.6 Hz, 8H), 4.43 (d, J = 56.6 Hz, 5H), 3.46 (d, J = 66.5 Hz, 0H), 1.96 (s, 8H), 1.53 – 0.34 (m, 44H) (see figure 12). The spectra clearly shows the aliphatic chains of the fatty acid being present and the starch protons at position 11 and 21 also clearly show on the spectrum at 4.43 ppm. However, the ester functional group does not show on the spectrum. IR spectroscopy does show the presence of the ester functionality (1731 cm⁻¹) and alcohol groups (3300 cm⁻¹ and 1340 cm⁻¹) (see figure F1). A slight shift of the ester band (1731 cm⁻¹) compared to the triglyceride (1740 cm⁻¹) is observed. The spectrum also clearly shows the aliphatic chains (2918 cm⁻¹ and 2844 cm⁻¹) with alkene functionalities (1640 cm⁻¹). The spectroscopic data strongly suggests the formation of fatty acid starch ester.



Figure 12:¹H-NMR of product of second scale up, after reacting starch and sunflower oil in DMSO.

Reaction at reduced temperature/Variation of catalyst

The reaction temperature was lowered with the intent of reducing autoxidation by residual oxygen in the reaction mixture[5]. The temperature was arbitrarily changed to 80°C in complement to bubbling the reaction mixture with nitrogen. Additionally different catalytic system was tested: cetyltrimethylammonium bromide with sodium hydroxide. As well as a reaction using TBD as catalyst was performed at this temperature to compare efficiency of these catalysts to each other. The reaction which used the CTAB/NaOH catalyst yielded 2.29 grams of product. A 21.1% weight increase was observed, which suggested a degree of substitution of 4.60%. The TBD reaction yielded 2.86 grams of product, a 49.0% weight increase was observed which indicates a degree of substitution of 10.7%. The calculation made for weight increase and degree of substitution both take into account the loss of product during purification and loss of transferring the reaction mixture. Lowering the temperature as expected seems to have lowered the degree of substitution. The effectivity of CTAB also does not appear to be as high as TBD. However, during purification of the CTAB product a small grain of undissolved NaOH was found, which could be responsible for a lowered yield.

Characterisation of the CTAB product.

¹H-NMR analysis yielded: ¹H-NMR (300 MHz, DMSO-d6) δ 5.20 (d, J = 69.9 Hz, 8H), 4.55 (s, 2H), 2.71 (s, 0H), 1.98 (s, 8H), 1.49 (s, 4H), 1.21 (s, 38H), 1.04 (d, J = 8.0 Hz, 6H). It is clear that fatty acid is present in the product with the aliphatic peaks at 1.49 ppm, 1.21 ppm and 1.04 ppm (figure 13). The secondary alcohol groups on position 4 and 18 found on the spectrum confirm that starch is also present in the product. However, no characteristic peaks were found for protons around the ester group. It is also clear that there is some CTAB in the product as well, seen by a singlet at 0.83 ppm and a large peak at 3.56 ppm that coincides with water in the product. The presence of CTAB in the product means that yield of product is probably lower than calculated. IR spectroscopy shows the presence of aliphatic chains (2817 cm⁻¹) with alkene functionalities (1635 cm⁻¹ and 704 cm⁻¹) (see figure G1). A peak at 1202 cm⁻¹ indicates that some ester groups are present. The small weight increase paired with smaller aliphatic peaks in both ¹H-NMR and IR spectroscopy confirm a low degree of substitution.



Figure 13: ¹H-NMR of product of starch and sunflower oil using CTAB as catalyst at 80°C.

Characterisation of the TBD product.

¹H-NMR analysis yielded the following: ¹H-NMR (300 MHz, DMSO-d6) δ 5.43 (d, J = 26.9 Hz, 8H), 5.08 (s, 2H), 4.56 (s, 2H), 3.60 (s, 4H), 1.94 (s, 8H), 1.47 (s, 4H), 1.21 (s, 38H), 0.81 (s, 6H). The aliphatic peaks (1.04 ppm, 1.21 ppm, 1.49 ppm and 1.98 ppm) indicate the addition of the fatty acid, with protons attached to the alkene groups are found in the doublet at 5.20 ppm (see figure 14). However this doublet has coincided with some peaks generated by protons in the starch peaks. The alcohol groups on the starch molecule are also clearly visible at 5.08 ppm and 4.56 ppm. Characteristic peaks for protons on position 23 and 42 are not found. The ¹H-NMR spectrum does not confirm the presence of an ester functional group. However, IR spectroscopy does show peaks at 1205 cm⁻¹ and 1731 cm⁻¹ which confirm an ester group being present (see figure H1). The presence of alcohol groups is confirmed by a broad peak at 3300 cm⁻¹ and by a peak found at 1080 cm⁻¹ which shows a

5_TBD_Prod_20190607153630 180 20190607150452 52358 3 1.94 147 ŝ 121 0.81 170 160 150 140 130 ¹H NMR (300 MHz DMSO- d_{1}) 5 5 43 (d. J = 26.9 Hz 8H), 5.08 (s 120 2H), 4.56 (s, 2H), 3.60 (s, 4H), 1.94 (s, 8H), 1.47 (s, 4H), 1.21 (s 38H), 0.81 (s, 6H). 39,59 (s) 110 0.81 -100 6,16 (s) 24",44' (s) 5.08 -90 2,12,30,31,50,51,53,54 (d) ,32',49',55" (s) 5,7,13,19 (s) 5.43 3.60 1.94 -80 4,14 (s) 25 ,34',35',36',37',38',45',46',47',48',56',57',58" (s) -70 4.56 1.21 -60 -50 40 28,33,34,35,36,37,38,45,46,47,48,56,57,58 25', 26', 27 -30 2,12,30,31,50,51,53,54 5,7,13,1 74" 44" -20 29'32'49'55 39.59 10 1.64 -0 1.45 0.33 -10.23-J 0.51 88 0.28

primary alcohol being present. The collected spectra indicate the formation of a fatty acid starch ester.

Figure 14: ¹H-NMR of product of starch and sunflower oil using TBD as catalyst at 80°C.

Bulk reaction

11.5 11.0 10.5 10.0

9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0

During preparation of IR samples for a starch and sunflower mix it was found that sunflower oil was able to dissolve starch in small quantities. Sunflower oil also readily dissolves the catalyst TBD. This prompted the question if a fatty acid starch ester could be synthesized by dissolving starch directly into sunflower oil. The advantages of not having to remove DMSO from the reaction mixture and being able to synthesize the product with just the reagents would be significant.

5

5.5 5.0 f1 (ppm)

56

4.5 4.0 3.5 3.0 9.41

2.5

6.21 88

2.0 1.5 1.0 0.5 0.0 -0.5

8

15.16 8

The bulk reaction yielded 2.34 grams of product, which had 24.1% weight increase. This would mean that 5.3% of all alcohol groups would have been substituted. However, this number is expected to be far lower, due to the oil being present in the filters their weight had increased significantly. While the reaction product had dried into the filters. This made determining the amount of product lost over filtration guite inaccurate. The product did not dissolve in THF, which hints that very little happened during the reaction conditions. ¹H-NMR spectroscopy yielded: ¹H-NMR (300 MHz, DMSO-d6) δ 5.43 (d, J = 31.1 Hz, 8H), 5.08 (s, 5H), 4.88 (s, 0H), 4.56 (s, 16H), 3.59 (s, 1H), 1.12 (d, J = 56.8 Hz, 0H) (see figure 15). When comparing the spectrum of the bulk product to starch it is clear that they are nearly identical, it seems that no reaction has occurred. However, there is a small peak at 1.12 ppm that could be from a fatty acid. Due to the small area under the peak it might have been a trace amount of sunflower oil, as the purification steps which involve dissolving the product in THF could not be done. IR spectroscopy of the product also shows a spectrum that is nearly identical to that of starch, but also shows peaks at 2916 cm⁻¹ and 2885 cm⁻¹ which indicate the presence of alkanes (see figure 11). It is unclear whether trace amounts of sunflower oil were still on the product or a small reaction had occurred. The collected spectra indicate either a small amount of fatty acid starch ester or some

-10

-1.0 -1.5



sunflower oil contamination, no significant amount of fatty acid starch ester had formed. The low solubility of starch in sunflower oil was most likely the reason.

Figure 15: ¹H-NMR of product of starch and sunflower oil using TBD as catalyst at 115°C.

Fatty acid starch ester films

As the films were being prepared by dissolving the product into DMSO, a viscous liquid formed that foamed during excessive stirring. The foam formation persisted even when the viscous solution was further diluted. The formation of foam can be explained by the structure of the fatty acid starch ester which has apolar fatty acid groups and polar backbone such as starch. This structure could explain the foaming properties as the fatty acid starch ester could function as a surfactant, which allowed bubbles to form. After addition of Borchers Oxycoat (Fe-Drier) the solution made with TBD (at 80°C) product formed a yellow solution, while the CTAB (at 80°C) product solution formed an orange solution. The suspension made out of the second scale up product maintained a milky colour after addition of Borchi® OXY-Coat 1101 on water. Films made with DMSO were dried for two weeks before obtaining a film, the film made with water required three days to dry. Due to the modifications of starch with the fatty acids, the solubility of the reaction product in DMSO had decreased. This caused granules of product to form in the film made with the more highly substituted reaction product (TBD @80C, figure 16) during the drying process. When removing the resulting film, the properties of the films made from products of the CTAB reaction and second scale up were very similar. Both formed a very hard layer, that could only be scraped off as a powder. The resulting films both had a light yellow colour. However, the film produced from the TBD reaction at reduced temperature had very different properties. When scraping the product out of the petri dish small flakes of a the film were obtained, the film was less coloured and more transparent. This difference in behaviour might be explained by the degree of substitution on the product. As the second scale up and CTAB reaction only had a degree of substitution of 6.0% and 4.60%, while the TBD product had a degree of substitution of 10.7%.

IR spectroscopy of the dried films gave a similar results, all spectra showed a large decrease or complete disappearance of the peaks at 1635 cm⁻¹ and 705 cm⁻¹ (see figure J1, J2 and J3). This indicates a decrease in alkene functionality in the molecule which indicates that the crosslinking was successful.



Figure 16: The resulting films, from left to right: TBD @80C, CTAB @80C and Scale up 2 @115C

Conclusion

A fatty acid starch ester was synthesized using sunflower oil and starch. The reaction was done at two different temperatures, 80°C and 115°C. The initial reaction used TBD as a catalyst and indicated that a direct transesterification is possible, as ¹H-NMR spectroscopy showed that some fatty acid had been attached to starch even after purification which extracted unreacted reagents. Putting the reagents, starch and sunflower oil, under reaction conditions revealed that both reagents are stable under the reaction conditions. However, the presence of oxygen interferes with the unsaturated fatty acids as autoxidation occurs at the reaction temperature which caused premature crosslinking which made the final product insoluble. Therefore, oxygen should be completely removed from the reaction system.

The scale up reactions both used TBD as catalyst and had a degree of substitution ranging from 6.0-13.5%. Both ¹H-NMR and IR spectroscopy clearly showed the formation of a fatty acid starch ester. Two reactions were performed at 80°C using two different catalysts, TBD and CTAB/NaOH. The TBD product had a degree of substitution of 10.7% while the CTAB product had 4.60% of all alcohol groups substituted. The CTAB product most likely had a lower degree of substitution as CTAB was still present in the product and sodium hydroxide had not fully dissolved into the reaction mixture. The bulk reaction product did have an increase in weight. However, ¹H-NMR and IR spectroscopy showed that little to no fatty acid was added to the starch molecules. This weight increase might be due unsuccessful purification of the product due to large amounts of sunflower oil being present. Determining the degree of substitution through IR and ¹H-NMR spectroscopy was not possible due to characteristic peaks of compounds coinciding in the spectrum which made quantification through these analytical methods impossible. The degree of substitution was determined by weight, which is considered to be quite inaccurate.

A suggestion for follow up research would be to use elemental analysis paired with a calibration curve that represents a carbon hydrogen ratio for several degrees of substitution. This method would allow for far more accurate determination of the degree of substitution.

Several films were created from products of the second scale up reaction, TBD at 80°C reaction and CTAB at 80°C reaction. IR spectroscopy of these films showed that most double bond within the film had disappeared, which indicated that the crosslinking was successful. Overall the transesterification of starch and sunflower oil was successful and a film was made. However, the degree of substitution on the product has to be higher to have any applications as a coating.

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Appendix



A (Spectra obtained from starting materials)

A1: IR spectrum of sunflower oil.



A2: IR spectrum of starch.



A3: IR spectrum of starch and sunflower oil.

B (Spectra obtained from first reaction)



B1:Predicted ¹H-NMR spectrum of TBD.







C (Spectra obtained from starch under reaction conditions)

C1: ¹H-NMR spectrum of reaction mixture before reaction conditions.



C2: ¹H-NMR spectrum of reaction mixture after reaction conditions.



D (Spectra obtained from sunflower oil under reaction conditions)

D1: ¹H-NMR spectrum of the reaction mixture before reaction conditions.



D2: ¹H-NMR spectrum of the reaction mixture after reaction conditions.



E (Spectra obtained from first scale up reaction + excel sheet for mass balance + sample calculation for degree of substitution)

E1: IR spectrum of the product of the first scale up.



E2: ¹H-NMR spectrum of ethanol residue 1



E3: ¹H-NMR spectrum of ethanol residue 2



E4 : ¹H-NMR spectrum of ethanol residue 3

Reaction flask	IN (theoretical)	IN (actual)	OUT			Oil Remai	Actual	Theoretic	al							
#		Amount (g)				#	Amount (g))								
Sunflower Oil	12,25	12,256	78,5054			Oil		12,25	5							
TBD	0,65	0,6535				DMSO		66	Amount o	of DMSO ca	n be lower	because r	not all starc	h and DMS	SO was add	led
DMSO + Starch	68,5	65,5959					70,217	78,25	ls lower if	fsunflowe	r oil is cons	sumed				
Total	81,4	78,5054	76,3439			Recov Oil	2,4195									
	0,04239562	2,394010949				Total	72,6365									
% weight incease	0,701960031	%														
			Prod Lost	in purifica	tion		Beaker 600	ml 1.2			THF disso	lving Beak	er (250 ml)			
Actions			Filters	Unused	Used	Diff		Clean	Used	Diff	Clean	Used	Diff			
Mixing Beaker (DMS	O +STARCH)		1	2,0772	2,5207	0,4435	1.2	188,904	188,963	0,059	96,1796	96,2518	i i			
In	Out		2	2,0735	2,4568	0,3833	1.	193,095	5 193,198	0,103						
68,5019	65,5959		3	2,0783	2,1623	0,084			Total	0,162						
			4	2,0674	2,1472	0,0798										
Leftover From R flask	(5	2,0835	2,9362	0,8527										
Used	Clean		6	2,0759	2,4168	0,3409										
158,5075	156,346		7	2,055	2,1017	0,0467										
Total	2,1615				Total	2,2309										
						Petri Dish										
	Recovered R Mix	Recover Materia	ls			Clean	Used	Diff								
		72,6365				129,544	131,0255	1,4815	5							
		3,8744														
	76,3439	76,5109				Extra oil r	emains reco	verd								
						First prec	ipitation									
						Clean	Used	Diff								
						162,528	164,749	2,221	L							
	Theorethical	Actual				Second pr	recipitation									
Starch	2,5	2,394010949				Clean	Used	Diff					1			
Dmso	66	63,20188905				162,4285	162,627	0,1985	5							
							Total	2,4195	5							
	Weight increase in p	roduct														
	1,480389051	g	61,83719			Total proc	duct made									
						Filters	2,2309									
Weight		wi				Beakers	0,162									
Linoleic Acid	280,452	5				Petri dish	1,4815									
Oleic Acid	282,468	6				Total	3,8744									
Palmitic Acid	256,43	30														
Stearic Acid	284,483	59														
	275,74465	100														
Amount of AUG	2,394010949		#fatty acid	d chains ad	ded (mole	s)										
Weight glucose	180,156															
#moles	0,013288544		0,005369													
#OH-groups	0,039865632															
	Degree of substitution	on #fattyacid/#OF	l-groups													
all OH	0,134669768	13,46697683														
Primary OH		40,4009305														

E5: Excel sheet used for calculating degree of substitution

Example Degree of Substitution calculation

Suppose 2.00 grams of starch were put into a reaction. If 10% of the reaction mixture is lost through transferring it from glassware. In total 3.00 grams of product was obtained, which also includes product lost in filters. As the filters were weighed before usage and dried and weighed again after usage.

 $2.00 * 0.9 = 1.80 \ gram$ Each AGU unit in starch has three free alcohol groups moles of $OH = 3 * \frac{1.80}{180.156} = 29.97 \ mmol$ Total weight increase = 3.00 - 1.80 = 1.20

The average molecular weight of a fatty acid (in sunflower oil) was calculated:

Weight		wi
Linoleic Acid	280,452	5
Oleic Acid	282,468	6
Palmitic Acid	256,43	30
Stearic Acid	284,483	59
	275,7447	100

moles of fatty acid attached =
$$\frac{1.20}{275.75}$$
 = 4.35 mmol
Degree of substitution = $\frac{4.35}{29.97}$ = 14.5%

Weight increase
$$= \frac{1.20}{1.80} = 66.7\%$$

E6: Sample calculation for degree of substitution



F (Spectra obtained from second scale up reaction + excel sheet for mass balance)

F1: IR spectrum of the product of the second scale up.



F2: ¹H-NMR spectrum of ethanol residue



F3: ¹H-NMR spectrum of ethanol residue 2

	Into flask	Out of flag	sk		Purificatio	on Steps			Dissolving	g in THF			
DMSO	49,65906	46,79069			1st Precip	itation			1st time d	issolving			
Starch	1,883094	1,774324			500 ml Beaker				100 ml Be	aker			
Sunflower Oil	9,217023	8,684636			Clean	Used	Diff		Clean	Used	Diff		
TBD	0,492923	0,464451			193,0865	193,1695	0,083		62,6316	62,6498	0,0182		
Total	61,2521	57,7141				oitation			2nd time	dissolving			
					Clean	Used	Diff		Clean	Used	Diff		
Dissolving all chem	ical				188,8905	188,9135	0,023		62,6356	62,644	0,0084		
100 ml beaker					3rd Precip	itation				Total	0,0266		
Clean	Used	Diff			Clean	Used	Diff						
62,6024	66,4766	3,8742			188,893	188,9135	0,0205		Filter #	Clean	Used	Diff	
						Total	0,1265		2	2,2103	2,408	0,1977	
Into beaker (100 ml)		Into flask						4	2,1374	2,3129	0,1755	
DMSO	52,8		49,65906						7	2,2779	3,1979	0,92	
Starch	2,0022		1,883094		Oil Remai	ns			8	2,346	2,613	0,267	
TBD	0,5241		0,492923		Precipitation solvent i		t is remove	ed			Total	1,5602	
Sunflower Oil	9,8		9,217023		EtOH rema	ains				Product ir	n Petri Dish		
Total	65,1263	g	61,2521		1000 ml fla	ask				Clean	Used	Diff	
		0,940513			Clean	Used	Diff			82,8106	83,3568	0,5462	
					254,795	313,75	58,955						
Residue in reaction	vessel				EtOH + TH	F remains			Total Proc	luct lost in	purification		
Clean	Used	Diff			Clean	Used	Diff		1,7133	g			
118,266	121,804	3,538			254,774	258,902	4,128		Total proc	l collected			
	0,942239				EtOH + TH	F remains	2		0,5462				
					Clean	Used	Diff		Total proc	I	Starch out c	of reaction	vessel
					254,774	254,937	0,163		2,2595		1,7743242		
Weight		wi				Total	63,246						
Linoleic Acid	280,452	5							Weight ga	in			
Oleic Acid	282,468	6							0,485176	g	27,344258	%	
Palmitic Acid	256,43	30											
Stearic Acid	284,483	59											
	275,7447	100											
Amount of AUG	1,774324		#fatty acid	chains ad	lded (mole	s)	Degree of	substituti	on				
Weight glucose	180,156						All OH	5,955065	%				
#moles	0,009849		0,00176				Primary O	17,8652	%				
#OH-groups	0,029546												

F3: Excel sheet used for calculating degree of substitution

G (Spectra obtained from CTAB at 80°C + excel sheet for mass balance for both CTAB and TBD reaction at 80°C)



G1: IR spectrum of the CTAB product.



G2: ¹H-NMR spectrum of ethanol residue



G3: ¹H-NMR spectrum of ethanol residue 2

CTAB 0,0560	TBD 0,0412		Percent	Percent	#2 CTAB 1: Percent	#1TBD 1 #2CTAB 1 Percent	#1 TBD Clean #2 CTAB 1 Percent	#1TBD Clean #2CTAB 1 Percent	Total 5 Starch/A Clean #1TBD 1 #2CTAB 1 #2CTAB 1	CTAB 5 Total 5 Starch/A Clean #1 TBD Clean #2 CTAB 1 #2 CTAB 1	TIBD TGA Total Starch/A #1TBD Clean #2 CTAB 1 #2 CTAB 1	DMSO TBD CTAB Total Starch// Clean #1TBD #2 CTAB Percent	Starch Starch Starch Starch Starch Starch Starch Starch/ITBB Starch/ITBD #11BD 142 CIean 144 CIe	# In beak Starch DMSO TBD CTAB CTAB Starch/I Total Starch/I #1TBD Clean #2CTAB 1 #2CTAB 1	# In beak Starch In beak DMSD TBD CTAB S Total Starch/I #1TBD Clean #2 CTAB 1	Starch Starch B# In beak Starch DMSO CTAB S Total Starch/A #1 TBD Clean #1 TBD 1 #2 CTAB 1 B Starch/A Clean 1 #2 CTAB 1 #1 TBD 1 #1 TB	# In beak Starch DMSO CTAB TBD CTAB Total Total #1TBD #12CTAB #2 CTAB Total Starch/i Clean #1 TBD #1 TBD #1 TBD Starch/i Clean	Starch CTAB # In beak Starch : DM/SO : TBD : TOTAB : Total : Total : Total : Total : Starch/i Clean : #1TBD : #2 CTAB : Percent :	# In beak Starch CTAB # In beak DMISO DMISO TBD CTAB S Total Starch/I #1TBD Clean #1TBD 1 #2 CTAB 1	# In beak CTAB Starch DMSO TBD TCTAB Total #1TBD #2 CTAB Starch/ Clean #1 TBD Elean Clean 1 1 Percent	TOTAL 6	Cat 1,0712 TOTAL 6 Starch CTAB ## In beak Starch 5 DMSO 5 DMSO 5 TBD 5 TAB 5 Total 5 Total 5 Total 5 Total 5 Total 5 Total 5 Total 1 # 1 PPC 1 Percent	Starch 1,893 Cat 1,0712 TOTAL 6 Starch CTAB # In beak Starch 1 CTAB 5 Total 5	DMSO 49,841 Cat 1,0712 TOTAL 6 Starch Starch CTAB 5 Total In beak Starch/ DMSO 1 TBD 5 Total 5 Total 5 Total 5 Clean 1 #1 TBD 1 #2 CTAB 5 Percent	NaOH 49,841 Starch 1,993 TOTAL 6 TOTAL 6 Starch Starch CrAB # In beak Starch/ DMSO 7 TBD 7 TBD 5 Total	Oil 49,841 DNAOH 49,841 Starch 1,893 Cat 1,0712 TOTAL 6 1,893 Cat 1,0712 TOTAL 6 Starch 5 Starch CTAB 5 Starch 9 DMSO 1 DMSO 5 Total 5 Total 5 Total 5 Total 5 Total 5 Total 1 BD 1 DMSO	# CTAB FI NaOH 1 NaOH 49,841 Cat 1,893 Cat 1,0712 TOTAL 6 Starch 1,0712 TOTAL 6 Starch 1,0712 TOTAL 6 Starch 1,0712 TOTAL 6 Starch 5 DMSO 5 DMSO 5 TBD 5 Total 5 Total 5 #1TBD Clean #1TBD 1 #2 CTAB 1
041587 5,604158697 %		257634 4.125763402 %	tage lost in transfer 257634 4.125763402 %	tage lost in transfer 257634 4.125763402 %	18,265 121,4 tage lost in transfer 257634 4.125763402 %	118,77 121,05 118,265 121,4 18,265 121,4 121,4 121,4 125,7634 125,763402 %	Used Diff 118,77 121,05 18,265 121,4 18,265 121,4 121,4 121,4 121,4 121,05 121,6 121,05 120,05 120,0	dmso beakers Used 121,05 118,77 121,05 121,4 18,265 121,4 18,265 121,4 13,265 121,4 13,265 121,4 121,4 121,4 125,763,402 %	5,9406 52,8056 5 dmso beakers Used 1118,77 121,06 1118,765 121,46 118,265 121,4 118,265 121,4 121,46 121,47	1,1349 1,071298403 1,5,9406 52,8056 5 dmso beakers Used Diff 118,77 121,05 121,05 118,265 121,4 1121,4 118,265 121,4 121,4 118,265 121,4 121,4 118,265 121,4 121,4 128,265 121,4 121,4 129,263 121,4 121,4 120,276,44 1125,753402 %	1,1349 1,071298403 15,5406 52,8056 5 'dmso beakers' Used Diff 118,77 121,05 121,05 118,265 121,4 121,4 128,265 121,4 121,4 128,265 121,4 121,4 128,265 121,4 121,4 128,265 122,4 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In b 5,2,8 49,84100421 In b 1,1349 1,071298403 In b 5,9406 52,8056 5 'dmso be akers Used Diff 118,77 121,05 In 121,05 118,265 121,05 In 121,4 118,265 121,4 In 121,4 118,265 121,4 In 121,4 118,265 121,45 In 121,4 118,265 121,45 In 121,4	rer In flask In b 2.0057 1.893207389 52,8 49,84100421 1.1,349 1.071298403 355,9406 s2,8056 5 (dmso beakers Used 121,05 118,77 121,45 118,77 121,45 118,77 121,45 118,77 121,45 118,77 121,45 121,65 121,45 12	In flask TBE 2,0657 1,893,297,88 52,8 49,84100421 1,1349 1,071,298403 55,9406 52,8056 52,8 10,071,298403 11,1349 1,071,298403 15,9406 52,8056 118,77 Used 118,77 121,05 118,77 121,05 118,76 121,165 118,76 121,26 118,76 121,46 118,76 121,46 118,76 121,46 118,76 121,47	ier In flask TBC 2,0057 1,83927388 In blask 52,8 49,84100421 1,1349 1,071298403 55,9406 52,8056 52,8 10,071298403 1,1349 1,071298403 55,9406 52,8056 51,9406 52,8056 118,77 Used 118,72 121,05 118,265 121,46 118,265 121,4 118,265 121,4 118,265 121,4	in flask TBE 2,0057 1.893297389 52,8 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All OH 4,596527494 % 1		Degree of cubetitutic CTAR	Degree of substitutio (TAR	#OH-group 0,031528 0,031970319	#moles AC 0,010509 0,010656773 C #OH-group 0,031528 0,031970319 Deerse of substitution (TAR TE	Weight gl 180,156 180,156 CI #moles Ad 0,010509 0,010656773 C #OH-group 0,031528 0,031970319 C Decrease of substitution TAR TE	Amount o 1,893297 1,919881588 #f Weight gl 180,156 180,156 C #moles A 0,010509 0,010656773 C #OH-groul 0,031528 0,031970319	CTAR TBD Amount 0 1.893297 1.919881588 #ff Weight glt 180,156 CI CI CI #moles Ad 0.010509 0.010565773 C CI CI #OH-group 0.031528 0.031970319 C CI CI	275,7447 100 CTAB TBD Amounto 1,89327 1,89327 1,91981588 #moles Ad 0,010509 #moles Ad 0,010509 #OH-group 0,031528 Decrease of substitutivity CTAB	Stearic Ac 284,483 59 275,7447 100 CTA TBD Amount 0 1,893297 1,919881588 #f Weight gli 180,156 180,156 #moles Ac 0,010509 0,031970319 #OH-group 0,031528 0,031970319	PalmiticA 256,43 30 Stearic AC 284,483 59 275,7447 100 CTAB TBD Amount o 1,893297 Just b 180,156 Weight glt 180,156 #MOH-group 0,031528 0,031970319 #Dezros of substituti (TAR 10	Oleic Acid 282,468 6 Palmitic A 225,433 30 Stearic Ac 284,483 59 Z75,7447 100 275,7447 Amount o 1,893297 1,919881588 #f Amount o 1,893297 1,919881588 #f Weight gli 180,156 120,1156 CI #moles Ac 0,010509 0,031970319 CI #OH-group 0.031528 0,031970319 CI	Linoleic A 282,452 S Olici A 282,468 6 Palmitic Aci 256,473 30 Stearic Ac 284,483 59 Z75,7447 100 CTAB TBD Amount 0 1,893297 1,919881588 #ff Weight gl 180,156 IS0,001656773 C #moles Ac 0,010509 0,001656773 C	Weight wi Linoleic A 280,452 5 Oleic Acid 282,463 30 Palmitic A 282,463 30 Stearic Ac 284,483 59 Z75,7447 100 CTAB TBD Amount 0 1,893297 1,9981568 #moles AC 0,010509 0,010565773 C #DH-group 0,031528 0,0313970319 C	Degree of substitution wi Weight wi Linoleic A 280,452 5 Oleic Acid 282,468 6 Palmitic A 256,43 30 Stearic Ac 284,483 59 Z75,7447 100 100 CTAB TBD 100 Weight glt 180,156 CI #moles AC 0.010509 0.031970319 #OH-group 0.031528 0.031970319	Degree of substitution wi Weight wi Linoleic A 280,452 5 Oleic Acid 282,468 6 Palmitic A 256,43 30 Stearic Ac 284,483 59 ZTS,7447 100 100 Krabit 1,939297 1,919881588 #f Amount o 1,893297 1,919881588 #f Weight glt 180,156 130,156 C1 #moles Ac 0,010509 0,031970319 0 #Decrea of substricture TCAR 170 100 100	21,10617 48,99356 Degree of substitution wi Weight wi Linoleic A 280,452 Oleic Acid 282,468 Oleic Acid 226,433 StearicA 2 225,7447 ZistaricA 284,483 59 StearicA 284,483 59 ZistaricA 284,483 59 Kinounto 1,893297 Hib 100 Weight glo 180,156 #moles Ac 0,0010556773 #OH-group 0,031528 Degree of substitution 1708	Weight increase % wi 21,10617 48,99356 Degree of substitution wi Unoleic A 280,452 5 Oleic Acid 282,468 6 Palmitic A 282,443 30 Stearic Ac 284,483 59 Zits,7447 100 275,7447 Amount 0 1,893297 1,919881588 Weight git 180,156 180,156 C1 Weight git 40,1528 0,031970319 0 Degree of substitution 100 100	0,399603 0,940618 Weight increase %	CTAB TBD 0.399603 0.940618 Weight increase % 21,10617 21,10617 48,99356 Degree of substitution wi Degree of substitution wi Unoleic A 280,452 Oleic Acid 225,43 Palmitic A 226,43 Stearic Ac 284,483 Veight TBD Amount 0 1,893297 100 275,7447 Weight git 180,156 130,156 Weight git 180,156 0.010509 #moles AC 0.010509 #moles AC 0.0313970319 #Degree of substitution CTAB	WEIGHT INCREASE CrAB TBD 0,399603 0,940618 0,399603 0,940618 Weight increase % 21,10617 21,10617 48,99356 Degree of substitution 100 Degree of substitution 228,468 Oleic Acid 226,453 Oleic Acid 225,7447 PalmiticA 225,7447 StearicAC 227,7477 Weight glv 180,156 Weight glv 180,156 #moles AC 0,0010565773 #OH-group 0,031570319 #Degree of substitution 174	1,893297 1,919882 WEIGHT INCREASE IDD 0,399603 0,940618 Weight increase % Increase % 21,10617 48,99356 Degree of substitution Mi Unoleic A 280,452 Olici Acid 282,468 Olici Acid 282,463 Stearic A 284,483 Stearic A 284,483 Stearic A 284,483 Amounto 1,893297 1,919881588 #f Weight B 180,156 Global B 0,031970319 #OH-group 0,031528 0,031970319	CTAB TBD 1,893297 1,91982 WEIGHT INCREASE ED CTAB TBD 0,399603 0,940618 Weight increase % 21,10617 21,10617 48,99356 Degree of substitution wi Unoleic A 280,452 Oleic Acid 282,483 Stearic Ac 284,483 275,7447 100 Amounto 1,893297 Amounto 1,893297 400+group 0,01056773 #OH-group 0,031970319 #OH-group 0,0313970319	STARCH IN FLASK TIBD L393297 1919822 VEIGHT INCREASE 191982 CTAB TBD 0,399603 0.940618 VVEIGHT INCREASE 2000 21,10617 48,93356 21,10617 48,93356 Degree of substitution wi Vieight 282,463 30 Oleic Acid 282,443 30 Stearic Ac 284,483 59 Stearic Ac 284,483 59 Amounto 1,893297 1,10981588 Weight git 180,156 130,156 CT Weight git 180,156 0,0313970319 #OH-group 0,031528 0,0313970319 0	STARCHIN FLASK CTAB TBD 1,8932.97 1,919882 WEIGHT INCREASE CTAB CTAB TBD 0,399603 0,940618 Weight increase % 21,10617 21,10617 48,99356 Degree of substitution wi Linoleic A 280,452 Oleic Acid 282,468 Palmitic A 225,7447 Amount o 1,893297 Amount o 1,893297 Weight git git 180,156 130,156 Weight group 0,031573 C	STARCH IN FLASK TARCH IN FLASK CTAB TBD 1,893297 1,919882	STARCH IN FLASK TBD I 393.297 1,393.297 1,393.287 CTAB TBD 0,395603 0,940618 Weight INCREASE CTAB TBD 0,395603 0,940618 Degree of substitution Degree of substitution Weight Wi Mi Degree of substitution Veight 282,452 5 0 of cital as 256,43 50 Dineic A 282,453 59 59 59 Stearic Ac 284,483 59 59 50 100 75,7447 100 75,7447 100 75,938 #f Amount 0 1,893297 1,919881588 #f Meight get 40,192,528 0,0313970319 40+ 6 #0H-group 0,031528 0,0313970319 0 6 71 71 71
10,66988 %	TBD				0,001449 0,003411	CTAB TBD 0,001449 0,003411	#fatty acid chains added (n CTAB TBD 0,001449 0,003411	#fatty acid chains added (n CTAB TBD 0,001449 0,003411	#fatty acid chains added (m CTAB TBD 0,001449 0,0034111	#fatty acid chains added (m CTAB TBD 0,001449 0,0034111	#fatty acid chains added (n CTAB TBD 0,001449 0,003411	#fatty acid chains added (n CTAB TBD 0,001449 0,003411	#fatty acid chains added (n CTAB TBD 0,001449 0,003411	#fatty acid chains added (r CTAB TBD 0,001449 0,003411	#fatty acid chains added (r CTAB TBD 0,001449 0,003411	#fatty acid chains added (r CTA8 TBD 0,001449 0,003411	#fatty acid chains added (n CTAB TBD 0,001449 0,003411	#fatty acid chains added (n CTAB TBD 0,001449 0,0034111	#fatty acid chains added (m CTAB TBD 0,001449 0,003411	#fatty acid chains added (m CTAB TBD 0,001449 0,003411	#fatty acid chains added (m CTAB TBD 0,001449 0,003411	#fatty acid chains added (m CTAB TBD 0,001449 0,003411	#fatty acid chains added (n CTAB TBD 0,001449 0,0034111	#fatty acid chains added (n CTAB TBD 0,001449 0,0034111	#fatty acid chains added (n CTAB TBD 0,001449 0,003411	#fatty acid chains added (m CTAB TBD 0,001449 0,003411	#fatty acid chains added (m CTAB TBD 0,001419 0,003411

G4: Excel sheet used for calculation of degree of substitution

H (Spectra obtained from TBD at 80°C)



H1: IR spectrum of the TBD product.



H2: ¹H-NMR of ethanol residue



H3: ¹H-NMR of ethanol residue 2



I (Spectra obtained from bulk reaction + excel sheet for mass balance)

I1: IR spectrum of the bulk product.



I2: ¹H-NMR of ethanol + Ether residue

	Into flask	Out of fla	sk		Purificati	on Steps		Filter #	Clean	Used	Diff						
DMSO	0	0			1st Preci	oitation		1	2,1404	3,3453	1,2049	Filters cor	ntained a lo	ot of oil,			
Starch	1,929357	1,922294			500 ml Be	eaker		3	2,1438	2,5068	0,363	3 from the bulk reaction.		on.			
Sunflowe	64,49131	64,25522			Clean	Used	Diff	5	2,1025	2,6306	0,5281						
TBD	0,505032	0,503184			188,91	1 188,9445	0,0335	6	2,3745	2,524	0,1495						
Total	66,9257	57 66,6807			2nd Prec	2nd Precipitation				Total	2,2455						
					Clean	Clean Used			Product ir	Petri Dish							
Dissolving	Dissolving all chemical				193,056	5 193,129	0,0725		Clean	Used	Diff						
	100 ml bea	aker				Total	0,106		37,5404	38,6022	1,0618						
	Clean	Used	Diff														
	56,0763	58,6031	2,5268						Total Proc	luct + lost i	n purificat	ion					
					Oil Rema	ins			2,3862	g							
	Into beake	er (100 ml)	r (100 ml)		0 ml) Into flask		Precipitation solve		t is removed		Total proc	collected					
			0		D EtOH + Et	20 remains			1,0618			Weight in	Weight increase %				
	Starch	2,0022		1,929357	7 1000 ml f	lask						24,13296					
	TBD	0,5241		0,505032	2 Clean	Used	Diff	Degree of	substituti	on							
	Sunflowe	66,9262		64,49131	l 161,59:	1 225,835	64,244	Weight		wi							
	Total	69,4525	g	66,9257	7 EtOH + TH	HF + Et2O re	mains	Linoleic A	280,452	5							
			0,963618		Clean	Used	Diff	Oleic Acio	282,468	6							
					161,6	161,786	0,166	Palmitic A	256,43	30							
	Residue ir	n reaction v	eaction vessel			Total	64,41	Stearic Ac	284,483	59							
	Clean	Used	Diff		Dissolvin	g in THF			275,7447	100							
	118,772	119,017	0,245		1st time	dissolving			СТАВ								
		0,996339			100 ml Be	eaker		Amount o	1,922294			#fatty acid	d chains ad	ded (mole	s)		
					Clean	Used	Diff	Weight gl	180,156								
					56,106	56,1416	0,0347	#moles A	0,01067			0,001682					
						Total	0,0347	#OH-grou	0,03201								
								Degree of	substituti	TBD							
								All OH		5,255705	%						
								Primary C	н	15,76712	%						

13: Excel sheet used for calculating degree of substitution

J (Spectra obtained from product films)



J1: IR spectrum of CTAB product film



J2: IR spectrum of TBD product film



J3: IR spectrum of scale up 2 product film.