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Adjusting Experimental Parameters to Modify Corn Starch with Glycidyl Methacrylate to Tune Specific Properties

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Chemical modification reactions are viable alternatives for improving the properties of starch because they allow the insertion of molecules that change the original behavior of the polymer. Glycidyl methacrylate (GMA) is an example of a vinylic molecule added to starch to decrease its hydrophilicity and improve mechanical properties. This modification reaction is affected by parameters like the volume of the modifier, time, and temperature. Furthermore, the correlation between these parameters might change the response of an individual parameter on the degree of substitution (DS), crystallinity (X_c), and molecular-weight-related parameters. This work evaluated how these effects and their correlations would affect DS, X_c, molecular weight, and polydispersity index (PDI) of starch. The results suggested that, while higher volumes of GMA and temperatures decreased the DS and X_c, the correlation between them increased the DS by 2.47, and X_c by 1.25. On the other hand, higher temperatures also favored the occurrence of hydrolysis. Also, although none of the parameter correlations had a significant effect on the assessed properties (at 95%), the statistical analysis confirmed that the correlation between two parameters changed the effect of individual parameters.

Keywords: chemical modification, factorial design, natural polymer, polysaccharide, physical-chemical characterization

Introduction

The material science field has considerably grown in the twentieth-first century because its materials are versatile, display good performances regarding their intended application, and tend to be friendly to the environment. This field benefits greatly from the availability and versatility of synthetic and, especially, natural and green polymers.

The excitement surrounding green polymers lies not only in their renewability, biodegradability, and relatively low cost[1] but also in their eco-friendly character, which is capable of preventing the buildup of waste.[2,3] Proteins and polysaccharides are examples of natural (therefore, green) polymers commonly used in materials science for the most varied applications like food coatings,[4] hydrogels,[5] scaffolds,[6] wound dressings,[7] and so on.

Starch is an example of a green polymer widely used for biomaterial production. This complex carbohydrate is composed of amylose and amylopectin, being amylose (α(1,4)-linked-D-glucose residues) a linear and hydrophilic polymer while amylopectin (formed by D-glucose residues with α (1,4) and α (1,6) linkages) is a branched and less hydrophilic polymer.[8] Despite being biocompatible, biodegradable, and non-toxic, the performance of starch-based materials is often compromised by the inter and intramolecular hydrogen bond formation, and by the high hydrophilicity of this polymer.[9]

Among the alternatives available for improving the properties of natural polymers like starch, one can mention methods of combination (like the formation of composites, and blends), and chemical modification reactions.[10] The formation of composites involves the addition of organic or inorganic particles like titanium dioxide (TiO_2),[11] and silicon dioxide (SiO_2)[12] to the polymer. Even though this method is efficient, it is not always suitable for all materials-

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Editor handled this article: Fernando C. Giacomelli (Associate)

producing techniques. For example, for electrospinning, the addition of solid particles to polymeric solutions as property enhancers is limited by the diameter of the needle and the solid particles. If the solid particles have a diameter that is higher than the diameter of the needle, the particles will clog the needle, preventing the electrospinning of the solution.

The formation of blends usually overcomes the limitation of needle-clogging mentioned before, for example. The polymer blended with starch act as a plasticizer, reducing the interaction between adjacent starch chains. Poly(lactic acid),[13] poly(vinyl alcohol),[14] and poly(caprolactone)[15] are examples of polymers blended with starch. Although this method usually succeeds in improving the properties of starch-based materials, chemical modification reactions are still the simplest and preferred alternative for minimizing the drawbacks of starch.[10]

Chemical modification reactions incorporate new molecules into the structure of the polymer, changing the solubility and other properties of starch. They allow the insertion of (*i*) molecules that increase or decrease the hydrophilicity of starch,[16] (*ii*) groups required for further reactions (e.g., vinyl groups for later crosslinking formation reactions via radical polymerization),[17] or (*iii*) bioactive molecules (e.g., folic acid),[18] for example. Acetylation, oxidation, and hydroxypropylation are examples of chemical modifications usually performed in starch.[10]

Glycidyl methacrylate (2,3-epoxypropyl methyl propenoate; GMA)[19] is an example of a less toxic[20,21] and relatively cheaper vinylic molecule used to increase the hydrophobic character of starch.[22] Its graftization to the hydroxyl group (OH) from starch decreases the water affinity originally presented by starch because it decreases the possibility of adjacent OH groups interacting with each other via hydrogen bond. Besides, the presence of a vinylic group allows the formation of crosslinking between adjacent starch chains, which is particularly appealing for the production of hydrogels,[23,24] for example.

The chemical modification reactions used for starch were already extensively studied. However, the literature reports usually focus on the effect that a particular parameter has on the final property of the modified starch and ignore the correlation between experimental parameters. The ignorance of correlations and the hasty attribution of a behavior to a particular parameter might lead to incorrect conclusions. For example, Pellá *et al.*,[25] evaluated how the statistical correlation between experimental parameters affected the solubility of an edible coating prepared by physically blending native cassava starch, casein, gelatin, and sorbitol. The statistical analysis confirmed that higher amounts of gelatin increased the solubility of the coatings

by 3.990, while higher amounts of casein decreased the solubility by 1.388. However, when statistically correlated, higher amounts of gelatin and casein considerably increased the solubility of the coatings (it increased by 9.256). It confirms that considering the correlation of two parameters is a matter of utmost importance when designing an experiment that involves the modification of a green polymer and that ignoring this correlation might compromise the efficiency of the developed material.

It is known that parameters like temperature and monomer concentration affect the graftization of GMA into starch.[26] Therefore, this work aimed to apply statistical comparisons to determine how the correlation between these graftization-related parameters (temperature, time, and volume of modifier) would truly affect the properties of the modified starch regarding the degree of substitution, the crystallinity degree, the molecular weight, and the thermal stability. These comparisons were expected to determine whether the correlation of two parameters would change the effect of the individual parameters or not, and, in positive cases, by how much the parameters determine the specific application of the material based on the assessed properties.

Experimental

Materials

Corn starch (number-average molecular weight (M_n) = 201.664 g mol^{-1}, weight-average molecular weight (M_w) = 1.886 × 10^6 g mol^{-1}, M_w/M_n = 9.354, as determined by size exclusion chromatography (SEC) analysis; degree of branching = 9.76%, as determined by ^{1}H nuclear magnetic resonance (NMR) analysis), glycidyl methacrylate (GMA; 99.8%), and deuterated dimethyl sulfoxide (DMSO-d_6) were acquired from Sigma-Aldrich (Burlington, USA). Propanone, hydrochloric acid (HCl; 37%), and dimethyl sulfoxide (DMSO) were acquired from Synth (Diadema, Brazil). Lithium chloride (LiCl) was acquired from Vetec Química Fina LTDA (Duque de Caxias, Brazil).

Methods

Optimization of the initial conditions

The initial goal of this work was to determine the minimum amount of time required to graft GMA into starch. For this experiment, 10 g of starch was solubilized in a solvent mixture composed of 100 mL of DMSO and 100 mL of distilled water under magnetic stirring. After solubilization, the solution temperature was increased to 90 °C, and the solution was gelatinized at this temperature for 15 min. Since the solvent mixture contained DMSO,

the gelatinization of starch (St) led to a solution rather than a high-viscosity gel. Subsequently, the pH was adjusted to 4 using a 0.1 M HCl solution, as described by Lima-Tenório *et al.*[17]

The graftization reaction started after the addition of 2 mL of GMA[17] into the gelatinized starch solution. The solution was kept under magnetic stirring at T = 60 °C. Aliquots (3 mL) were collected after specific times (t = 1, 2, 3, 4, 5, 6, 8, 24, and 50 h), precipitated in propanone, vacuum-filtered, and the filtered precipitate was redissolved in distilled water. This process (precipitation followed by redissolution) was performed three times.

The final material was transferred into a desiccator coupled to a vacuum pump and dried for 24 h (T ca. 25 °C), nitrogen-frozen, and lyophilized (24 h; T = −55 °C) to produce the dry samples. They were named St-x, in which St refers to the starch molecules with GMA grafted into them, and x specifies the duration of the graftization reaction (x = 0, 1, 2, 3, 4, 5, 6, 8, 24, or 50 h). The graftization of GMA into St occurred already after 1 h of reaction, as confirmed by Fourier transform infrared (FTIR) analysis (Figure S1, Supplementary Information (SI) section) and discussed in the SI section.

Starch modification with GMA (^{GMA}St)

The effect of the reaction time, the volume of GMA, and temperature over the graftization of GMA into St were evaluated using an experimental design 2^3 with 3 central points, totalizing 11 experiments. Starch was solubilized using the same conditions described in "Optimization of the initial conditions" sub-section. The graftization reaction was performed using the conditions presented in Table 1.

Table 1. The volume of GMA, temperature, and time of reaction, evaluated in the 2^3 factorial design with 3 central points

Level	Factor		
	Reaction time / h	Volume of GMA / μL	Temperature / °C
Lowest (−1)	2	631	60
Central (0)	3	770	70
Highest (1)	4	911	80

GMA: glycidyl methacrylate.

At the end of the graftization reaction, 150 mL of propanone was added (dropwise) into the beaker containing the solution, forming a precipitate. This precipitate was vacuum-filtered, transferred to a beaker, and dissolved in water. Propanone was added to the beaker to precipitate the material one more time. This washing process (precipitation in propanone and solubilization in water) was performed three times.

The final product was precipitated in propanone one last time, vacuum-filtered, and transferred to Petri dishes. The material was kept in a desiccator coupled to a vacuum pump for 24 h at room temperature (T = (25 ± 2) °C) to remove possible remaining propanone molecules. Subsequently, the material was nitrogen-frozen, lyophilized (24 h; T = −55 °C), and stored in a refrigerator (T ca. 8 °C).

The samples were named ^{GMA}St X-Y-Z, where ^{GMA}St refers to starch modified with GMA, X refers to the modification time (X = 2, 3, or 4 h), Y refers to the volume of GMA (Y = 630, 770, or 911 μL), and Z refers to the temperature used for the modification reaction (Z = 60, 70 or 80 °C). The central point samples still present the letters a, b, or c in their name merely to differentiate them from each other.

Characterizations

FTIR

The chemical modification of St with GMA was evaluated by FTIR. The analyses were performed in a PerkinElmer Equipment (Waltham, USA) using the attenuated total reflection (ATR) mode, from 4000 to 600 cm^{-1}, performing 128 scans for each sample, reaching a resolution of 4 cm^{-1}.

The obtained data were normalized, and the baseline was also subtracted for each FTIR spectra to ensure a more accurate comparison between the samples.

^{1}H NMR

^{1}H NMR analyses were used to confirm the modification of St with GMA, and to determine the degree of substitution (DS) of each sample. For the analysis, ca. 20 mg of each ^{GMA}St X-Y-Z sample was placed in an individual NMR tube containing 1 mL of DMSO-d_6. The tubes were placed in an ultrasound (40 kHz, Odontobras Ultrasonic Cleaner 1440 D, São Paulo, Brazil) for 60 min.

The analysis was performed using sodium 3-(trimethylsilyl) propionate (TPS; 2,2,3,3-d_4) as the internal standard (0 ppm), and the spectra were recorded in a Varian spectrophotometer Mercury Plus BB (Palo Alto, USA), operating at a frequency of 300 Hz, pulse width of 90° (PW = 90°), and recycle time of 30 s.

The obtained data were used to determine the degree of branching (DB) presented by the starch used in the experiments, and the degree of substitution (DS). The DB was determined using equation 1, in which α-1,4 refers to the area under the peaks of chemical shift between 5.60 and 5.21, and α-1,6 refers to the area under the peaks of chemical shift between 4.95 and 4.83.[27] The integration was performed using end-points as the baseline.

$$DB(\%) = \frac{\alpha\text{-}1,6}{(\alpha\text{-}1,4) + (\alpha\text{-}1,6)} \times 100 \qquad (1)$$

The DS was determined as described by Abdul Hadi *et al.*,[28] using equation 2, which A_{CH3} refers to the area under the CH_3 peak from GMA,[17] and A_{glu} refers to the area of the protons from the glucose unity.[29]

$$DS = \frac{A_{CH_3}}{A_{glu}} \qquad (2)$$

Size exclusion chromatography (SEC)

Pure starch (St) and the modified starch samples (^{GMA}St X-Y-Z) were analyzed by size exclusion chromatography to determine the number-average molecular weight (M_n), weight-average molecular weight (M_w), and the polydispersity index (PDI; M_w/M_n). The analysis was performed in a Viscotek SEC-Max VE2001 equipment, with RI-Viscotek VE3580 detector, Viscosimetric, and Light Scattering-modulo 270 Dual detector, both from Malvern Instruments (Malvern, United Kingdom), using aqueous columns (SHODEX SB806M-HQ).

The samples were dissolved in DMSO for 24 h before the analysis. Next, 200 µL of solution (concentration equivalent to 3.0 mg mL^{-1}) was injected into the equipment. The analysis was performed at 40 °C and using a mobile phase consisting of water/LiCl 0.1 M (flow rate of 1.0 mL min^{-1}). The absolute molecular weight, expressed in Daltons, was obtained by integrating the signals. A poly(ethylene oxide) (PEO) calibration curve was used as the standard.

X-ray diffraction (XRD)

The XRD analysis was performed in a diffractometer (Shimadzu 6000, Kyoto, Japan) using monochromatic radiation Cu Kα (λ = 1.5405 Å), a voltage of 40 kV, and a current of 30 mA. The measures were performed at a scanning rate of 2° min^{-1}, at diffraction angles (Bragg's angle; 2θ) ranging from 5 to 40°.

The crystallinity of St and the ^{GMA}St X-Y-Z samples was determined as described by Lopez-Rubio *et al.*[30] Initially, the obtained data were normalized and the baseline was subtracted. The peaks observed in the diffractogram were deconvoluted, and the area of each peak was determined by integration. The crystallinity was determined using equation 3, where A_{C_i} refers to the area of each peak i, and A_t is the total area under the diffractogram.

$$X_c = \frac{\sum_{i=1}^{n} A_{c_i}}{A_t} \qquad (3)$$

Differential scanning calorimetry (DSC)

The DSC analysis was performed in a Shimadzu DSC-60 differential scanning calorimeter (Kyoto, Japan). Weighted samples (m = (6.55 ± 0.23) g) were placed in aluminum crucibles and heated from 28 to 200 °C at a heating rate of 10 °C min^{-1} in an inert atmosphere produced using nitrogen (N_2; flow rate of 50 mL min^{-1}).

The enthalpy involved in the thermal events was determined by integrating the area under the curve using the end-points straight line as the baseline.[31]

Statistical comparisons

The statistical analysis was performed using Statistica[32] and Minitab.[33] The data were evaluated by analysis of covariance (ANOVA) and analysis of effects, and the statistical results were presented in Pareto charts.

Results and Discussion

### Chemical modification of starch with glycidyl methacrylate

Figure 1 displays a mechanistic suggestion for the reaction of starch and GMA. The reaction was performed in an acidic medium. Therefore, the first step towards the modification reaction involved the protonation of the epoxy ring from GMA. Epoxides are known for their high reactivity, caused by the high tension in the three-member heterocycle.[34] The reactivity increases when the epoxy-ring has a protonated oxygen because it creates a better leaving group (alcohol rather than an alkoxide) for the nucleophilic substitution reaction.[35] It allows a weak base such as starch to act as the nucleophile, attacking the epoxy ring. This attack might occur in either carbon atoms from the ring. However, the formation of a partial positive charge over a secondary carbon (Cf in Figure 1) leads to a more stable intermediate than a primary carbon (Cg in Figure 1).[34,35] Hence, even though the reaction might lead to two products, the major product will be the one obtained by the attack represented in A (Figure 1). After the nucleophilic attack, the loss of a proton from the protonated starch ensures the formation of stable products (A and B) and regenerates the catalyst.

Physical-chemical characterization

The final product, it is, the modified starch (^{GMA}St), was characterized by FTIR. Figure 2a displays the obtained spectra. The spectra were grouped according to the reaction time to facilitate data visualization. The spectra presented characteristic starch bands as, for example, the stretching of OH, CH sp^3, and C−O at 3343,[36] 2930, and 999 cm^{-1},[36,37]
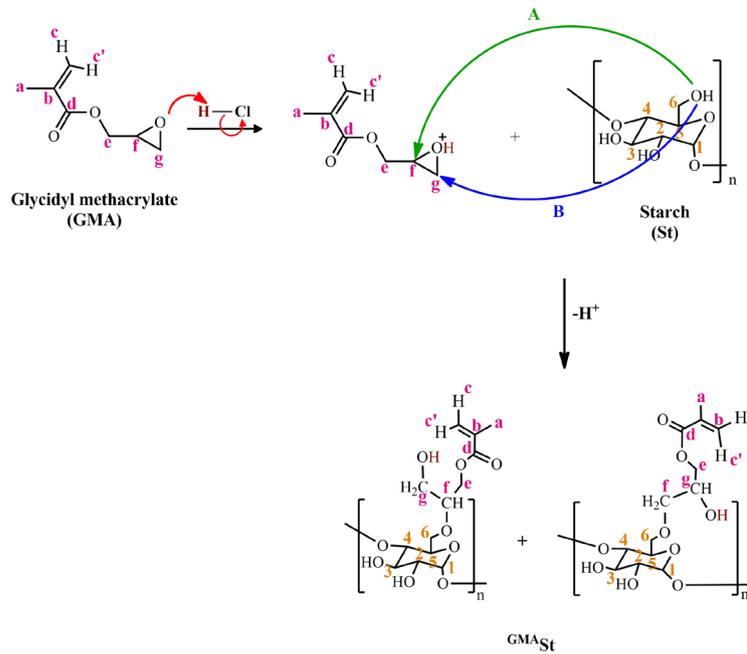
Figure 1. Mechanistic suggestion for the modification of starch with glycidyl methacrylate in an acidic medium.
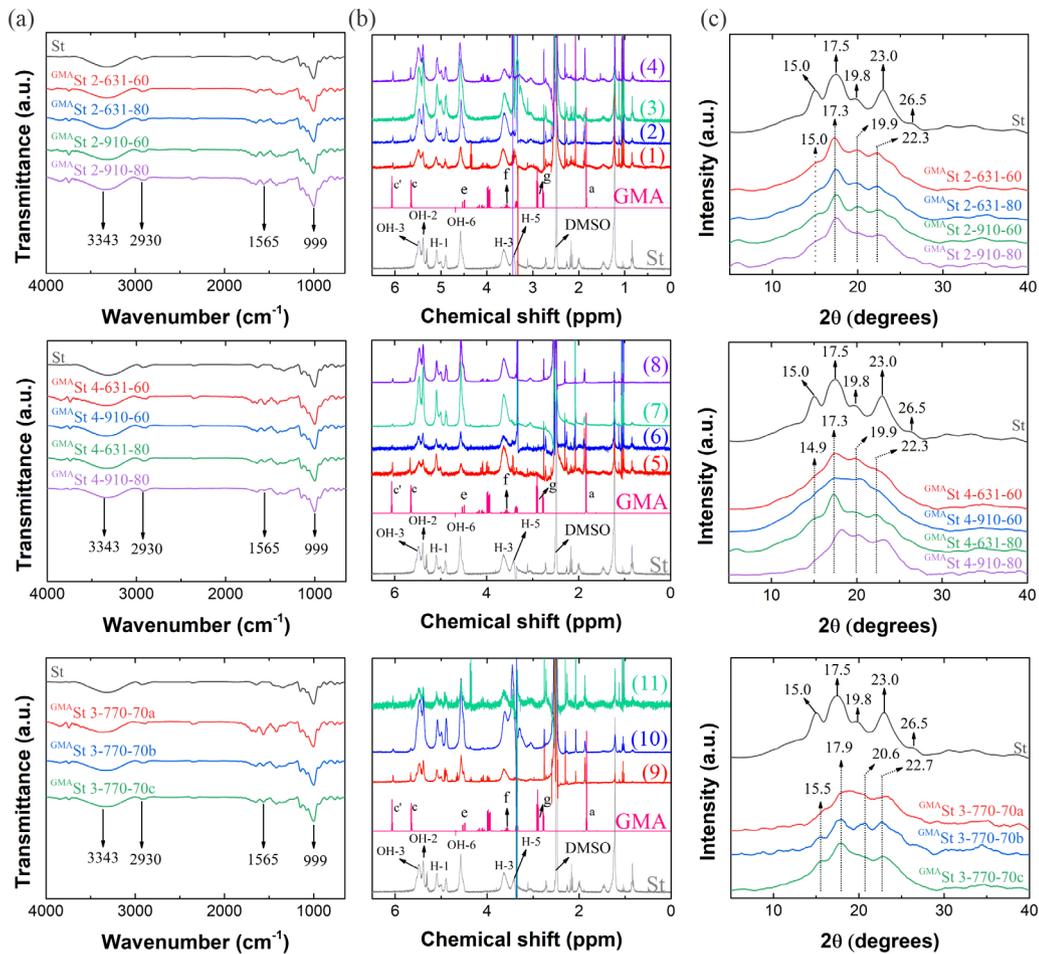
Figure 2. (a) FTIR-ATR and (b) ¹H NMR spectra, and (c) X-ray diffractograms of the different GMASt X-Y-Z experimental conditions. The obtained data was grouped according to the modification time (2, 4, or 3 h) to facilitate data visualization. In the NMR spectra, the numbers specify the samples: (1) ᴳᴹᴬSt 2-631-60, (2) ᴳᴹᴬSt 2-631-80, (3) ᴳᴹᴬSt 2-910-60, (4) ᴳᴹᴬSt 2-910-80, (5) ᴳᴹᴬSt 4-631-60, (6) ᴳᴹᴬSt 4-910-60, (7) ᴳᴹᴬSt 4-631-80, (8) ᴳᴹᴬSt 2-910-80, (9) ᴳᴹᴬSt 3-770-70a, (10) ᴳᴹᴬSt 3-770-70b, (11) ᴳᴹᴬSt 3-770-70c.

respectively. The band 1565 cm^{-1} confirms the presence of GMA in the final product. It describes the axial deformation of the C=C bond from GMA.[8,38]

The results confirm the successful modification of starch with GMA in all the evaluated conditions. However, it is not possible to establish which of the two possible products was the major one by FTIR. Also, the low intensity of the GMA bands on the IR spectra suggests that only a small amount of GMA molecules grafted into starch.

Pure starch, GMA, and the [GMA]St X-Y-Z samples were also characterized by ¹H NMR analysis. Figure 2b displays the obtained results. Characteristic starch peaks appeared between δ 3.15 ppm and δ 5.50 ppm. The peaks between δ 3.15 and 3.67 ppm come from the hydrogens (CH-2, 6; Figure 1) from the glucose unity while the peaks between δ 4.58 and 5.50 ppm come from the hydrogens from hydroxyl groups and CH-1.[29] In the GMA spectra, the signal at δ 1.92 ppm refers to protons Ha from the CH$_3$ group. The peaks at δ 2.60 and 2.80 ppm refer to the protons from the epoxy-ring (Hg and Hf, respectively). The vinylic protons appeared at δ 5.80 ppm (Hc) and δ 6.10 ppm (Hc').[17]

As described in "Chemical modification of starch with glycidyl methacrylate" sub-section, the modification of St with GMA occurs via a ring-opening mechanism. Therefore, the peaks δ 2.60 and 2.80 ppm were no longer seen in the spectra of the [GMA]St X-Y-Z samples. The ring-opening should lead to the observance of two new peaks at δ 4.20 and 4.70 ppm.[17] However, the peak at δ 4.20 ppm could not be separated from the noise in some of the spectra due to its low intensity, and the peak at δ 4.70 ppm overlapped with the signals from the starch protons. Nevertheless, the observance of vinylic hydrogen peaks in the [GMA]St X-Y-Z spectra confirms the modification of St with GMA. The Hc and Hc' peaks appeared at δ 5.60 and 6.04 ppm, respectively.[17] The low intensity of these peaks in the [GMA]St X-Y-Z spectra in comparison to the GMA spectra also reinforces that only a few GMA molecules successfully grafted into starch.

X-ray diffraction analysis was used to assess the effect that grafting GMA into starch had on the semicrystalline structure of starch. Figure 2c presents the X-ray diffractograms obtained for all the experimental conditions. Pure starch (St) presented characteristic diffraction peaks at 2θ = 15.0, 17.5, 19.8, and 23.0°. The diffractogram pattern presented by St is typical of A-type semi-crystalline starch.[39,40] This polymorph has amylose packed in a double-helical conformation,[41] crystallized in orthogonal unit cells.[42] Moreover, the absence of a peak at 2θ = 6.3° excludes the possibility of the B-type polymorph.[41]

The XRD results confirmed that the incorporation of GMA into St changed the original crystalline structure

presented by St. The shift observed for the peak originally at 2θ = 23° (which shifted to 2θ ca. 22), the disappearance of the peak at 2θ = 26.5°, and the broadening of some peaks strongly suggest that the modification reaction increased the amorphous character of St. Nevertheless, the rupture of St granules during the gelatinization step (before the modification reaction)[43] also contributed to the increase of the amorphous character of the samples. Some of the samples ([GMA]St 2-631-60, [GMA]St 2-631-80, [GMA]St 2-910-60, [GMA]St 2-910-80, [GMA]St 4-631-80, and [GMA]St 3-770-70c) presented a shoulder at 2θ ca. 6°. This peak indicates the retrogradation of starch to the B-type polymorph.[44]

The average molecular weight (M_n, M_w) and the polydispersity index of the samples were determined by SEC (Table 2). The number-average molecular weight (M_n) of all samples but [GMA]St 2-631-80 and [GMA]St 4-631-80 increased after the modification, confirming the graftization of GMA into starch. The M_n of the aforementioned samples, on the other hand, decreased. Theoretically, the graftization of GMA into starch was only supposed to increase M_n due to the formation of a chemical bond with a new molecule (GMA molecules). Therefore, this decrease in M_n suggests that the acidic medium used in the reaction might have favored the occurrence of a hydrolysis reaction (in the oxygen atom bonding two starch glucose units) simultaneously to the modification reaction with GMA. Hence, even if the GMA molecules were successfully grafted into starch, the hydrolysis would change the extension of the starch chains, decreasing the M_n of the samples (number of chains of the same molecular weight).

The changes in the polydispersity index of all samples reinforce the hydrolysis theory. For example,

Table 2. Number-average molecular weight (M_n), weight-average molecular weight (M_w), and the polydispersity index (PDI; M_w/M_n) of pure starch and the [GMA]St X-Y-Z samples

Sample		M_n / (kg mol^{-1})	M_w / (kg mol^{-1})	PDI
0	St	201.664	1886.0	9.354
1	[GMA]St 2-631-60	265.278	1238.0	4.668
2	[GMA]St 2-631-80	131.803	1716.0	13.020
3	[GMA]St 2-910-60	224.414	1770.0	7.885
4	[GMA]St 2-910-80	226.993	1469.0	6.469
5	[GMA]St 4-631-60	268.982	2325.0	8.645
6	[GMA]St 4-910-60	256.022	2248.0	8.781
7	[GMA]St 4-631-80	176.665	1281.0	7.253
8	[GMA]St 2-910-80	215.943	1569.0	7.266
9	[GMA]St 3-770-70a	255.100	2147.0	8.415
10	[GMA]St 3-770-70b	237.161	2213.0	9.331
11	[GMA]St 3-770-70c	201.367	1845.0	9.161

samples ^GMASt 2-631-60 and ^GMASt 2-631-80 presented, respectively, the lowest (4.668) and the highest (13.020) PDI values. It suggests that, for the reaction performed for 2 h using 631 μL of GMA, a higher temperature favored the formation of largely polydisperse chains (favored the hydrolysis). However, for the samples ^GMASt 4-631-60 and ^GMASt 4-631-80, the PDI value was not too sensitive to the temperature used in the reaction, leading to closer PDI values (8.645 and 7.253, respectively).

The aforementioned results suggest that exposing the polymer for longer reaction periods at a higher temperature favored the graftization reaction instead of hydrolysis. A higher temperature would fasten the solubilization of GMA into the aqueous medium. One shall not forget that the first modification step involved the deprotonation of the reactive OH groups from starch. The results suggest that once the proton is transferred to GMA, the hydrolysis reaction could no longer occur, and the graftization becomes the preferred reaction. Furthermore, the higher temperature would also increase the probability of GMA molecules presenting the minimum required energy to react with the OH groups from starch.[45]

The literature does not report the competition between the hydrolysis and graftization reaction of GMA into starch using conditions similar to the ones presented in this work.

Thermal stability

Differential scanning calorimetry was used to evaluate the changes in thermal transitions of St after grafting the GMA molecules into it. Figure 3 presents the obtained results. Corn starch usually presents an endothermic peak at T ca. 70 °C, referring to the phase change caused by the dissociation of the amylopectin chains during gelatinization, which was previously arranged in double helices, followed by the collapse of the crystalline structure caused by the temperature employed in the process.[43,46] However, the endothermic event observed in the thermograms does not refer to this particular transition because starch had already been gelatinized (during the modification step) and no longer had its original crystalline structure to lose.

The broad endothermic peaks centered at ca. 100 °C in Figure 3 provide information about the thermal stability of each modified starch sample. Regardless of the experimental conditions, the ^GMASt X-Y-Z samples presented very similar thermal behavior. This result suggests that, even though the graftization of GMA molecules into St changed the stability of the non-modified polymer, the change was not strongly affected by the extent of the modification reaction. It also suggests that the destruction of the crystalline structure of starch had a more significant effect on the thermal stability than the graftization of GMA into the St chains itself.
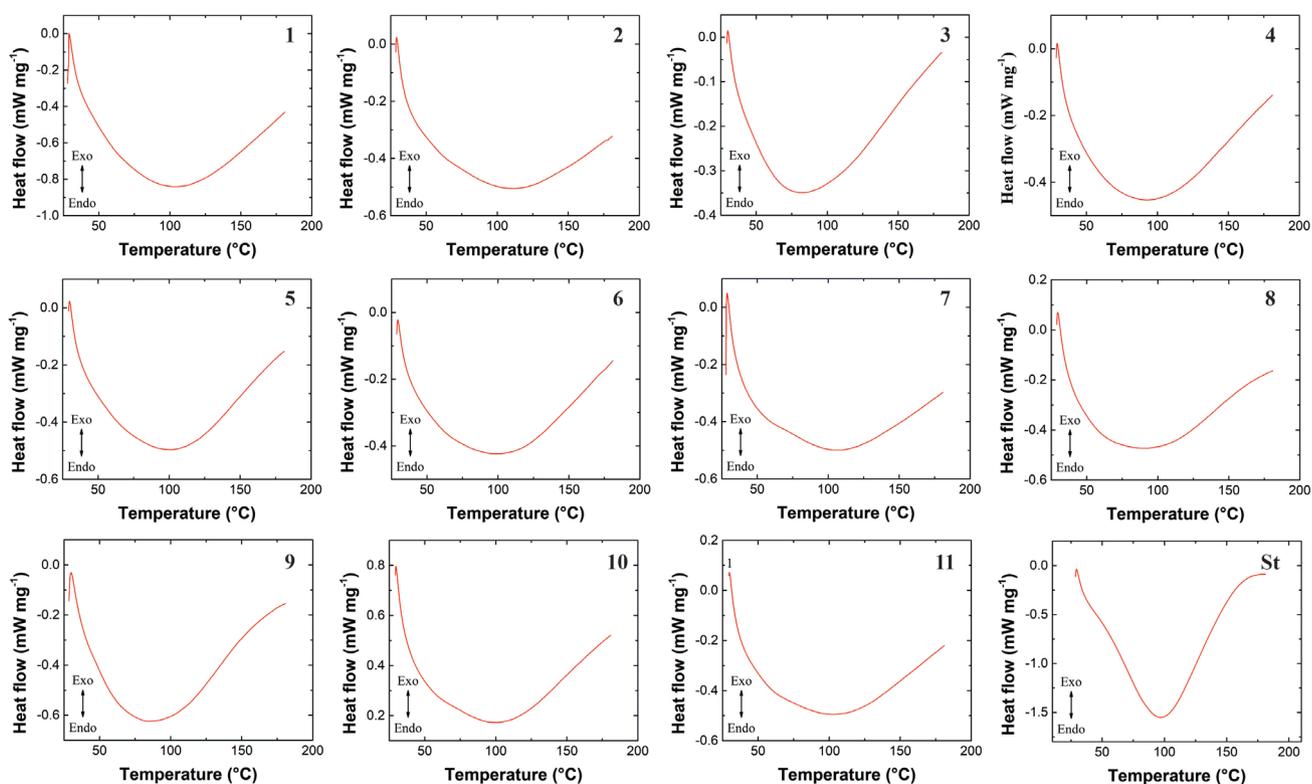
Figure 3. DSC thermograms of (1) ^GMASt 2-631-60, (2) ^GMASt 2-631-80, (3) ^GMASt 2-910-60, (4) ^GMASt 2-910-80, (5) ^GMASt 4-631-60, (6) ^GMASt 4-910-60, (7) ^GMASt 4-631-80, (8) ^GMASt 2-910-80, (9) ^GMASt 3-770-70a, (10) ^GMASt 3-770-70b, (11) ^GMASt 3-770-70c.

The DSC results were used to calculate the enthalpy involved in the endothermic event observed in the thermograms. Table 3 presents the onset temperature (T_o; the beginning of the thermal event), the peak temperature (T_p; the center of the thermal event), and the melting temperature (T_m; the ending of the thermal event). The enthalpies involved in the endothermic event observed in Figure 3 were smaller for the modified samples than for pure starch (St), ranging from 32.3 J g^{-1} (^{GMA}St 2-910-60) to 65.2 J g^{-1} (^{GMA}St 2-631-60). These values confirm that the modification affected the crystallinity of the samples because the higher the crystallinity, the higher the energy that must be absorbed by the sample to melt it.[47]

Table 3. The enthalpy (ΔH) and the endotherm temperatures (onset temperature (T_o) peak temperature (T_p) and melting temperature (T_m)) of St and the ^{GMA}St X-Y-Z samples

Sample		T_o / °C	T_p / °C	T_m / °C	ΔH / (J g^{-1})
0	St	29.3	97.0	177.0	77.0
1	^{GMA}St 2-631-60	28.9	103.7	180.0	65.2
2	^{GMA}St 2-631-80	29.1	111.7	180.9	38.9
3	^{GMA}St 2-910-60	29.9	82.0	180.3	32.3
4	^{GMA}St 2-910-80	29.5	92.9	180.9	40.6
5	^{GMA}St 4-631-60	29.9	100.8	180.9	44.3
6	^{GMA}St 4-910-60	29.4	99.4	181.0	35.9
7	^{GMA}St 4-631-80	28.8	106.1	181.0	41.8
8	^{GMA}St 2-910-80	29.6	90.9	180.7	44.9
9	^{GMA}St 3-770-70a	30.0	85.6	180.3	50.0
10	^{GMA}St 3-770-70b	29.7	100.5	180.7	52.1
11	^{GMA}St 3-770-70c	29.8	103.3	181.0	46.0

The enthalpies calculated in this work are considerably higher than the values observed by Wootton and Bamunuarachchi.[48] They obtained an enthalpy of gelatinization of 5.2 cal g^{-1} (21.76 J g^{-1}) for dry corn starch heated at a rate of 8 °C min^{-1}. The different heating rates and the water content in the samples analyzed in this work might justify the differences in the enthalpy values.

The behavior observed in the thermograms corroborates the XRD results (Figure 2c), confirming that the modification reaction changed the semi-crystalline structure of starch into a more amorphous structure. While more amorphous structures tend to be attractive for materials in which a viscoelastic behavior is expected, higher crystallinities are desired for structures with enhanced strength, for example.[49]

Statistical analysis

Statistical analyses allow a better understanding of how each parameter and the combination of two parameters

affect the final material. It provides information about the experimental conditions needed to achieve the desired property, for instance. The NMR and XRD results were used to produce quantitative information (degree of substitution and crystallinity degree, respectively) regarding the statistical effect that each evaluated parameter had over the final properties presented by ^{GMA}St X-Y-Z samples. Table 4 shows the results obtained from the NMR and XRD data.

Table 4. The degree of substitution (DS) of hydroxyl groups from starch by GMA molecules and the crystallinity degree (Xc) of the pure and modified starch samples

Sample		Degree of substitution	Xc
0	St	0	1.00
1	^{GMA}St 2-631-60	0.023	0.98
2	^{GMA}St 2-631-80	0.009	0.86
3	^{GMA}St 2-910-60	0.005	0.87
4	^{GMA}St 2-910-80	0.025	0.91
5	^{GMA}St 4-631-60	0.077	0.89
6	^{GMA}St 4-910-60	0.018	0.81
7	^{GMA}St 4-631-80	0.009	0.88
8	^{GMA}St 2-910-80	0.017	0.85
9	^{GMA}St 3-770-70a	0.036	0.93
10	^{GMA}St 3-770-70b	0.009	0.89
11	^{GMA}St 3-770-70c	0.042	0.78

The results obtained from the NMR and XRD data confirm that the modification of St with GMA occurred differently for each sample. It also confirms that the experimental parameters affected the modification in different ways. The data referent to the samples from the experimental design (^{GMA}St X-Y-Z) was analyzed by analysis of variance (ANOVA) and effects to determine how each parameter, individually or combined, affected the modification reaction. Tables S1 to S10 (SI section) resume the obtained data, and Figure 4 displays the Pareto charts obtained from the data presented in Tables 2 and 4.

Pareto charts provide information regarding the major causes behind an observed behavior.[50] In this case, it was used to evaluate the effect of the experimental parameters (volume of GMA, time, and temperature of reaction) over the DS and the crystallinity of the ^{GMA}St X-Y-Z samples (Figure 4). If an interaction is statistically significant at 95% ($p > 0.05$), the bar crosses the red line in the Pareto chart.

According to the statistical analysis, neither the individual parameters nor their combination had a significant effect on the degree of substitution presented by the ^{GMA}St X-Y-Z samples. The statistical analysis indicated
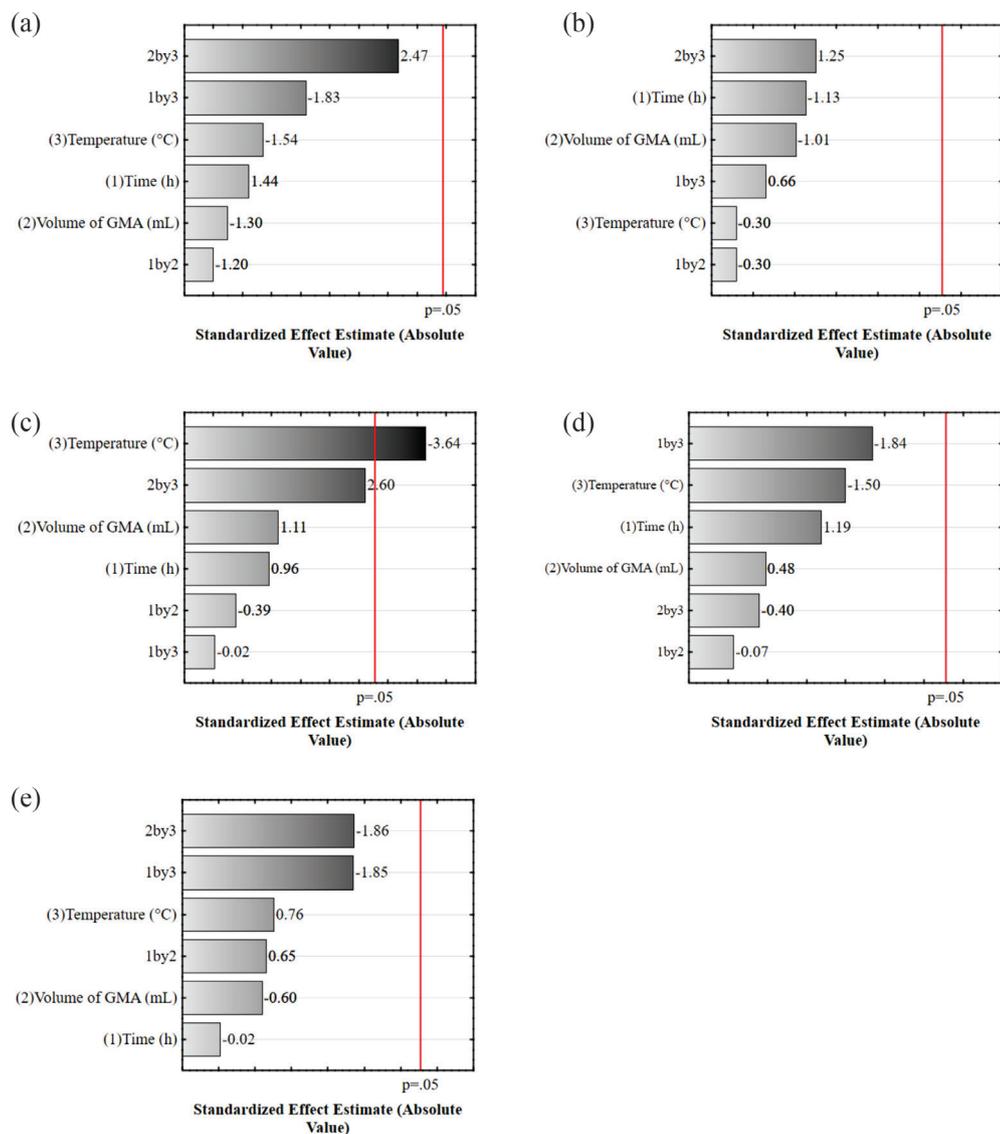
Figure 4. Pareto charts of the statistical effect of the parameter time (h; 1), the volume of GMA (μL; 2), and temperature of reaction (°C; 3) over the (a) degree of substitution, (b) crystallinity degree, (c) M_n, (d) M_w, and (e) PDI of the ^{GMA}St X-Y-Z samples.

that, although the individual parameters volume of GMA and temperature, at the highest assessed level, decreased the DS, their combination (parameters 2 by 3; Figure 4a) increased the DS by 2.47. The higher temperature increases the kinetic energy of the molecules in the solution. When combined with more GMA molecules in the medium, it increases the probability of a starch and a GMA molecule colliding with each other with the ideal energy to react,[45] justifying the DS increase.

The second parameter combination that got closer to being significant regarding the DS was the parameters temperature and time (parameters 1 by 3; Figure 4a). In this case, even though the higher reaction time increased the DS by 1.44 and the higher temperature decreased the DS by 1.54, their combination decreased the DS by 1.83. Since the molecules would have longer periods to react and higher

kinetic energy than they would have at a lower temperature, the DS was supposed to increase. The competition between the modification reaction and the hydrolysis of the St chains might justify this result. The hydrolysis would lead to the formation of smaller chains. Considering that the formation of new chemical bonds requires not only the collision between two molecules of ideal energy but also the appropriate orientation,[45] a longer reaction time might allow the newly formed smaller St chains to assume a conformation that increases the hindrance towards the reactive hydroxyl group from St,[51] restricting the access of GMA molecules to the grafting center.

The degree of substitution influences the stability of microparticles produced using emulsion polymerization. Li *et al.*[52] evaluated the effect of the degree of substitution of octenyl succinic anhydride onto starch. They noticed

that the higher DS increased the hydrophobicity, the stability of the particles within the assessed storage period, the encapsulation efficiency, and it also increased the bioaccessibility of the drug used in their experiment. The authors still observed that a higher DS decreased the size of the oil droplet formed in the medium. In another literature report, Chugh et al.[53] assessed the DS effect on corrosion inhibition using chitosan modified with cinnamaldehyde. They confirmed that the sample with the highest DS was the most effective one in inhibiting the corrosion of mild steel under acidic conditions.

Regarding the crystallinity of the [GMA]St X-Y-Z samples (Figure 4b), the parameters (individual or combined) did not have a significant effect on this property either. Comparing the individual parameters, the results suggest that, on the highest level, all three parameters decreased the crystallinity of the polymer. Nevertheless, when combined, the parameters volume of GMA and temperature (2 by 3), and time and temperature (1 by 3) increased the crystallinity of the samples by 1.25 and 0.66, respectively. Since the modification reaction competed with the hydrolysis of the St chains (as confirmed by the changes in the PDI values; Table 2), the observed crystallinity increase might be a consequence of the formation of smaller and, possibly, more linear chains. It would facilitate the packing of these chains due to the smaller free volume between them.[54] However, regardless of the experimental condition, all [GMA]St X-Y-Z samples presented lower crystallinity values than pure St for the reasons already mentioned in "Physical-chemical characterization" sub-section.

Demina et al.[55] produced a composite based on poly(lactic acid) and calcium phosphate with enhanced mechanical properties. The authors attributed the mechanical improvement to the nucleation effect promoted by the calcium phosphate which consequently increased the crystallinity of the polymer. This material with enhanced mechanical properties is a good candidate for bone fixation implants.

The poly(lactic acid) nanotube produced by Smith et al.[56] is another example of a material in which crystallinity played a remarkable role considering the intended application. The crystalline nanotubes presented a high piezoelectric coefficient (150% bigger than the coefficient of the amorphous nanotubes), which is attractive for biomedical purposes because piezoelectric materials detect and apply forces compatible with the dimensions of the cells. Besides, they also confirmed that the adhesion of human dermal fibroblasts onto the material was a crystallinity-dependent factor: the higher the crystallinity, the higher the adhesion. These literature reports reinforce the importance of having control over the properties of a material to achieve a successful performance concerning the intended application.

The SEC results confirmed that the experimental conditions not only grafted GMA molecules into St chains but affected the length of these chains (Table 2). The Pareto chart indicated that the temperature had a significant effect ($p < 0.05$) on M_n, decreasing its value by 3.64 (Figure 4c). Since the definition of M_n is the molecular weight of all chains divided by their number,[54] the occurrence of hydrolysis increased the number of polymeric chains of smaller molecular weight, therefore, decreasing the M_n values.

The combination of the parameters temperature and volume of GMA (2 by 3) almost had a significant effect on M_n. In this case, even though the high temperature favors hydrolysis, the combination of a higher level of temperature and volume of GMA increased the probability of two molecules colliding at the ideal energy to react. It corroborates the DS results.

The experimental parameters did not have a significant effect on M_w (Figure 4d). Unlike M_n, M_w considers how each fraction contributes to the final molecular weight.[54] The combination of a higher reaction time and higher temperature (1 by 3) almost had a significant effect over M_w. It decreased M_w because the longer exposure of the St chains to the acidic medium favored the hydrolysis reaction, leading to the formation of smaller chains with possibly a more regular distribution of molecular weight.

The aforementioned results agree with the effects observed in the PDI values (Figure 4e). Although none of the individual parameters nor their combination had a significant effect on the PDI values, the combination of higher levels of volume of GMA and temperature (2 by 3) and reaction time and temperature (1 by 3) led to more homogeneously length-distributed polymer chains for the reasons mentioned in "Physical-chemical characterization" sub-section. Comparing the correlations to the results of the individual parameters, a higher temperature would increase the PDI (Figure 4e). It reinforces that the effect of an individual parameter might be affected by a second variable in an experiment and that incorrect conclusions might be reached by ignoring this correlation.

Even though most of the values chosen for parameters assessed in this work did not lead to significant differences regarding their effect on the evaluated properties, this statistical analysis provides information about how to control the experimental conditions to achieve a particular characteristic. For example, more GMA molecules will graft into starch if the reaction occurs at a high temperature and using higher volumes of GMA. It also confirmed that

the use of high temperatures increases competition between hydrolysis and graftization.

Du *et al.*[57] produced a low-molecular-weight zwitterionic copolymer based on tris hydroxyethyl allyl ammonium bromide (THAAB), 2-acrylamide-2-methyl propane sulfonic acid (AMPS), and acrylamide (AM) to increase wellbore stability. In this case, control over the molecular weight was required to prevent the twisting and breaking observed for high molecular weight polymers exposed to high-salt environments. The synthesized copolymer presented high-temperature resistance besides enhanced inhibition properties, making this copolymer very useful for drilling purposes.

The literature reports the effect of chain polydispersity on the diameter of chitosan-based nanoparticles. The microparticles produced using the high molecular weight and high polydisperse chitosan presented diameters of ca. (18.7 ± 12.3) μm while the diameter of the microparticles produced using low-molecular-weight chitosan was (1.62 ± 0.38) μm.[38,58] Even though both microparticles were suitable for biological applications, the control over molecular weight and PDI would allow the production of particles with a narrower size distribution, preventing deviations from desired behaviors.

Regarding the modification parameters, the obtained results suggest that the degree of substitution did not directly influence the crystallinity of the samples. However, the results also suggest that the same parameter combination that increased the DS (volume of GMA and temperature) increased the crystallinity of the samples as well. The crystallinity of the samples was more affected by the hydrolysis of the St chains than by the graftization of GMA into St. Therefore, the volume of GMA and the temperature used in the experiment are the most important parameters to be properly adjusted when designing a material.

The statistical comparisons confirmed that the individual parameters influence the properties of the modified starch. However, defining experimental conditions based only on the individual parameters would not be the most appropriate option because the combination of two parameters changed the chemical potential of the reaction. Therefore, the ideal experimental conditions must be defined considering the correlation between two parameters instead of considering them individually. It would truly help choose the appropriate conditions to achieve the properties that best suit the intended application of the modified polymer.

The use of starch modified with GMA for hydrogel purposes was reported by Lima-Tenório *et al.*[17] They successfully grafted GMA into starch using a base-catalyzed reaction and blended it with *N,N'*-dimethyl acrylamide, and sodium acrylate to produce pH-responsive hydrogels whose swelling reached ca. 34 g g^{-1}. Despite allowing the graftization of GMA into starch, the base-catalyzed reaction favors the competition between the ring-opening mechanism and the nucleophilic addition to the carbonyl group from GMA.[17,23,59]

Conclusions

This work evaluated how the parameters time (2, 3, or 4 h), the volume of modifier (631, 770, or 910 μL), and temperature (60, 70, or 80 °C) affected the graftization of GMA into starch. FTIR and ^{1}H NMR analyses confirmed the occurrence of a modification reaction. However, no quantitative information could be obtained from the FTIR analysis because no band had a constant IR absorbance after the modification reaction. The degree of substitution, calculated using the NMR spectra, ranged from 0.005 to 0.077 (for samples (^{GMA}St 2-910-60 and ^{GMA}St 4-631-60, respectively). Statistical analysis indicated that, even though the individual parameters temperature and volume of GMA decreased the DS, their correlation increases the DS. XRD analysis confirmed that the graftization of GMA into St changed the crystallinity of the polymer. Even though all the experimental conditions presented crystallinity values lower than pure St ($X_c = 1\%$), ranging from 0.78 to 0.98% (^{GMA}St 3-770-70c and ^{GMA}St 2-631-60, respectively) the combination of higher temperatures and volumes of GMA increases the crystallinity, while, individually, the temperature and the volume of GMA decreased the crystallinity. The number-average (M_n) and weight-average (M_w) molecular weight were determined by size exclusion chromatography. The results indicated a decrease in the M_n and M_w values of the ^{GMA}St X-Y-Z samples in comparison to pure starch. It confirms that the graftization reaction competed with the hydrolysis of the starch chains, decreasing the length of the St chains. The statistical analysis confirmed that higher temperatures increased the PDI of the starch chains, higher volumes of GMA, and graftization time decreased the polydispersity of the chains. However, the correlation confirmed that higher temperatures, the volume of GMA, and graftization time decreased the PDI altogether. DSC analysis confirmed that the graftization of GMA into starch changed the crystallinity of the samples, but it did not significantly affect their thermal stability. Even though the values chosen for the assessed parameters did not lead to significant differences in the evaluated properties, it provided information on how to combine the conditions to adjust the properties that best fit the intended application.

Supplementary Information

Supplementary data (FTIR results, ANOVA, and analysis of effects) are available free of charge at http://jbcs.sbq.org.br as a PDF file.

Acknowledgments

Author Contributions

Michelly C. G. Pellá was responsible for conceptualization, formal analysis, investigation, data curation, writing-original draft, review and editing, visualization; Andressa R. Simão for formal analysis, writing - review and editing; Marcos Roberto Maurício for formal analysis, writing - review and editing; Douglas C. Dragunski for formal analysis; Rafael da Silva for supervision; Adley F. Rubira for conceptualization, supervision, writing original draft, review and editing.

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