Effect of degree of substitution of carboxyl groups on cross-linking of carboxymethyl starch

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Abstract: Cross-linked carboxymethyl starch of different degrees of substitution-DS (DS, 0.14, and 0.49) was synthesized and investigated the effect of DS on cross-linking of starch derivatives. Native corn starch was first carboxymethylated in an alkaline condition of the alcoholic medium using sodium chloroacetate as a carboxymethylating agent. The aqueous Carboxy Methyl Starch (CMS) solutions (15 and 50%, w/w) were then irradiated by electron beam (EB) radiations at 1, 2, 3, 5, 10, 20, and 50 kGy dosages and underwent radiation cross-linking, resulting in a Cross-linked Carboxymethyl Starch (CCMS). The gel content to be estimated in the CCMS of different DS (0.14 and .049) with 50% (w/w) CMS was 1.3 and 19.2%. These are the maximum degrees of cross-link achieved with 50% (w/w) aqueous CMS irradiated at 5 and 2 kGy dosages. Our results showed that the cross-linking of CMS depends on the DS of carboxyl groups and the concentration of CMS. Maximum cross-linking observed with CMS of DS, 0.49, and 50% (w/w) concentration demonstrates that the increased DS and concentration of CMS are favorable for cross-linking of CMS.

Keywords: CCMS; Degree of substitution; Gel content; Electron beam dosage.

Introduction

Polymeric gels have found a broad range of applications over the last few years[1]. In general, these are the materials that can absorb or retain liquid or fluid greater than their dry weight and cannot release the retained liquid in liquid or fluid form but only by diffusion through the gel membrane as water vapor or moisture[2]. These polymeric materials have found extensive commercial applications as a sorbent in personal care products such as infant diapers, feminine hygiene products, and incontinence articles for fluid absorption [3, 4] and for binding of odors [5]. Polymers gels are also applicable to maintain the moisture in dry and arid regions by releasing water to the root of plants[6]. Furthermore, their use as cosmetic, medical care products, and contact lenses[7, 8] can be potential fields of applications in the medical sector. Similarly, hydrogels are also used for the removal of toxic metal/metalloids and dyes from water and wastewaters[9]. The high absorbing capability and improved biocompatibility due to high water content make these hydrogels applicable in several fields. These are used in drug delivery systems[10], soil conditioning[11], and other non-hygienic applications[12, 13]. Polymeric gels are formed by cross-linking the functionalized polymers of hydrophilic

nature either by utilizing multifunctional chemical additives or by radiation sources. Since some of the chemical cross-linking agents are toxic to the biotic environment and cross-linking method itself also produces additional sludge, which may cause secondary environmental pollution by releasing toxicants in soil, air, and water[14]. As an alternative to these chemical cross-linking agents, radiation sources of polymer modification via crosslinking the functionalized polymeric materials may be an excellent method for the synthesis of the polymeric gels. In radiation cross-linking, polymeric materials are subjected to cross-linking by radiation sources rather than chemical additives. The products obtained as such are readily biodegradable[15] in the natural environment without releasing any hazardous materials. Hence, the radiation source of polymer modification is considered safe from the view of environmental protection.

In the present work, native corn starch was carboxymethylated and then cross-linked the aqueous CMS solutions at 15 and 50% (w/w) concentration by EB radiation of various dosages (1-50 kGy). The gel content (cross-linked mass) of CCMS was estimated by measuring the insoluble portion of the dried sample after immersion in distilled water and then evaluated the effect of DS on the cross-linking of CMS. Characterization of the samples for functionality and the morphological study was carried out by FT-IR and WAXS analysis.

Materials and method

Materials

The corn starch and chemicals used in this work (ethyl alcohol, sodium hydroxide, sodium chloroacetate, hydrochloric acid, acetone, and methyl alcohol) were of analytical reagent grade and purchased from Sigma-Aldrich (St. Louis, MO, USA).

Synthesis of carboxymethyl starch

Carboxymethyl starch (CMS) was synthesized in an alkaline condition of the alcoholic medium using sodium chloroacetate as a carboxymethylating agent[16, 17]. For the synthesis of CMS, 100 g (0.617, mol) of native corn starch was suspended in 300 mL ethyl alcohol (99.9%). The starch-ethanol suspension was vigorously stirred at room temperature (RT) for 30 min and 49.2 mL of 50% aqueous sodium hydroxide solution (24.6 g, 0.615 mol) was added drop-wise during 15 minutes, maintaining at RT for another 20 min. Then the aqueous solution of sodium chloroacetate (71 g, 0.609 mol SMCA in ~90 mL of distilled water) was gradually added into the reaction mixture and stirred for another 30 minutes at RT. The temperature of the reaction bath was raised to 40° C and stirred for another 30 minutes. The carboxymethylation reaction was performed for 110 minutes. The product was filtered off, dissolved the residue in approximately 200 mL of distilled water, and neutralized the solution with dilute hydrochloric acid (0.1 M). The CMS solution was then precipitated with ethyl alcohol to obtain the CMS in pure form. The precipitate was filtered and the residue was dried at 50°C in an oven overnight. For multi-step carboxymethylation, the carboxymethyl starch obtained at the first step of carboxymethylation was further carboxymethylated for the second cycle following similar procedures as in the first step with the molar ratio of 1:1:1, respectively for CMS:NaOH:SMCA.

Electron beam irradiation

The aqueous solution of CMS at 15 and 50% (w/w) concentrations were prepared by dissolving powdered CMS in distilled water at room temperature with a stirrer. The solutions of CMS were then poured into polyethylene bags (~ 6 x 7 mm) without eliminating the air from the sample and sealed the bags using the electrical pressing device. The bags were exposed to EB radiation at 1, 2, 3, 5, 10, 20, and

50 kGy dosages to crosslink the CMS paste. Irradiation was carried out in EB Tech Daejeon, Korea by utilizing the following irradiation parameters: acceleration energy, 2.5 MeV; beam current 1 mA for samples exposed to 1-3 kGy and 3.9 mA for the samples irradiated to 5-50 kGy dosage and the dose per pass was 1 kGy.

Characterization of CCMS

Carboxymethylation in the native starch was confirmed by spectral analysis using Fourier transform infrared (FT-IR) spectroscopy. The FT-IR spectra of NS and CMS were acquired on a Perkin Elmer spectrophotometer (PARAGON 1000 pc Perkin Elmer, Norwalk, CT, USA), using potassium bromide (KBr) discs prepared from powdered samples mixed with dry KBr. Morphology of the NS and CMS of different DS was studied by the Wide-Angle X-ray Scattering (WAXS) analysis.

The WAXS is the most widely used method to provide information on crystallographic structure, atomic position, size in a unit cell, and to some extent, chemical composition as well as chemical stoichiometry[18]. The method specifically relies on the Braggs peak scattered to wide angles. The diffraction pattern determines the chemical composition or phase composition of the film and the size of the crystal. The scattering experiment was carried out using the rotating anode type Rigaku generator (RU200, Rigaku Tokyo, Japan) operated at 70 kV and 45 mA with Cu Ka radiation. After irradiation, air-dried CCMS samples (0.5 g for each) were exposed with 25 mL distilled water at RT for 48 h. The insoluble part of the sample was then recovered from the water by filtration using 0.45 µm pore size of filter paper and dried in an oven at 50° C. The gel content was estimated gravimetrically using the following equation[19, 20].

$$Gel\ Fraction\ (\%) = \frac{W_d}{W_i} \times 100 \quad \text{........ (1)}$$

Here W_i is the initial weight of the dried sample after irradiation and W_d is the weight of the dried insoluble part of the sample recovered after treated with water.

Back titration method[21] was used to determine the DS of the carboxyl group on AGU of starch. The sodium salt of CMS (Na-CMS) was first converted to H-CMS by treatment with dilute hydrochloric acid (5 M HCl) solution. The H-CMS (0.5 g) was dissolved in 20 mL of 0.2 M NaOH solution in a 100 mL volumetric flask and the solution in the flask was filled up to the mark with distilled water. The required volume of solution (25 mL) was transferred to an Erlenmeyer flask and diluted by the addition of 50 mL of distilled water. The excess amount of NaOH was back titrated with standard 0.05 M HCl. The titration was repeated three times and the average value of the HCl volume consumed during titration was used for calculations. A blank solution without sample was also titrated and the DS was calculated using the following equation mentioned in the literature[16].

$$DS = \frac{162 \times nCOOH}{m_{ds} - 58 \times nCOOH} \quad \text{.... (2)}$$

Here, 162 g/mol is the molar mass of an anhydro-glucose unit (AGU); $nCOOH$ (mol) is the amount of COOH calculated from the obtained value of the equivalent volume of know concentration of NaOH, 58 g/mol is the net increase in the mass of an AGU for each carboxymethyl group substituted, and $m$ds (g) is the mass of dry sample calculated from known sample mass ms (g) and the water content, W_{water} %.

$$mds = \frac{1 - W_{water}}{100} \times mds \quad \text{.....(3)}$$

The amount of COOH being equal to,

$$nCOOH = (V_b - V).C_{HCL}.4 \ \ ...... (4)$$

Here, V_b (mL) is the volume of HCl used for the titration of the blank V (mL) is the volume of HCl used for titration of the sample, C_{HCl} (mol/dm³) is the HCl concentration, and 4 is the ratio of the total solution volume (100 mL) and the volume taken for titration (25 mL).

Results and discussion

The carboxymethyl starch (CMS) of DS, 0.49 was synthesized by following the multi-step carboxymethylation technique, while the CMS of DS 0.14 was obtained in an attempt using sodium chloroacetate as a carboxymethylating agent.

In multistep carboxymethylation, the DS of the carboxyl groups obtained in the first step of carboxymethylation (DS, 0.20) increased to 0.49 in the second step of carboxymethylation. Here DS is the average number of carboxyl groups introduced in the anhydroglucose unit (AGU) of starch. In this work, the values of DS determined by the back titration method were 0.49 and 0.14 for two different carboxymethylated samples. Aqueous solutions of carboxymethyl starches at two different concentrations (15 and 50%, w/w) were then cross-linked by electron beam (EB) radiation of various dosages (1-50 kGy) to obtain the CCMS. The expected chemical structure of the CCMS is depicted in Figure 1.

The morphological structure of the native starch and the CMS of two different DS of carboxyl groups (DS, 0.49 and 0.14) were investigated using WAXS patterns. The XRD technique has been widely used for material identification, texture analysis, crystallinity determination, and several

other applications[22].

As depicted in Figure 2, crystalline structures observed in the WAXS pattern of native starch are changing towards amorphous form in the carboxymethyl starches, which is apparent in the modification of starch through carboxymethylation. The intensity of the peaks observed close to 6, 15, 17, and 22 indicates that the CMS has amorphous structures (Figure 2)[19].
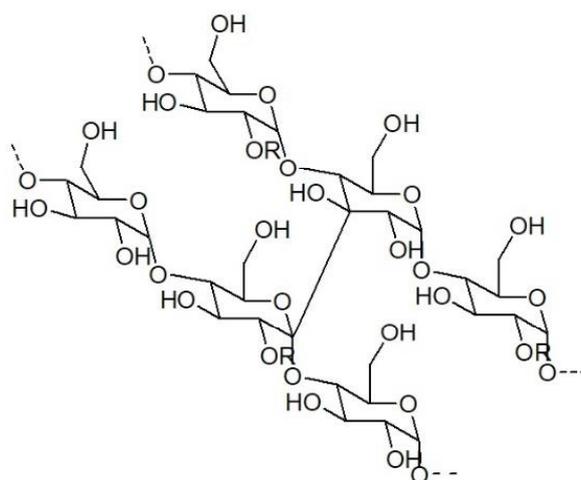
Figure 1: Proposed structure of CCMS (R: the CH₂COONa) [17].

The amorphous solids are considered to be disordered crystalline solids[23], thus originating from the corresponding crystalline state. This is similar to the structural change observed when native starch is subjected to carboxymethylation in an alkaline condition[20]. Structural consistency observed in the structures of two different DS (DS, 0.49 and 0.14) of carboxyl groups (Figure 2, b and c) obviously demonstrates that the carboxyl groups are stable against WAXS patterns.

Carboxymethylation in the starch was confirmed by spectral analysis using FT-IR spectroscopy. Figure 3 shows the FT-IR spectra of the native starch and the CMS of DS, 0.49 and 0.14. In the FT-IR spectrums, CMS displayed additional absorption peaks at 1746 and 1742 cm⁻¹ respectively with the CMS of DS 0.49 and 0.14. These additional absorption peaks obtained with carboxymethyl starches with respect to the native starch are evidence of the introduction of the carboxyl group in the starch molecule[17].
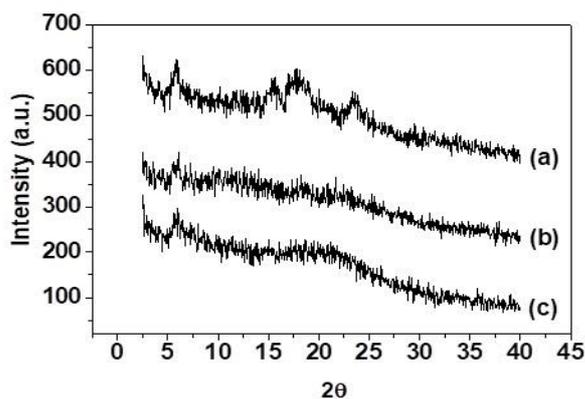
Figure 2: WAXS patterns of (a) native starch (b) CMS of DS, 0.49 (c) DS, 0.14.

Figure 3: FT-IR spectra of I and II (a) native corn starch, I and II (b) CMS of DS, 0.49 & 0.14.

Additionally, the O–H stretching peak shifted to lower wavelength at around 3300 cm^{-1} [24]. Absorption peaks at around 1608 cm^{-1} and 1432 cm^{-1} indicate the substitution of $CH_2COO^-Na^+$ group on starch molecule chain during carboxymethylation[24].

The spectra at around 1125 cm^{-1} owing to the C-O band stretching and the absorption band appeared near 575 cm^{-1} is due to anhydroglucose ring stretching vibration[25]. A low intensity band observed at around 1625 cm^{-1} is probably due to the water present in the amorphous phase[26].

The maximum gel content estimated in the CCMS of different DS (DS, 0.49 and 0.14) of CMS was 19.2 and 1.3%. These are the maximum cross-linked masses obtained when 50% (w/w) aqueous CMS solutions of DS, 0.49, and 0.14 were irradiated by EB radiation of 5 and 2 kGy dosages. While the gel content of CCMS with 15% (w/w) aqueous solution of CMS at 1-50 kGy dosages was extremely low. At low concentration ($\leq$ 15%, w/w) of CMS solution, starch chains disintegrate into small fractions due to scission of acetal linkages, resulting in lower gel content in CCMS. Low cross-linking observed with 15% (w/w) concentration of CMS at 1-50 kGy dosage is also due to scission of polymer chains. The aqueous

solution of polymers when irradiated to EB radiations simultaneously undergo cross-linking and degrade to low molecular weight polymeric fractions[26]. In general, polymer degradation intensely increases with a decrease in the concentration of polymer solution. Whereas increased concentration of polymers positively affects cross-linking, and yield high cross-linked mass in the samples. As shown in figure 4, 50% (w/w) aqueous solution of CMS (DS, 0.49 and 0.14) when irradiated to EB radiations yield, 19.2 and 1.3% cross-linked masses. But it was very low when CMS solution of 15% (w/w) concentration was exposed to the radiation source. These results clearly state that cross-linking of CMS depends on the concentration of CMS solution and also on the DS of carboxymethyl groups.

Similar work was undertaken to obtain the cross-linked carboxymethyl derivative of polysaccharides using electron beam radiation sources as a function of the degree of substitution of carboxyl group[28] and concentration of the sample in aqueous media[29] also manifest, the dependence of cross-linking on the degree of substitution of functional group and the concentrations of the sample. Effective cross-linking can be achieved on exposure to the aqueous CMS sample of 50-60% (w/w) concentration to a suitable dosage of EB radiation[26]. Besides the concentration of CMS

solution and the DS of the carboxyl group, EB dosages also affect the gel content of CCMS. As depicted in figure 4, maximum cross-linking (19.2%) was obtained when 50% (w/w) CMS solution (DS, 0.49) was irradiated at 2 kGy dosages. While, with 15% (w/w) concentration of CMS solution (DS, 0.14), maximum cross-linking (1.3%) was achieved at 5 kGy. From this result, we suggest that cross-linking of CMS also depends on the dosage of EB radiation. Cross-linking in polysaccharides is primarily due to intra-molecular bond formation between polymer chains. Since the established mechanism of polymer cross-linking by radiation sources has not been clearly identified. Nevertheless, irradiation of polysaccharides leads to the breakdown of the ordered system of intermolecular as well as intra-molecular hydrogen bonds, particularly the C-H bonds, resulting in the formation of polymer radicals with free hydrogen atoms[30]. These polymer radicals immediately bond with each other forming a cross-linked structure of a polymer network (Figure 5).
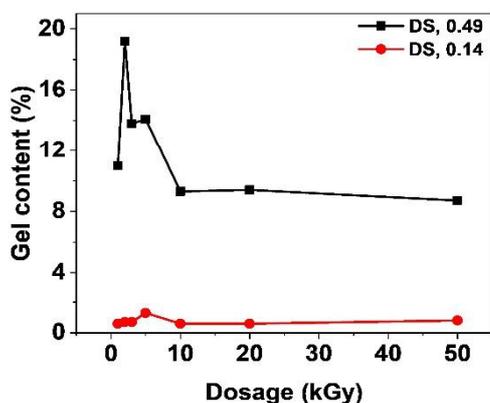
Figure 4: Gel content vs EB dosage for 50% (w/w) aqueous CMS of DS, 0.49 and 0.14.

EB radiation produces free radicals that induce molecular change and fragmentation in the starch chain[31]. This property of free radical formation has been suggested as a major mechanism for overcoming physico-chemical changes in starch molecules by reducing water solubility[29].

During radiation treatment, glycoside bonds in glucose molecules break down into micro-molecules with small chains[32]. The findings also suggest that the crystalline phase decreases with the distribution of amylose and amylopectin in starch granules[33]. Figure 5 shows the production of free radicals in starch molecules after exposure to radiation treatment. Free radicals lead to cross-linking in the starch matrix.
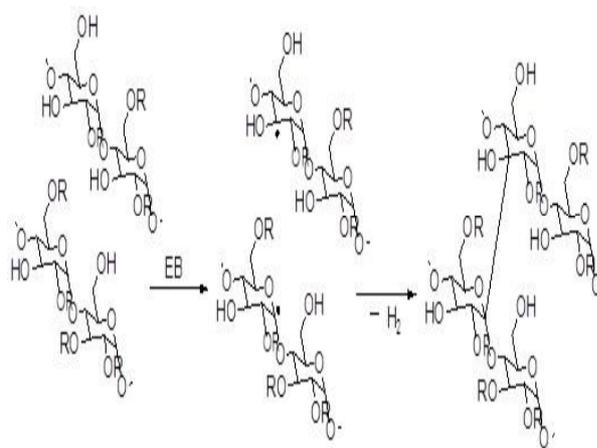
Figure 5: Proposed mechanism of cross-linking (R: the CH_2COONa)[34].

Conclusions

Native corn starch was carboxymethylated using sodium chloroacetate as carboxymethylating agent and cross-linked the carboxymethyl starch using electron beam radiation to obtain a cross-linked carboxymethyl starch (CCMS). The samples (NS and CMS) were analyzed for functionality and morphological characterization using FT-IR spectra and WAXS patterns. Our results demonstrate that the cross-linking in carboxymethyl starch depends on the degree of substitution of the carboxyl group and concentration of CMS. Electron beam dosage also influences the cross-linking of carboxymethyl starches.

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