



Cross-linking of starch with 1, 2, 3, 4-diepoxybutane or 1, 2, 7, 8-diepoxyoctane

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Abstract

Water-soluble and native potato starches were modified with 1, 2, 3, 4-diepoxybutane (DEB) or 1, 2, 7, 8-diepoxyoctane (DEO). Optimal reaction conditions and the effectiveness of the cross-linkers were determined on the basis of yields of water-soluble and insoluble material. The presence of the introduced groups was confirmed by NMR spectroscopy in solution as well as in solid state. According to multi-angle laser light-scattering method, the weight average molecular masses of the water-soluble fractions prepared from water-soluble starch were larger ($M_w = 471$ kDa using DEB; $M_w = 931$ kDa with DEO) in comparison with the starting material ($M_w = 291$ kDa). Due to high molecular mass only new anomeric signals were observed by ¹H NMR in solution. Maximal yields of insoluble parts were 18 or 50% for DEB or DEO. On native starch the yields of insoluble products decreased with increasing concentration of cross-linker and NaOH in comparison to unmodified material. Solubilized components were dialyzed and we suppose that starch was degraded under these conditions. This material is not suitable for the modification when the goal is to increase the yield of insoluble part. With water-soluble starch the initial first order rate constants of cross-linking with E, DEB and DEO were determined with rheometer. From the relation of complex shear modulus and time by linear regression method at 20–70 °C, first-order rate constants of the same order of magnitude were calculated. The corresponding activation energies of the process are decreasing in the order: E (131 kJ/mol) > DEO (105 kJ/mol) > DEB (55 kJ/mol). We consider these results in agreement with the differences in the structure of the cross-linkers and the mechanism of cross-linking. Both cross-linkers gave spherical particles when cross-linked by the water-in-oil emulsion method with dimensions 60–610 (10% ≤ 192; 50% ≤ 294; 90% ≤ 425) μm, when cross-linked with DEB or 250–730 (10% ≤ 278; 50% ≤ 392; 90% ≤ 548) μm, when DEO was used.
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1. Introduction

Cross-linking chemistry is a well-known method used to prepare starch derivatives. One of the commonly used cross-linkers is epichlorohydrin (E; Rutenberg & Solarek, 1984; Šimkovic, Laszlo, & Thompson, 1996). However, interest is growing in preparation of starch derivatives with new properties and other cross-linkers. Diglycidylether-type diepoxy compounds have been used as cross-linkers of dextran (Mislovičová, Petro, & Berek, 1993; Petro, Berek, & Novák, 1994) and hyaluronic acid (Yui, Okano, & Sakurai, 1992). Diepoxybutane has been used for

the cross-linking of hyaluronic acid (Laurent, Hellsing, & Gelotte, 1964). In this paper, the usefulness of 1, 2, 3, 4-diepoxybutane (DEB) and 1, 2, 7, 8-diepoxyoctane (DEO) for the cross-linking of starches will be investigated. The procedure is similar to previous studies on the modification of starches (Šimkovic et al., 1996) and hyaluronic acid (Šimkovic, Hricovíni, & Šoltés, 2000) with epichlorohydrin and 1, 3-bis(3-chloro-2-hydroxypropyl)imidazolium hydrogensulfate on polygalacturonic acid (Šimkovic, Hricovíni, & Sasinková, 2002). The water-soluble, respectively, insoluble fractions have been analysed with solution-state and solid-state NMR spectroscopy. Some water-soluble fractions were analysed with multi-angle laser-light scattering (MALS) technique. The reaction conditions were optimised in terms of yields of water-soluble or insoluble

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material. Under optimised conditions also cross-linking kinetics were studied using rheometer as well as spherical particles prepared using water-in-oil emulsion method.

2. Experimental

2.1. Materials

Water-soluble Paselli SA-2 and native potato starches (Avebe, The Netherlands) were used as starting materials. All other chemicals were of commercial grade. The mixtures were stirred for 24 h at room temperature in closed glassware. The compositions (the molar ratios of reactants) are listed in Tables 1 and 2. The molar content of starch refers to the number of moles of anhydroglucose residues. Reaction was stopped by transferring the material into dialysis tubing (12–14 kDa MWCO, Spectrum). After dialysis the water-soluble and water-insoluble fractions were separated on a fritted glass funnel (G4, Koch-Light) and lyophilised. The yields of soluble and insoluble material (in %) were calculated on the basis of theoretical highest yield (yield = (weight of product/weight of starch with theoretical highest yield) × 100). Most of the experiments were run in parallel and the error was less than 5%.

For emulsion cross-linking Paselli SA-2 starch (3.24 g, 0.02 mol) was dissolved in 18 ml of water (1 mol) containing NaOH (0.8 g, 0.02 mol). After addition of DEB or DEO (1.72 g, 0.02 mol or 2.84 g, 0.02 mol) the mixture was homogenized and 36 ml of paraffin oil containing Span[®] (0.5 g; Aldrich) was added. The mixture was stirring at 20,000 rpm for 30 s and than at 1,200 rpm at room temperature for 24 h and subsequently washed with 20% ethanol on plastic sieve (\varnothing 65 μ m) to minimize the amount of oil content. Samples were stored in water for particle-size measurements.

2.2. Analysis

The light scattering measurements were performed by a multi-angle laser-light scattering (MALS) photometer

Dawn DSP-F in 90% DMSO/water solvent at 25 °C (Wyatt, 1993). The MALS photometer uses a vertically polarized laser of 632.8 nm of wavelength and simultaneously measures the intensity of the scattered light at eighteen fixed angles (ranging from 21.6 to 147.8°). Instrument was calibrated using toluene. Angular normalization was performed by measuring the scattering intensity of the narrow, low molecular mass Pullulan standard ($M = 12.2$ Kg/mol). This was assumed to behave as an isotropic scatterer. The experimental data were elaborated by a Zimm-Plot and were fitted with a second order polynomial for the angular variation and a first-order polynomial for the concentration. The exact amount of polymer was weighed in a vial with the necessary solvent to reach the desired concentration. The solution was heated at 150 °C for 30 min and after cooling slowly to room temperature, each solution was filtered through a 0.45 μ m nylon filter. All samples were completely soluble and clear solutions were obtained. The specific refractive index increments, $dn/dc = 0.074$ ml/g, of starch with respect to the used solvent at 25 °C was obtained from the literature (Chen & Wyatt, 1999). The particle size distribution was determined as described before (Yilmaz, Jongboom, van Soest, & Feil, 1999). High-resolution solution-state NMR spectra were recorded at 300 and 500 MHz on a Bruker spectrometers with a 5 mm inverse broadband probe with a shielded z-gradient at 40 °C. The ¹H and ¹³C chemical shifts were referenced to internal TSP. Two-dimensional techniques, COSY, HSQC and HMBC were used to determine the ¹H and ¹³C chemical shifts; the 2D HSQC experiment was performed in the phase-sensitive pure-absorption mode. In the HSQC and HMBC experiments, carried out at 7 T, the spectral widths were typically 1800 Hz (¹H) and 10000 Hz (¹³C), respectively. Other details of NMR experiments were described previously (Šimkovic et al., 2002). ¹³C CP/MAS and high-power decoupling (HPDEC) NMR experiments were run on AMX400 Bruker instrument.

The rheology experiments were run on Bohlin cylindrical rheometer in oscillation mode (frequency: 5 Hz; amplitude: 40%) using a C 25 cylinder with 21.79 g cm torsion bar at 20–70 °C. The homogenized mixtures at

Table 1
Results of cross-linking of water-soluble starch

Sample #	Moles of reactants ^a			Yield (%)	
	Cross-linker	NaOH	H ₂ O	Soluble	Insoluble
1	None/0	0	11/99	75	0
2	Diepoxybutane/0.010/4.1	0.01/1.9	1/86.2	66	0
3	Diepoxybutane/0.010/3.5	0.10/16.3	1/73.5	64	0
4	Diepoxybutane/0.010/3.0	0.20/28.1	1/63.2	41	18
5	Diepoxyoctane/0.001/0.7	0.01/2.0	1/89.3	77	0
6	Diepoxyoctane/0.005/3.4	0.01/1.9	1/86.8	30	48
7	Diepoxyoctane/0.010/6.6	0.01/1.9	1/83.9	16	50

Amount of starch used in all experiments was 0.01 mol AGU.

^a Second number is in g/100 g of mixture.

Table 2
Results on cross-linking of native potato starch

Sample #	Moles of reactants ^a			Yield (%)	
	Cross-linker	NaOH	Water	Soluble	Insoluble
1	None/0	0	11/99	0	72
2	Diepoxybutane/0.01/4.1	0.01/1.9	1/86.2	1	74
3	Diepoxybutane/0.01/3.0	0.20/28.1	1/63.2	6	51
4	Diepoxyoctane/0.01/6.6	0.01/1.9	1/83.9	0	75
5	Depoxyoctane/0.01/4.9	0.20/27.5	1/62.0	2	42

Amount of starch used in all experiments was 0.01 mol AGU.

^a Second number is in g/100 g of mixture.

molar ratio of starch: cross-linker: NaOH: H₂O = 0.01: 0.01: 0.01: 1 were transferred into the tempered rheometer hardware and covered with paraffin oil and immediately the oscillation was started. Each 60 s phase, viscosity, complex shear modulus, storage modulus and loss modulus were measured. The initial rate constants of cross-linking were calculated by the linear regression method from the function $\ln(1/G^*) = f(t)$, where G^* is the complex shear modulus in Pa and t is time in sec. Only regression coefficients higher than 0.95 were accepted and only values obtained during first 500 s were used. From the rate constants activation energies were calculated using the Arrhenius equation by the same method (Šimković et al., 1986).

3. Results and discussion

3.1. Modification of the water-soluble starch with diepoxybutane

When water soluble starch was dissolved in water without adding chemicals and dialyzed, only 75% of the material was recovered after freeze-drying of the part, which remained in dialysis tubing (Table 1, sample 1; recovery of starch referred to the dry mass). As the dialysis tubing used is permeable for sugars with a DP smaller than circa 80, this indicates that about 25% of the SA-2 starch sample has a DP < 80. Possibly, this fraction is the most accessible for the cross-linkers. The recovered sample was characterized by static off-line light scattering method (using the Zimm Plot). The weight average molecular mass was $M_w = 291,000$ g/mol and the gyration radius was $R_g = 24.4$ nm.

When the original SA-2 was modified, the yield of the starch derivative was 66% at a molar ratio of starch: DEB: NaOH: H₂O = 0.01: 0.01: 0.01: 1 (sample 2). A similar yield was obtained after a tenfold increase in the amount of sodium hydroxide (sample 3). This sample had $M_w = 471,000$ g/mol and $R_g = 23.8$ nm. Although the M_w increased after cross-linking, the average dimensions in water of the obtained derivative molecules compared to the

starting material were not increased. A further increase to 0.2 mol of NaOH (sample 4) resulted in preparation of insoluble material (18% yield). The amount of water-soluble material was still larger (41% yield). This could be explained by relatively short length of the epoxybutyl-bridge. It might result in a preferential attack of the same polysaccharide chain prior to cross-linking with an unmodified starch chain and the possible interaction between $-O^-$ formed from first epoxy ring and the second epoxy ring. The difference in mass balance of dialysed material could also be the result of alkaline degradation with increasing amount of NaOH.

3.2. Modification of water-soluble starch with diepoxyoctane

With DEO the reaction conditions could be more easily adapted towards the bifunctionally linked product, resulting in the preparation of insoluble material (Table 1, samples 5–7). At the ratio of starch: DEO: NaOH: H₂O = 0.01: 0.01: 0.01: 1, the yield of water-soluble material was only 16% and the amount of insoluble residue was 50% (sample 7). The fact that the sum of yields of both components is lower than 100% might be due to degradation of starch under used reaction conditions. The water-soluble material (sample 7) had $M_w = 931,000$ g/mol and significantly larger gyration radius ($R_g = 110.7$ nm), compared with the sample obtained with DEB at the same reactant ratios. It is probably due to much larger interaction radius of the octyl- in comparison to butyl-group. At lower amounts of DEO 0 or 48% of insoluble residue were prepared (samples 5 and 6). Under identical reaction conditions more insoluble material was obtained with DEO than with DEB. The fact that more insoluble material is obtained with DEO could be the result of the difference in spacer dimensions or number of cross-links versus mono-ether linkages.

3.3. NMR analysis of water-soluble products

The SA-2 starch material (Table 1, sample 1) gave similar ¹H-NMR, COSY and HMBC spectra in D₂O as

obtained previously (Nilsson, Bergquist, Nilsson, & Gorton, 1996). The intensity ratio of the signals at 5.35 and 4.95 ppm (1–4 and 1–6 glycosidic linkage) was 23: 1. This corresponds to 4% (molar based) degree of branching. The HSQC experiment gave $^{13}\text{C}/^1\text{H}$ chemical shifts at 101.0/5.36 (C-1/H-1), 72.9/3.61 (C-2/H-2), 74.4/3.93 (C-3/H-3), 78.2/3.63 (C-4/H-4), 72.3/3.81 (C-5/H-5), and 61.7/3.81 (C-6/H-6) ppm. ^1H -NMR spectrum of hydroxybutyl-starch (Table 1, sample 3) gave in the anomeric region two new signals at 5.67 and 6.02 ppm (not observed in sample 1). Ratios of the intensities of all anomeric resonances (4.95, 5.38, 5.67 and 6.02; the first two originating from the unmodified sample 1) were: 0.059: 1: 0.034: 0.034. This corresponds to the degree of etherification (DE) of 0.09 for both positions. The COSY spectrum showed correlation of H-1' signal at 5.67 to H-2' at 3.45 ppm. The later signal corresponded to signal at 72.1 ppm in the HSQC experiment (Fig. 1) and was not observed in the HSQC experiment of the starting material. In the complementary HMBC experiment (Fig. 1) the signal at 3.45 ppm correlates with signal at 75.3 ppm; the later belonging to R–O-part of the cross-linking group. The substitution in position 6 can be ruled out as any new signals were observed close to C-6 or H-6 resonances. Analogically for hydroxyoctyl-starch (Table 1, sample 7), ^1H -NMR spectrum contained two new signals at 5.48 and 5.61 ppm. Ratios of the intensities of anomeric resonances at 4.95, 5.34, 5.48 and 5.61 ppm were: 1: 9: 2: 3 and correspond to the DE of 0.40 or 0.60. Signal at 5.61 ppm (H-1') showed scalar interaction with H-2' at 3.46 ppm in the COSY spectrum. This signal correlates with the signal at 72.1 ppm in HSQC spectrum (Fig. 2). Signal at 3.46 ppm in the HMBC experiment (Fig. 2) is analogical triplet as observed in the HMBC experiment on Fig. 1. In addition there are other signals due to more complicated environment for the 2, 7-dihydroxyoctyl cross-linking bridge. As all the data known from literature on analogical starch modification are with monoepoxy compounds and favour position 2 (Wilke & Mischnick, 1997; Richardson,

Nilsson, Berquist, Gorton, & Mischnick, 2000) and there were no new ring signals due to substitution observed, we believe these two new anomeric signals are related to substituents at 2 and 3 positions. The substitution at OH-3 might be related to intramolecular reaction of this group with the second epoxy-group of cross-linker already bonded at C-2. Higher degrees of the substitution for DEO support a greater reactivity of this cross-linker under identical conditions. The decrease in mass recovery of soluble fractions (compare samples 3 and 7) in relation to the increase of degree of etherification could be explained with the preferential accessibility of the dialyzable fraction determined in sample 1. This was totally recovered as a part of the soluble fraction for samples 2 and 3. We think that the soluble fractions are predominantly just mono-functionally derivatized and to much lower extent in comparison to insoluble fractions. The DE's of the remaining water-soluble fractions were not determined as their values were lower as those mentioned as well as due to their unproved origin due to unsuitable molecular mass for NMR experiments in solution.

3.4. Modification of native starch

Only 72% of native potato starch, prior to cross-linking, was recovered as insoluble residue after dialysis (Table 2, sample 1). The recovered part contained no water-soluble material. This means 28% was dialyzed through the tubing. The material, which passed the dialysis membrane, most likely contains some impurities (like salts and ash) and small carbohydrates. The smaller carbohydrates, similarly as with Paselli SA-2 sample, are probably somewhat more accessible for the cross-linkers. After modification of the original native potato starch at an equi-molar ratio of NaOH: cross-linker (sample 2), only 1% soluble material is found. The yield of insoluble fraction increased only slightly (74% yield) in comparison with sample 1. An increase in the amount of NaOH from

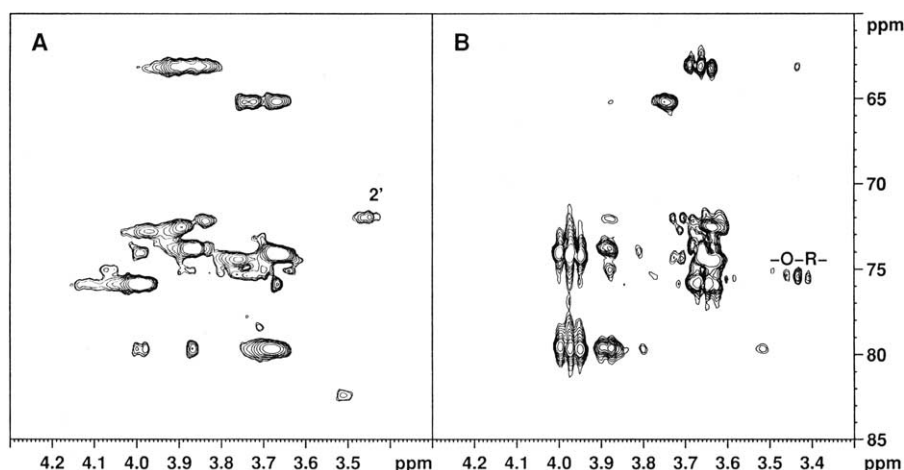


Fig. 1. The HSQC (A) and HMBC (B) spectra of hydroxybutyl-starch (Table 1, sample 3).

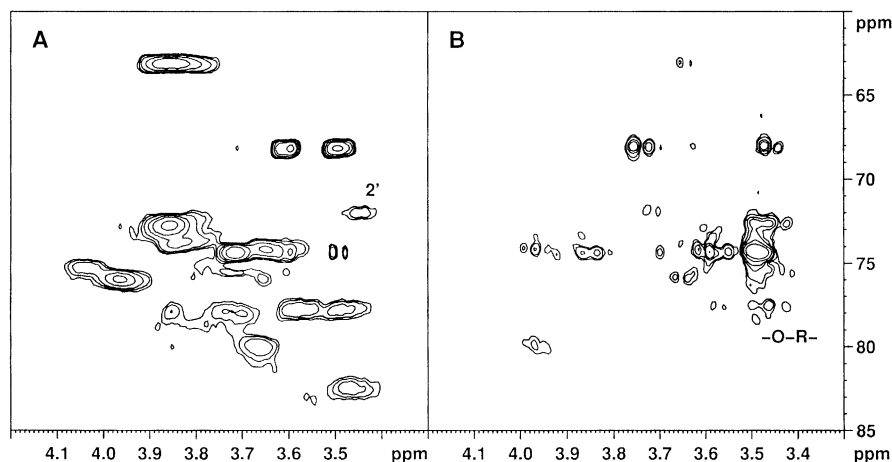


Fig. 2. The HSQC (A) and HMBC (B) spectra of hydroxyoctyl-starch (Table 1, sample 7).

0.01 to 0.2 mol resulted in a decrease of the yield of insoluble material, while the amount of the water-soluble material for both cross-linkers was negligible (samples 2–5). This is probably due to two reasons: hydrolysis of epoxy rings of diepoxides and solubilization of native starch with increased alkali concentration.

3.5. The solid state NMR analysis of insoluble products

The SA-2 starch fraction (Table 1, sample 1) analysed in the solid state with CP/MAS NMR experiment gave signals at 101.3 (C-1 of glucose unit), 81.4 (C-4), 72.3 (C-2, C-3, and C-5), and 61.3 ppm (C-6). On the hydroxybutyl-starch (Table 1, sample 4) a very similar spectrum with broad signals at 101.4, 80.1, 70.4, and 59.4 ppm were measured. The HPDEC experiment on the same sample gave only two broad signals at 70.3 and 59.4 ppm. Because they were broad, we assume they originate from polysaccharide and not from the dihydroxybutane bridge. The water-insoluble part of sample cross-linked with diepoxyoctane (Table 1, sample 7) had starch signals at 104.3, 82.6, 74.0 and 63.9 and two additional signals at 28.42 and 35.60 ppm in ^{13}C CP/MAS NMR spectra. The latter resonances originate from two types of the CH_2 groups in the middle part of dihydroxyoctane bridge. The signals from the CHOH groups in the bridge are overlapped with broad starch signals.

HPDEC and CP/MAS solid-state NMR spectra of native starch samples 1 and 2 (Table 2) were identical showing only signals of the unmodified starch. We assume that the DE is too low and the molecular mass of native potato starch is unsuitable for this type of experiments. However, CP/MAS experiment gave two new signals at 32.3 and 25.4 ppm on sample cross-linked with diepoxyoctane (Table 2; sample 4). The chemical shifts values of these new signals are close to those values measured on hydroxyoctyl-starch (Table 1, sample 7) in D_2O for the CH_2 cross-linking group. HPDEC experiment gave only

starch signals at 100.6, 80.6, 70.5 and 59.6 ppm, which were also present in CP/MAS spectrum. The remaining substituent signals are probably hidden under the broad starch signals.

3.6. Rheology kinetics

The first-order rate constants were calculated on the basis of experiments run in a Bohlin rheometer (Table 3). These constants fit with the first order criteria and are interpreted as initial rates of cross-linking. We have used them to measure/compare the rates of cross-linking of three different cross-linkers. The criterion of relation of the samples rheometrics with initial stage of cross-linking reaction was the fitting with calculated regression coefficients (>0.95).

Table 3
Kinetic parameters of cross-linking of starch with E, DEB and DEO

Cross-linker	Temperature (°C)	Rate constant $\times 1000$ (s^{-1})	Activation energy (kJ/mol)
E	26	0.102	131
	30	0.205	
	40	1.071	
	50	5.059	
	60	21.776	
DEB	20	0.128	55
	30	0.236	
	31	0.429	
	32	0.612	
	33	1.777	
	34	3.507	
DEO	30	0.070	105
	35	0.441	
	36	1.364	
	37	4.454	
	38	15.113	

Complex shear modulus was chosen instead of viscosity, storage or loss modulus parameters, because the values for this parameter fitted the regression coefficient the best in initial stage of the process. All three cross-linkers were run under identical conditions, chosen as optimal on the basis of the above listed experiments (Table 1; molar ratio of starch: cross-linker: NaOH: H₂O = 0.01: 0.01: 0.01: 1). The determined values of rate constants are in the same order of magnitude, which indicate the similar mechanism. The highest value of activation energy was determined for E (131 kJ/mol) and might be due to different structure than of DEB and DEO. The smallest value for DEB (55 kJ/mol) indicates that the opening of one epoxy ring might affect the other more easily than on DEO (105 kJ/mol), where the epoxy groups are separated by four CH₂ groups. According to the activation energies both epoxy-compounds are more reactive cross-linkers than E. These results indicate that there are relations between cross-linking of macromolecules and determined rheometrics values in diluted solutions (Barbosa-Cánovas, Kokini, Ma, & Ibaraz, 1996). As these values are time-dependent it is hard to compare them with values run on different rheometers due to different geometry and approach. To the best of our knowledge there are no data available on chemical mechanism studies on rheometer.

3.7. Water-in-oil cross-linking

The formation of emulsion of water-in-oil particles by initial high-speed stirring and fixation of the spherical particles by cross-linking of water-soluble starch resulted in suspension formation and was proved by measuring of their dimensions. Due to the presence of equal volumes of water and oil, the concentrations of starch, NaOH, and cross-linker were doubled. All particles were spherical with diameters 60–610 (10% ≤ 192; 50% ≤ 294; 90% ≤ 425) μm when cross-linked with DEB or 250–730 (10% ≤ 278; 50% ≤ 392; 90% ≤ 548) μm when DEO was used. This shows the relation between the length of spacer group and the dimensions of the particle, as the smaller particles were produced with DEB than with DEO under identical conditions. These findings are only preliminary and require further research as much smaller particles were prepared when other types of cross-linkers were used (Dziechciarek, van Soest, & Philipse, 2002).

4. Conclusions

Modification of the water-soluble potato starch with DEB and DEO in aqueous/NaOH solutions, results in production of mono-functionally and bi-functionally linked water-soluble and insoluble materials. More NaOH is needed to prepare the insoluble material with DEB than with DEO. On native starch the amount of insoluble material was decreasing with increasing amount

of NaOH and cross-linker and the modification could be proved by solid state NMR only when DEO was used. Rheology kinetics indicate higher reactivity of both cross-linkers in comparison to E. Spherical particles prepared from water-in-oil suspension indicate relation between dimensions of particles and the length of the spacer group.

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