



## Catalytic synthesis of sulfated polysaccharides I: Characterization of chemical structure



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### ABSTRACT

In the present study, sulfated derivatives of *Artemisia sphaerocephala* polysaccharide (SASP) with high degree of substitution (DS) were synthesized by using 4-dimethylaminopyridine (DMAP)/dimethylcyclohexylcarbodiimide (DCC) as catalyst in homogeneous conditions. It was found that DMAP/DCC showed marked improvement in DS of sulfated samples. Compared to sulfated derivatives without catalyst, the DS of SASP increased from 0.91 to 1.28 with an increment in dosage of DMAP from 0 to 10 mg. The influence of DMAP/DCC on the DS of sulfated derivatives was depended on the content of DMAP. The effect of DMAP might be due to its strong coordination to the hydroxy group. The results of FT-IR and X-ray photoelectron spectroscopy (XPS) indicated that  $-\text{SO}_3^-$  group ( $\text{S}^{6+}$ , binding energy of 172.3 eV) was widely present in sulfated polysaccharide molecules.  $^{13}\text{C}$  NMR results indicated that C-6 substitution was predominant for sulfated polysaccharide when compared with other positions. In the sulfation reaction, a sharp decrease in  $M_w$  was observed. DMAP/DCC was an effective catalyst system in sulfated modification of polysaccharide.

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### 1. Introduction

Polysaccharides have been widely studied for their chemical properties and biological activities in food and pharmaceutical industry [1,2]. Most studies have demonstrated that biological activities of polysaccharides increased by molecular modification [3,4]. Among the natural and synthetic biopolymers, sulfated polysaccharides represent a class of macromolecules of particular interest. It possesses many different or stronger biological activities in comparison with non-sulfated polysaccharide, such as anti-virus, antitumor, immune enhancement, hypoglycemic activity and

antioxidant capacity [5–7]. The activity of sulfated polysaccharides also depends on structural parameters such as the degree of substitution (DS), the average molecular mass, the position of sulfation and solution conformation [8].

In general, the sulfated modification of polysaccharide is carried out in acid solution and/or organic reagents. Sulfated modification is carried out slowly and needed longer reaction time in strong acid solution, which lead to the degradation of polysaccharides and losing the original biological activities. To solve this problem, catalyst was added into the reaction for increasing the reaction rate and decreasing the depolymerization of polysaccharide [5]. Since Steglich and Hofle reported that 4-dimethylaminopyridine (DMAP), facilitated acylations to a far greater extent than pyridine. Over the years, DMAP has been shown to catalyze a wide variety of reactions like carbamylation, silylation, sulfonylation and phosphorylation of amino and hydroxy groups [9]. DMAP is also widely used in esterification reactions due to the strong nucleophilicity for many organic reactions such as regioselective acylation of 6-O-protected octyl  $\beta$ -D-glucopyranosides and synthesis of electrophilic alkenes [10]. Dimethylcyclohexylcarbodiimide (DCC) has been used as an esterification agent, removing water and driving the reaction to completion. An advantage of DMAP/DCC system is

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that it does not require a toxic metal catalyst. It is reported that the system has been applied to synthesis of graft copolymers [11]. It is well known that the structure of chemical modified polysaccharides is depended on the reaction conditions [12]. This suggests that upon interaction with reaction reagent, sulfated polysaccharide under-goes complex structural changes that subsequently modify the biological activities.

It is the purpose of our present investigations to explore systematically the chemical structure of sulfated polysaccharide using DMAP/DCC as catalyst. In our previous studies, solution conformation and antioxidant activities of sulfated derivatives of *Artemisia sphaerocephala* polysaccharide were reported [13]. Size exclusion chromatography combined with multi-angle laser light scattering (SEC–LLS), circular dichroism (CD), methylene blue (MB) and congo red (CR) spectrophotometric method and atomic force microscopy (AFM) results confirmed that the degradation of ASP and  $-\text{SO}_3\text{H}$  groups improved significantly the stiffness of the chains due to the electrostatic effect. The extended chain conformation was beneficial to enhance the biological activity of sulfated polysaccharides.

In the present study, sulfated derivatives of ASP (SASP) with high DS were prepared by using DMAP/DCC as catalyst. The chemical structure of SASP was analyzed by FT-IR, X-ray photoelectron spectroscopy (XPS) and  $^{13}\text{C}$  NMR spectroscopy. SEC–LLS was employed to determine the molecular weight and molecular weight distribution. The purpose of this research was to probe into the effectiveness of DMAP/DCC system improving the DS of sulfated polysaccharides.

## 2. Materials and methods

### 2.1. Materials

Chlorosulfuric acid (CSA), pyridine (Py) and formamide were purchased from Jingchun Industry Co. Ltd. (Shanghai, China). 4-Dimethylaminopyridine (DMAP), dimethylcyclohexylcarbodiimide (DCC), L-Ara, D-Xyl, D-Lyx, D-Man, D-Glc and D-Gal were purchased from Sigma Chemical Co. (USA). All chemicals used in this work were of analytical or better grade without further purified.

ASP was extracted and purified according to our previous studies. The composition of ASP was L-Ara, D-Xyl, D-Lyx, D-Man, D-Glc and D-Gal (1:4.2:45.9:9.7:11.4) with the molecular weight ( $M_w$ ) of  $1.42 \times 10^5$  Da [14].

### 2.2. Sulfated modification of ASP

#### 2.2.1. Preparation of sulfating reagent

Chlorosulfuric acid (CSA) was dropped one by one in anhydrous pyridine (Py) filled in three-necked flask, under agitating and cooling in ice water bath [15]. All determinations were completed in 40 min.

#### 2.2.2. Sulfation reaction using DMAP/DCC as catalyst

ASP (500 mg) was suspended in anhydrous formamide (30 mL) at room temperature with stirring for 30 min, and the sulfating reagents were added dropwise. Then, DMAP and DCC were added. For comparison, sulfation reactions without DMAP and DCC were also studied. The mixture was stirred for 3 h at different temperatures (Table 1). After the reaction, the mixture was cooled to room temperature and the pH value was adjusted to 7–8 with 2 mol/L NaOH solution. The mixtures were precipitated with ethanol (95%), washed, redissolved in water, and then dialyzed (molecular weight cutoff 8–12 kDa) against tap water for 48 h and distilled water for 24 h to remove pyridine, salt and potential degradation products. Sulfated ASP (SASP<sub>1</sub>–SASP<sub>5</sub> for samples without catalyst and SASP<sub>cat1</sub>–SASP<sub>cat7</sub> for samples using catalyst) with different DS

(Table 1) were collected. The samples were stored in a dry box under room temperature till use.

The sulfur contents of SASP were determined by Element Analysis (EuroVector EA3000, Leeman). A calibration curve was constructed with sodium sulfate as standard. The degrees of substitution (DS) was calculated according to the equation [14]:

$$\text{DS} = \frac{1.62 \times \text{S}\%}{32 - 1.02 \times \text{S}\%} \quad (1)$$

Phenol–sulfuric acid method was employed for the measurement of carbohydrate contents of ASP before and after modification, using glucose as standard [16].

All the data were repeated 3 times and shown in mean  $\pm$  S.D.

### 2.3. Characterization of chemical structure

#### 2.3.1. FT-IR analysis

FT-IR spectra were recorded with KBr pellets on Thermo Nicolet iS10 FT-IR. Sixteen scans at a resolution of  $4 \text{ cm}^{-1}$  were averaged and referenced against air.

#### 2.3.2. X-ray photoelectron spectroscopy (XPS)

For chemical composition analysis, samples were characterized using XPS (PHI 5702, USA) with a focused monochromatic Al K $\alpha$  source (1486.7 eV) for excitation. The pressure in the chamber was below  $2 \times 10^{-9}$  Torr (i.e.  $2.67 \times 10^{-7}$  Pa) before the data were taken. The voltage and power of the anode were 15 kV and 200 W, respectively. The take-off angle was set at  $45^\circ$ . The pass energies for survey and multiplex scans were 117.40 and 23.50 eV, respectively. Data analysis was carried out with Multipak software which was provided by the manufacturer. For quantitative chemical composition analysis, two samples in each group were acquired and the survey scans were performed in two different areas on each sample.

#### 2.3.3. NMR spectroscopy

$^{13}\text{C}$  NMR experiments were recorded on a Bruker Avance DPX-400 spectrometer (operating frequency of 100.593 MHz). Samples were deuterium-exchanged several times by freeze drying from  $\text{D}_2\text{O}$ , and then examined in  $\text{D}_2\text{O}$  at  $25^\circ\text{C}$ . The chemical shifts were expressed in ppm relative to the resonance of the internal standard  $\text{Me}_4\text{Si}$ .

#### 2.3.4. Molecular weight determination

SEC–LLS measurements were carried out on size-exclusion chromatograph combined with multi-angle laser photometer (MALLS,  $\lambda = 690 \text{ nm}$ ; DAWN EOS, Wyatt Technology Co., USA). Ultrahydrogel™ column ( $7.8 \times 300 \text{ mm}$ , Waters, USA) was used as SEC instrument. An optilab refractometer (Dawn, Wyatt Technology Co., USA) was simultaneously connected. The polysaccharides samples with desired concentrations were prepared and optical clarification of the samples was achieved by filtration into a scattering cell. The injection volume was 50  $\mu\text{L}$  and the flow rate was 0.5 mL/min (mobile phase of ultrapure water). The refractive index increment ( $dn/dc$ ) value of the sample was determined by using an optilab refractometer at 690 nm ( $25^\circ\text{C}$ ) to be 0.147 mL/g.

## 3. Results and discussion

### 3.1. Sulfation of ASP using DMAP/DCC as catalyst

It was reported that controlling the reagent amount was better than controlling the reaction temperature to get sulfated polysaccharide derivatives with high DS [17]. Five sulfated derivatives of ASP were obtained by varying the ratio of CSA to pyridine without catalyst (Table 1). The DS of the samples varied from 0.51 to 0.91. SASP<sub>5</sub> had the highest DS of 0.91 with the yield of 513 mg. With the

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