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Preparation and characterization of sulfated galactomannan from guar gum: Optimization of reaction conditions by BBD and molecule conformational studies

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ABSTRACT

Sulfated polysaccharides exert significant biological activity which is depend on degree of substitution (DS), weight average molecular weight (M_W), substitution position and molecular conformation. In the present study, commercial guar gum was purified and its sulfated derivate (SGG) was prepared by Box–Behnken statistical design. Results of FT-IR and X-ray photoelectron spectroscopy (XPS) indicated that $-SO_3^-$ groups were widely present in SGG molecules. A conformation transition from a compact chain conformation of branched clusters to a random coil conformation was observed in size exclusion chromatography (SEC) combined with laser light scattering (SEC–LLS) analysis. It could be attributed to a comprehensive effect of molecule weight, sulfo groups and galactose substitution pattern in the sulfated derivatives.

1. Introduction

Guar gum (GG) is a natural nonionic high molecular weight (100–1000 kDa) galactomannan. It consists of (1→4)-linked β-D-mannopyranose units with α-D-galactopyranose units connected to the mannose backbone through (1→6) glycosidic linkages. The main chain is randomly substituted by galactose units at a mannose to galactose ratio of 1.8–1.0 [1]. The repeating unit of guar gum is shown in Fig. 1. GG is a widely applied industrial polysaccharide. In the pharmaceutical sector, its functional properties are of primary importance for controlling the release of drugs. It also possesses anticancer activities in the treatment of colorectal cancer [2,3].

Generally, the properties of GG is dependent on its chemical structure, such as chain length, availability of *cis*-OH groups, steric hindrance, degree of polymerization and additional substitutions, which result in considerable variations in their functional characteristics [4]. Recently, chemical modifications of polysaccharides by esterification and oxidation are generally done for preparing custom-made derivatives with desirable functional attributes [5]. Sulfated polysaccharides have complex groups of macromolecules with a wide range of important biological properties. The sulfation of polysaccharides cannot only enhance the water solubility but also change the chain conformation, resulting in the alteration of their biological activities [6]. Many studies have confirmed that the sulfated polysaccharides exerted potent biological properties in comparison with non-sulfated polysaccharides, such as anti-coagulant, anti-virus, anti-oxidant and anti-tumor activities [7].

In early studies, sulfated GG (composition of GG: 85.2% of carbohydrates and 7.1% of protein) was prepared using SO_3/formamide complex in the appearance of chlorosulfuric acid (CSA) [4]. It was found to have anti-inflammatory properties. For the random nature of the substitution, the least substituted sections of the GG molecules showed the greatest tendency to associate [1]. However, their results were not related to any aspects of structure information on sulfated GG. Therefore, the present work aimed to prepare sulfated GG and study the polysaccharide structure. Box–Behnken design (BBD) of response surface methodology (RSM) was employed to optimize the reaction conditions. Furthermore, we attempted to study the solution properties of GG and its sulfated derivative in aqueous solution using size exclusion chromatography (SEC) combined with laser light scattering (SEC–LLS). X-ray photoelectron spectroscopy (XPS) analysis was used to study the surface chemical composition and structure of polysaccharide molecules. We hope this work can provide valuable information to further understand chain conformation properties of sulfated biopolymers.

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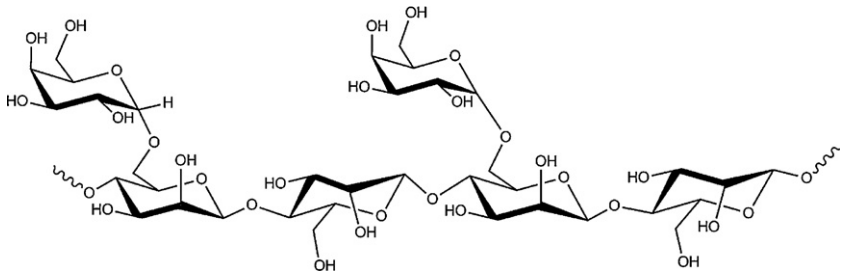

Fig. 1. The monomeric structure of guar gum.

2. Materials and methods

2.1. Purification of GG

The protein of commercial GG (obtained from *Cyamposis tetragonolobus*, protein content was 5%, number average molecular weights was 2.0×10^5 g/mol) was removed by Sevage method joined papain according to the earlier report in our laboratory [8]. The purity of GG collections was monitored under ultraviolet (UV) light at 280 nm. The ratio of mannose-to-galactose was about 2.5.

2.2. Sulfated modification of GG

2.2.1. Preparation of sulfating reagent

Chlorosulfuric acid (CSA) was added dropwise in anhydrous pyridine filled in three-necked flask under agitating and cooling in ice water bath [7]. Preparation of sulfating reagent was completed in 40 min.

2.2.2. Sulfation reaction

GG (500 mg) was suspended in anhydrous formamide (5 mL) at room temperature while stirring for 30 min and the sulfating reagents were added dropwise. The mixture was stirred for various durations and/or temperatures as designed by BBD shown in Tables 1 and 2. After reaction, the mixture was cooled to room temperature and the pH was adjusted to 7–8 with 2 mol/L NaOH solution. The mixtures were precipitated with EtOH (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. Seventeen SGG (SGG_1–SGG_{15}) with different degree of substitution (DS) were collected after lyophilizing and kept in dryness box.

The sulfur contents of SGG were determined by reported method [7]. A calibration curve was constructed with sodium sulfate as standard. DS was calculated according to the equation. S%, sulfur content determination by elemental analysis.

$$\mathrm{DS} = \frac{1.62 \times \mathrm{S}\%}{32 - 1.02 \times \mathrm{S}\%} \tag{1}$$

2.3. Box–Behnken design of reaction conditions

Box–Behnken statistical design was used to statistically optimize the formulation parameters and evaluate main effects, interaction effects and quadratic effects of the formulation ingredients on the DS of SGG. According to the principle of Box–Behnken design, ratio of CSA to pyridine, reaction time and reaction temperature were taken as the variables tested in a 15-run experiment to determine their optimum levels. As shown in Table 1, the three factors chosen for this study were designated as X_1, X_2, X_3 and prescribed into three levels, coded +1, 0, −1 for high, intermediate and low value, successively. Test variables were coded according to the following equation:

$$x_i = \frac{X_i - X_0}{\Delta X} \tag{2}$$

where x_i was the coded value of an independent variable; X_i was the actual value of an independent variable; X_0 was the actual value of an independent variable at centre point; and ΔX was the step change value of an independent variable. DS of SGG was taken as response. For predicting the optimal point, a second-order polynomial model was fitted to correlate relationship between independent variables and response (polysaccharide yield). For the three factors, the equation was

$$Y = A_0 + \sum_{i=1}^{3} A_i X_i + \sum_{i=1}^{3} A_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} A_{ij} X_i X_j \tag{3}$$

where Y was the response variables (DS of SGG), A_0, A_i, A_{ii}, A_{ij} were the regression coefficients of variables for intercept, linear, quadratic and interaction terms, respectively. X_i and X_j were independent variables ($i \neq j$).

Analysis of the experimental design and data was carried out using SAS software (Version 8.0). Analyses of variance were performed by ANOVA procedure. The fitness of the polynomial model equation was expressed by the coefficient of determination R^2 and its statistical significance was checked by F-test at a probability (P) of 0.001, 0.01 or 0.05. The significances of the regression coefficients were also tested by F-test.

Table 1
Levels and code of variable chosen for Box–Behnken design.

Variables	Symbol		Levels		
	Coded	Uncoded	−1	0	1
Ratio of CSA to pyridine	x_1	X_1	1/4	2	4
Reaction time (min)	x_3	X_3	120	180	240
Reaction temperature (°C)	x_4	X_4	40	60	80

Table 2
Box–Behnken design and the response values for the DS of SGG.

Run	CSA/pyridine	Reaction time (min)	Reaction temperature (°C)	DS
1	0	1	−1	0.787
2	1	1	0	0.75
3	0	0	0	0.907
4	−1	0	1	0.681
5	−1	1	0	0.793
6	0	0	0	0.906
7	1	0	−1	0.617
8	0	1	1	0.783
9	0	−1	1	0.904
10	0	−1	−1	0.669
11	1	−1	0	0.656
12	−1	0	−1	0.989
13	−1	−1	0	0.85
14	0	0	0	0.905
15	1	0	1	0.714