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Synthesis and characterization of dextrin monosuccinate

Xiuting Hu [a], Benxi Wei [a], Bao Zhang [a], Xueming Xu [b], Zhengyu Jin [a,b,*], Yaoqi Tian [a,**]

[a] The State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi 214122, China
[b] School of Food Science and Technology, Jiangnan University, Wuxi 214122, China

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ABSTRACT

Reaction conditions, including reaction solvents, reaction time, reaction temperature, and molar ratio of succinic anhydride (SA) to anhydroglucose units (AGU) in dextrin, were investigated for preparing dextrin monosuccinate with high degree of substitution (DS). The results showed the optimum conditions as follows: Solvent, dimethyl sulfoxide; reaction temperature, 50 °C; reaction time, 16 h; and molar ratio of SA to AGU in dextrin, 6:1. Under these conditions, the maximum DS reached 2.64. The chemical structure of dextrin monosuccinate was identified using FT-IR and ^{13}C NMR. The FT-IR data indicated the absorption bands of esters and carbonyl acids at 1726 and 1574 cm^{-1}. Signals at 173.13, 171.81, 28.79, and 28.61 ppm in ^{13}C NMR spectrum were ascribed to carbons in ester, carbonyl acid, and methylene. These data suggest that the prepared dextrin succinate was monoester with functional carbonyl acid groups and could be used in polymer therapy as drug carriers.

1. Introduction

In recent years, polymers have been widely used in polymer therapy as carriers of small-molecular drugs and protein or peptide drugs to increase the solubility, stability, and half-time of activity in vivo through slowly releasing (Duncan, 2003, 2011). Both synthetic and natural polymers are explored as drug carriers, but currently almost all polymers used clinically are synthetic polymers, especially polyethylene glycol (Chapman, 2002; Vicent et al., 2005). However, natural polymers are much safer than synthetic polymers, since the former are biodegradable. Therefore, natural polymers have advantages as drug carriers. Among the natural polymers, dextrin can be readily degraded by amylase in vivo. Additionally, icodextrin is a routine clinical drug as the peritoneal dialysis solution (Peers & Gokal, 1998), suggesting that dextrin is non-toxic and non-immunogenic. These properties made dextrin a feasible carrier candidate for the preparation of polymer–bioactive agent conjugates, which was also confirmed by the group of Professor Duncan (Duncan, Gilbert, Carbajo, & Vicent, 2008; Ferguson & Duncan, 2009; Hardwicke et al., 2011; Hreczuk-Hirst, Chicco, German, & Duncan, 2001a). Polymer–drug conjugates, including dextrin–doxorubicin and dextrin–zidovudine conjugates, were prepared and reported to increase the duration of activity through slowly releasing (Hreczuk-Hirst, Chicco, et al., 2001a; Wannachaiyasit, Chanvorachote, & Nimmannit, 2008). On the other hand, polymer–protein conjugates, such as dextrin–rhEGF and dextrin–phospholipase A_2 conjugates, were proved to be more stable than the nature protein drugs and had better properties for their application (Ferguson & Duncan, 2009; Hardwicke et al., 2010, 2011; Treetharnmathurot et al., 2009).

To develop dextrin as a water soluble drug carrier, it was first necessary to introduce reactive functional groups for attachment of pedant moieties. It was reported that polysaccharide activation could be achieved by periodate oxidation (Bruneel & Schacht, 1993a), chloroformate activation (Bruneel & Schacht, 1993b), or succinoylation (Bruneel & Schacht, 1994; Vermeersch & Schacht, 1986). Among these methods, succinoylation was the most widely used one to activate dextrin. In detail, dextrin was first modified into dextrin monosuccinate to introduce carboxylic acid groups. Secondly, dextrin–bioactive agent conjugates were synthesized by dextrin monosuccinates and bioactive agents through reactions between carboxylic acids and amino groups. In theory, the more carboxylic acid groups in dextrin monosuccniate, the higher efficiency reached for preparation of dextrin–bioactive agent conjugates. This indicated that synthesis of dextrin monosuccinate with high degree of substitution (DS) was an essential step to prepare dextrin–drug and dextrin–protein conjugates. Many reports, involving in application of dextrin in polymer therapy, focused on the synthesis process and properties of dextrin–bioactive agent conjugates. In terms of literature about starch modification, little attention is being played to starch monoesters (Tay, Pang, & Chin, 2012), although the preparation of

* Corresponding author at: The State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi 214122, China. Tel.: +86 510 85913299; fax: +86 510 85913299.
** Corresponding author. Tel.: +86 510 85328571.
E-mail addresses: jinlab2008@yahoo.com (Z. Jin), yqtian@jiangnan.edu.cn (Y. Tian).

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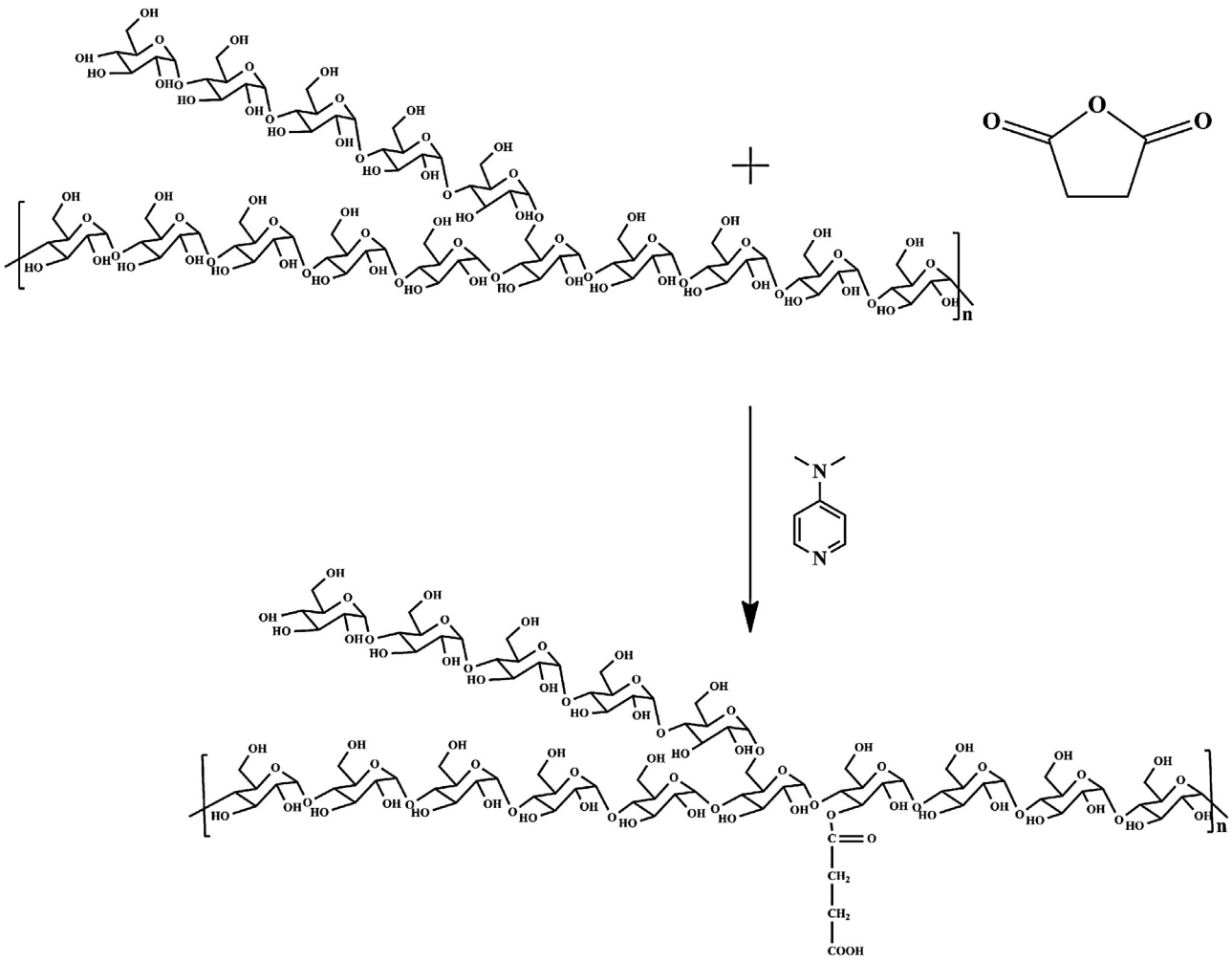

Fig. 1. Reaction of dextrin with SA with DMAP as the catalyst.

starch esters has been frequently reported (Bai & Shi, 2011; Singh, Kaur, & McCarthy, 2007). Therefore, little information about the preparation of dextrin monosuccinate is known.

This work tried to investigate the synthesis process of dextrin monosuccinate to obtain dextrin monoester with high DS. The reaction conditions, affecting the DS of dextrin monosuccinate, were investigated. The chemical structure of dextrin monosuccinate was then identified by Fourier-transform infrared spectroscopy (FT-IR) and ^{13}C nuclear magnetic resonance analysis (^{13}C NMR).

2. Experimental

2.1. Materials

Dextrin (M_w = 15.9 kDa, from maize starch), succinic anhydride (SA), 4-dimethylaminopyridine (DMAP), N,N dimethylformamide (DMF), and dimethyl sulfoxide (DMSO) were purchased from Sinopharm Chemical Reagent Co., Ltd. All other chemicals and reagents were of analytical grade unless otherwise stated.

2.2. Synthesis of dextrin monosuccinate

The method reported in the previous report (Bruneel & Schacht, 1994) was used to prepare dextrin monosuccinate. One gram of dextrin (6.2 mmol anhydroglucose units (AGU)) and 0.62 g of SA (6.2 mmol) were dissolved in 25 ml of DMF. The solution was thermostatted at 40 °C and DMAP (0.28 g) was added with constantly stirring. The reaction mixture was kept at 40 °C for 4, 8, 12, 16, 20, and 24 h. The reaction was stopped by precipitation in a fivefold volume of ether. The precipitation was redissolved in distilled water and further purified by dialysis against double distilled water over 48 h. The solution was finally freeze-dried to yield dextrin monosuccinate. When DMSO was used as the solvent, the procedure was similar except that the reaction product was precipitated by a mixture of ethanol and ether (1:1, v/v).

To obtain the optimum temperature for preparing dextrin monosuccinate, reactions were carried out at different temperatures of 30–70 °C for 16 h in DMSO. In addition, reactions were carried out using different molar ratios of SA to AGU (1:1, 2:1, 3:1, 4:1, 5:1, and 6:1) to investigate effect of the molar ratio of SA to AGU on the DS of dextrin monosuccinate.

2.3. Determination of degree of substitution (DS)

The direct titrimetric method was used to determine the DS (Liu, Zhang, Li, Yue, & Sun, 2010; Stojanović, Jeremić, Jovanović, & Lechner, 2005). The appropriate amount of dextrin monosuccinate was dissolved in 50 mL distilled water. Then, the solution was titrated by the 0.05 M NaOH solution with 3–4 drops of phenolphthalein solution as the indicator. The DS was calculated by using the following equation:

$$DS = \frac{162 \times (V_{NaOH} \times C_{NaOH})}{m - 100 \times (V_{NaOH} \times C_{NaOH})}$$

where 162 g/mol is the molar mass of an AGU, 100 g/mol is the net increase in the mass of an AGU for each succinoyl substituted, m is the weight of the sample analyzed, V_{NaOH} is the volume of standard NaOH solution consumed in the titration, and C_{NaOH} is the molarity of standard NaOH solution.

2.4. Fourier-transform infrared spectroscopy (FT-IR)

The FT-IR spectra of the dextrin and dextrin monosuccinate were collected between 4000 cm^{-1} and 500 cm^{-1} (mid infrared region) on a FT-IR spectrophotometer (5DXC FTIR, Nicolet Co., US) with 256 scans at a resolution of 4 cm^{-1}. Dextrin and dextrin monosuccinate were ground with spectroscopic grade potassium bromide (KBr) powder and then pressed into 1 mm pellets. A blank disc was used as the background.

2.5. ^{13}C nuclear magnetic resonance analysis (^{13}C NMR)

^{13}C NMR spectra of dextrin and dextrin monosuccinate were carried out on a BrukerAV400 MHz (Bruker, Rneinstetten, Germany) at 333.1 K (Arranz, Sanchez-Chaves, & Ramirez, 1992). Samples (30–50 mg) were dissolved in DMSO-d_6 (0.5 mL) with tetramethylsilane (TMS) as an internal standard.

2.6. Statistical analysis

Statistical analysis was performed using ORIGIN 8.0 program (OriginLab Inc., USA). Data were expressed as means ± standard deviations of at least three determinations on one sample for each time period and analyzed by a one-way analysis of variance (ANOVA).

3. Results and discussion

3.1. Preparation of dextrin monosuccinate

To develop dextrin as a drug carrier, succinoylation of dextrin represented one of the most versatile transformations as it provided dextrin access to a variety of valuable properties (Hreczuk-Hirst, German, & Duncan, 2001b; Vermeersch & Schacht, 1986). After succinoylation, the modified dextrin made carboxyl acid groups introduced via an ester linkage, and dextrin was more water-soluble, which made dextrin a good drug carrier. As shown in Fig. 1, SA reacted with O—H groups of dextrin to form the monoester. This reaction was catalyzed by DMAP, which was a useful nucleophilic catalyst for esterifications with anhydrides. The effects of the solvent, the reaction time, the reaction temperature, and the molar ratio of SA to AGU in dextrin on DS of dextrin monosuccinate were listed as follows.

Fig. 2 shows the kinetic results for the reactions between dextrin and SA using DMF or DMSO as the solvent. With the DMSO as the solvent, the DS of dextrin monosuccinate reached the maximum value at about 0.88 after reaction for 16 h. While the reaction was carried out in DMF, the maximum DS of dextrin monosuccinate reached 0.18 after 24-h reaction. Evidently, DS of dextrin monosuccinates prepared in DMSO was significantly higher and reached the maximum value much faster than in DMF. These results indicated that DMSO was more efficient than DMF as the solvent for preparing high-DS dextrin monosuccinate. This phenomenon could be interpreted by the greater solubility of dextrin in DMSO than in DMF.

Fig. 2 shows that the DS of dextrin monosuccinate was significantly affected by reaction time. An increasing of the reaction time from 4 h to 16 h in DMSO led to an increment in the DS of the products from 0.51 to 0.88, thereafter DS almost stayed the same

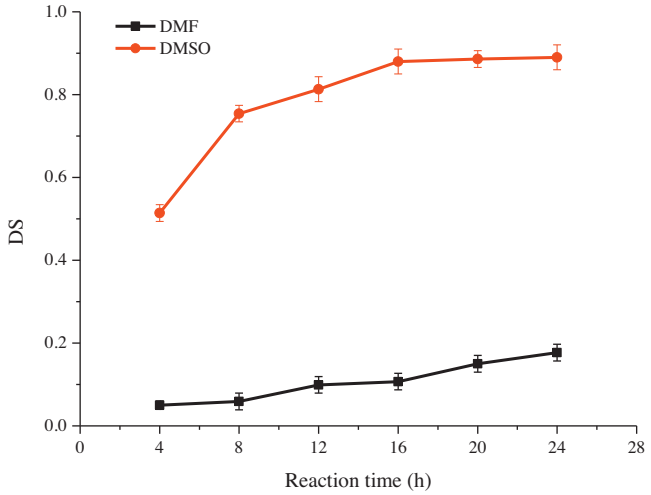

Fig. 2. Time–DS curves for synthesis of dextrin monosuccinate in DMF and DMSO (other conditions: reaction temperature, 40 °C; the molar ratio of SA to AGU in dextrin, 1:1) (data shown in this figure, Figs. 3 and 4 were expressed as means ± standard deviations of at least triplicate).

value when reaction time was prolonged to 24 h. This result indicated that the sufficient reaction time facilitated the esterification of dextrin with SA, whereas prolonged duration might lead to the potential occurrence of side reactions. Hence, in order to save time and energy, the 16-h reaction was sufficient to produce a high-DS product in DMSO.

The effect of the reaction temperature on DS of dextrin monosuccinate is shown in Fig. 3. There was a significant increase in the DS as the reaction temperature increased from 30 °C to 50 °C, and a maximum DS of 0.91 could be obtained at 50 °C. It was possible that higher reaction temperatures improved the compatibility of the reaction ingredients, swellability of dextrin, diffusion of the etherifying reagent, and mobility of the reactant molecules. However, DS decreased to 0.57 when the reaction temperature further increased from 50 °C to 70 °C, which was probably due to the side reactions at high temperatures, such as decomposing of ester linkages and formation of full ester.

Fig. 4 demonstrates the effect of the molar ratio of SA to AGU in dextrin on the DS of dextrin monosuccinate. The DS of the products remarkably increased from 0.91 to 2.45 when the molar ratio of SA

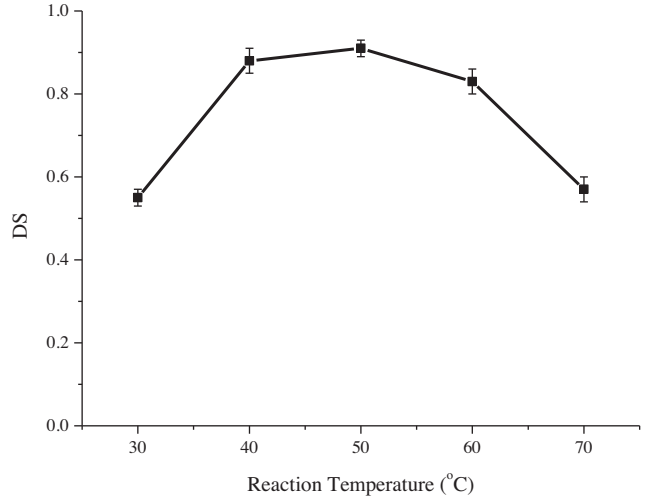

Fig. 3. Effect of the reaction temperature on the DS of dextrin monosuccinate (other conditions: reaction solvent, DMSO; reaction time, 16 h; the molar ratio of SA to AGU in dextrin, 1:1).

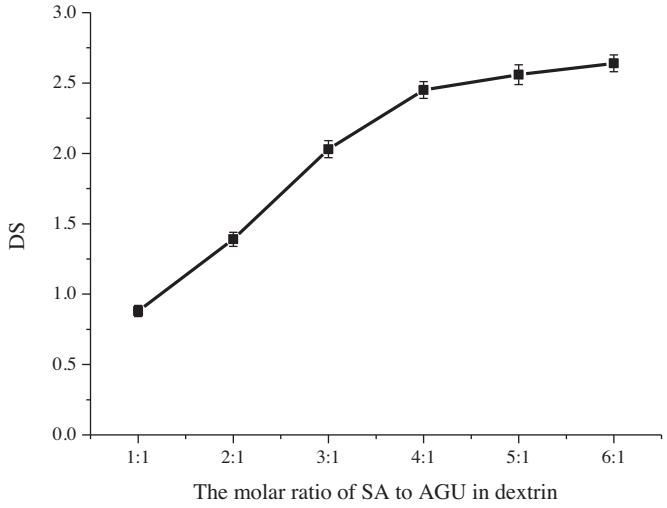

Fig. 4. Effect of the molar ratio of SA to AGU in dextrin on the DS of dextrin monosuccinate (other conditions: reaction solvent, DMSO; reaction temperature, 50 °C; reaction time, 16 h).

to AGU in dextrin increased from 1:1 to 4:1, and slightly increased from 2.45 to 2.64 with SA:AGU increasing from 4:1 to 6:1. This increase in DS could be interpreted by the greater availability of SA molecules to dextrin molecules at the higher concentration of esterifying agent. These results indicated that higher molar ratio of SA and AGU could be employed to produce dextrin monosuccinates with higher DS, but the effect of high SA ratio on improving DS of dextrin monosuccinate was limited.

3.2. FT-IR analysis

Fig. 5 illustrates FT-IR spectra of dextrin and dextrin monosuccinate. The FT-IR spectrum of dextrin showed prominent absorption bands at 3386 cm^{-1} (for O—H stretching vibration), 2927 cm^{-1} (for C—H stretching vibration), 1638 cm^{-1} (for H—O—H bending), 1157 cm^{-1} (for C—O stretching vibration), and 1028 cm^{-1} (for C—O—C stretching vibration) (Fig. 5a). Compared with the spectrum of dextrin, the spectrum of dextrin monosuccinate provided characteristics absorption bands at 1726 cm^{-1} and 1574 cm^{-1} (Fig. 5b). The former band at 1726 cm^{-1} was indicative of absorption by carbonyl groups in carboxyl acids and esters. According to the previous reports (Jayakumar, Balaji, & Nanjundan, 2000; Liu et al., 2008), the absorption by carbonyl bond in esters and carboxylic acids often gave characteristic peaks at about 1750 cm^{-1} and about 1712 cm^{-1}, respectively, and the two bands were strongly overlapped, which resulted in the peak appearing at 1726 cm^{-1}. The existence of carboxyl acid was confirmed by the absorption band at 1574 cm^{-1}, which was attributed to antisymmetric stretching of carboxylic anions (Peng, Ren, Zhong, & Sun, 2011; Yoshimura, Matsuo, & Fujioka, 2006). These results indicated that the succinoylated dextrin synthesized in this study was dextrin monosuccinate, which remained the carboxylic acid groups.

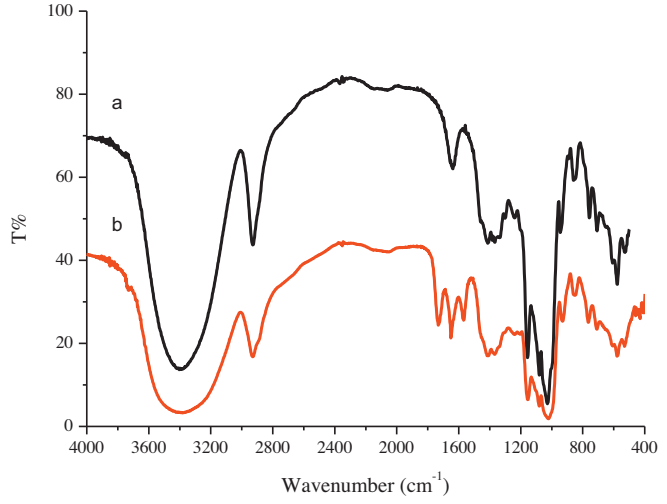

Fig. 5. FT-IR spectra of (a) dextrin and (b) dextrin monosuccinate.

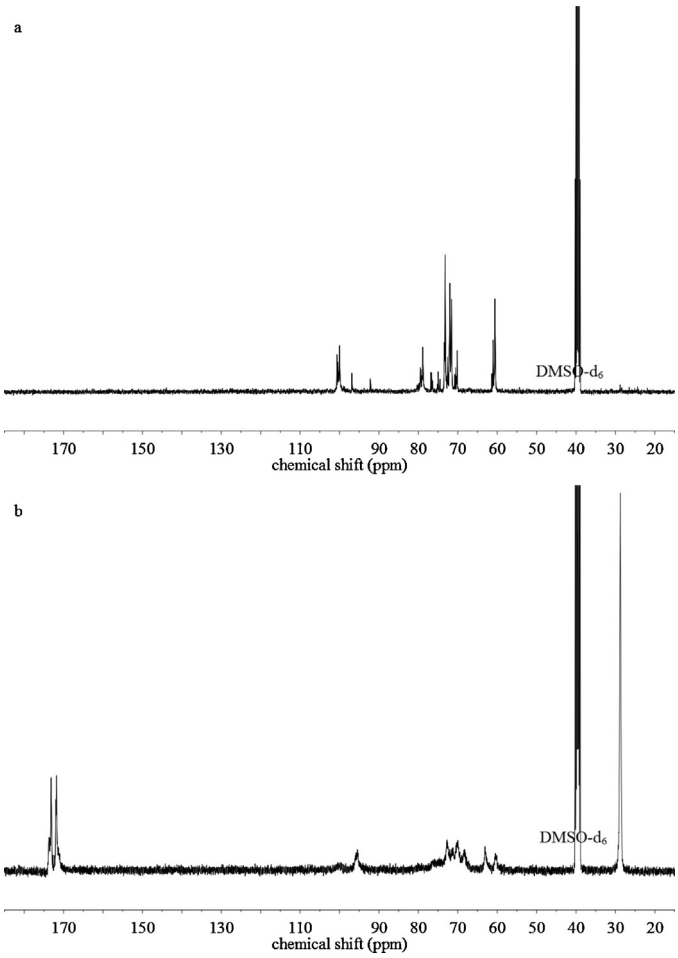

Fig. 6. ^{13}C NMR spectra of (a) dextrin and (b) dextrin monosuccinate.

3.3. ^{13}C NMR spectroscopy analysis

Fig. 6 shows representative ^{13}C NMR spectra of native dextrin and dextrin monosuccinate. In the ^{13}C NMR spectrum of dextrin, all noticeable signals were distributed in the region between 55 ppm and 105 ppm for the carbons of dextrin (Fig. 6a). The chemical shifts δ = 100.03 ppm (C1), 78.88 ppm (C4), 76.83–70.19 ppm (C2, C3, C5), and 60.59 ppm (C6) verified the dextrin molecule. After succinoylation, the region of carbon signals extended to 25–175 ppm (Fig. 6b). The signals at 173.13, 171.81, 95.45, 68.28, 72.69, 70.01, 63.09, 60.37, 28.79, and 28.61 ppm appeared in the ^{13}C NMR spectrum of dextrin monosuccinate. The chemical shifts of carbons in the dextrin skeleton changed significantly, suggesting that succinoylation changed the chemical environment of carbons in the dextrin skeletons. Moreover, the signals at 173.13 ppm and 171.81 ppm were ascribed to carbonyl carbons of the carboxylic acids and esters, respectively. The ^{13}C NMR spectrum of succinoylated dextrin also showed the signals of carbons in methylene groups at 28.79 and

28.61 ppm. Similar results were obtained in the succinoylation of dextran and cellulose in the previous reports (Arranz et al., 1992; Liu, Zhang, Li, Yue, & Sun, 2009), which both demonstrated the monoester by ^{13}C NMR technology. These results also confirmed the structure of dextrin monoester.

4. Conclusions

In conclusion, synthesis of dextrin monosuccinates using dextrin and SA as substances could be efficiently carried out in DMSO with DMAP as the catalyst. The factors, such as reaction time, reaction temperature, and molar ratio of succinic anhydride to anhydroglucose units in dextrin, showed important influences on the DS of the products. FT-IR and ^{13}C NMR analysis confirmed the chemical structure of dextrin monosuccinate. Dextrin monosuccinate with the maximum DS at 2.64 could be obtained, and thus the chemical and physical properties of this important biomacromolecule can be more desirable for polymer therapy.

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