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# A Review of Plant Sulfated Polysaccharides and their Relations with Anticoagulant Activities

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## Abstract

Heparin is an important therapeutic agent for the prophylaxis and treatment of thrombosis. Although effective, heparin can cause, among other adverse effects, bleeding and thrombocytopenia, being obtained from bovine lungs and porcine intestines, causing concern about contamination with pathogenic animal agents. This study aimed at reviewing the literature on the relationship between the sulfated polysaccharides isolated from plants and anticoagulant activity. Based on articles published between 2009 and 2016 was found that sulfation of polysaccharides can potentiate its biological activity, which is, in turn, influenced by the degree of substitution obtained, in the reaction, of the position of the sulfate group in the molecule or the final molecular weight. Thus, it is evident that the interest in the use of plant sulfated polysaccharides in the therapeutic field has increased, demonstrating the relevance of research in the field of drugs and materials.

**Keywords:** Plant polysaccharides; Sulphation; Anticoagulant activity; Thrombosis; Heparin

## Introduction

Since 1940, heparin, which is a naturally sulphated polysaccharide of animal origin, has been the drug of choice for treatment and prevention of venous thrombosis and thromboembolism [1]. However, this is an expensive substance, with limited supply and can cause bleeding and thrombocytopenia. In the search for effective anticoagulants such as heparin, algae have been the focus of growing interest in biomedicine because they are naturally sulfated and some studies have confirmed this activity.

Polysaccharides are one of the most important components of all living beings. They are present in a wide variety of organisms such as plants, animals and microorganisms in different forms and structures. They are long chain polymers of mono, di, oligo sugars linked by glycosidic bonds [2]. It is known that the introduction of sulfate group with a suitable degree of substitution can confer biological activity to a polysaccharide such as anticoagulant, antioxidant, antiviral and anti-inflammatory action [1].

Given this reality, sulphated polysaccharides have been increasingly studied as anti-thrombotic agents and anticoagulants as an alternative to heparin. These polymers can be naturally or chemically sulphated and their sources can be seaweed, mushrooms, vegetables, and others. Thus, chemical modification of a polysaccharide is usually performed to improve the biological properties and/or create new functional properties. In particular, sulphation, where the hydroxyl of the polymer structure group is substituted by sulphate group, can impart a significant effect on the physiological functions thereof, and the increase or decrease of the degree of substitution and molecular weight can further enhance this effect (Figure 1) [3].

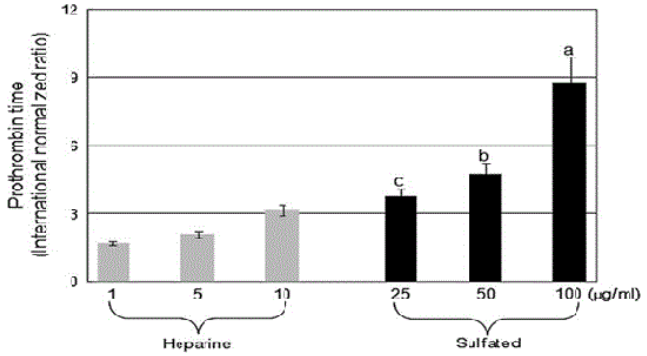


**Figure 1:** Comparison of anticoagulant activities of heparin and sulfated pectin [6].

The main sulfation method performed is O'Neill's method, where the polysaccharide is initially solubilized in formamide and then pyridine is added to this solution. The amount of formamide and pyridine used is determined according to the optimal adaptation of the methodology. The formamide increases the dissolution of polysaccharide and remains inert in the reaction, while the pyridine, being a strong organic base, may nucleophilically attack the polysaccharide, weakening the H-O bond and facilitating the entry of the sulfate group. The mixture is then placed in an ice bath for the addition of chlorosulfonic acid dropwise, under stirring, at a ratio of 10 moles of acid for each mole of free hydroxyl polysaccharide. After the reaction, the mixture is cooled to room temperature and the pH is adjusted with NaOH solution. Subsequently it is dialyzed against water to remove the pyridine salt and potential degradation products. Immediately after, the lyophilization is carried out [2]. The degree of

sulfation, established based on the sulfur content is what determines the percentage of sulfated polysaccharide.

In this review article, the objective is to show, the artificial sulfation plant polysaccharides can be an alternative to obtain substances with anticoagulant biological effect, and that chemical structure of the polysaccharides, such as the monosaccharide composition, molecular weight (Mw), degree and pattern of substituting groups and the sulfation method to play a vital role in influencing the anticoagulant activity.

Gracher [2] carried out the sulfation of the polymer composed of 100% mannose (Mn), with a molar mass of 500 mg, by the O'Neill method, giving the sulfated polysaccharide (PS) Mn-S1 molar mass 965 mg. The degree of substitution (DS) obtained was 1.66. Analysis of the molar mass of the two polysaccharides, crude and sulfated, was performed using size exclusion chromatography (HPSEC). The presence of the sulfate group was found to increase the molar mass of the starting molecule. In this work, the increase in the molar mass of the molecule was proportional to the increase in the anticoagulant effect since it was verified that Mn-S1 contains more extensive signs of overlapping than those of Mn, in addition, the increase of coagulation time happened because to activities of the thrombin in the coagulation cascade was inhibited. Comparing with heparin, the anticoagulant effect of Mn-S1 was lower, but it was found that using Mn-S1 at higher concentrations a similar effect can be achieved. Other studies that worked on chemical sulfation following the O'Neil method also demonstrated an increase in the biological activity of the sulfated molecule [4,5].

In the Kuzhim study, artificial sulfation of the polysaccharides of *Gossipium hirsutum* L was performed, obtaining a molecular weight (Mw) of 22.0 kDa and a substitution degree of 0.8-1.8. In the same study, the chemical sulfation of polysaccharides of *Helianthus tuberosus* as carried obtaining molecular weight of 8.0 kD and substitution degree of 0.6-1.6 and the chemical sulfation of pectin extracted from *Abies sibirica* L obtaining molecular weight of 24.0 kDa and degree of substitution within 0.8-1.1. The results showed that the sulfated polysaccharide of *Gossipium hirsutum* L, which obtained a higher degree of substitution, presented higher anticoagulant activity, followed by sulfated pectin, which, in turn, presented higher molecular weight. Kuzhim further states that there is an optimal degree of substitution to achieve the maximal biological response, which varies according to the type of polysaccharide [3].

In Bae's article the chemical sulfation of citrus pectin was carried out by the method of Huang et al. [6]. In this method the pectin was mixed with formamide and chlorosulfonic acid at a temperature of 80-90°C for 4 hours. The precipitate, obtained by the addition of propylene oxide (50 ml), was collected and then suspended in distilled water, and soon after the pH was adjusted to 10-11. The resulting sample was dialyzed against distilled water for 24 hours and then lyophilized. The incorporation of sulfate groups in the pectin structure is probably responsible for the anticoagulant activity, since the native pectin did not present this activity. The sulfated derivative of pectin significantly prolonged prothrombin time and activated partial thromboplastin (TAP and TTPA), which can be explained by the anionic characteristics of the sulfated pectin, which interact with the positive charge of the coagulant proteins, increasing the anticoagulant activity. A decrease in the viscosity of the sulfated pectin was also observed, which may be justified by the increase in the negative charge and consequently also the repulsion between the molecules increased [6].

Another method of sulfation that obtained good results in improving anticoagulant activity was used by Muschina [4]. In this paper the preparation and characterization of the sulfated galactomannans of Fenugreek gum, Guar Gum, Tara gum and Carob Gum are described. In this study, chemical sulfation was carried out using piperidine-N-sulfuric acid (PSA) or the sulfation reagent complex SO3 ASA-pyridine in dimethyl formamide (DMSO) under temperatures higher than 85°C to result in low galactomannan Molecular weight and under temperatures below 85°C to result in high molecular weight galactomannan. The degree of substitution (DS) of sulfated galactomannans ranged from 0.7 to 1.4. In this work, 17 types of different sulfated galactomannans with different degrees of substitution were obtained. By analyzing the sulfated samples, it was found that the sulfated polysaccharides with higher molecular weight and DS had better antiviral and anticoagulant activity. This result indicates that DS plays an important relationship with the biological activities of high molecular weight polysaccharides [4].

Drozd studied the relationship between the degree of sulfation and the anticoagulant activity of the starch extracted from *Solanum tuberosum*, with a molecular weight between 25,000-30,000 Da and a degree of sulphation of 0.4-2.5 and the inulin obtained from *Helianthus tuberosus*, with molecular weight between 7000-8000 Da and sulphation degree of 0.6-1.6. The antithrombotic activity of sulfated starch reached 16.8-70.0 IU/mg and the antithrombotic activity of sulfated inulin was 5.5-11.4 IU/mg. The increase in anticoagulant activity was proportional to the increase in the degree of sulfation for both polysaccharides [7].

In the Guo study, artificial sulfation of the glucan isolated from the aqueous extract of *Hedysarum polybotrys* (SHG) was performed in this work, and the increase in the DS of the molecule was proportional to the increase in the anticoagulant effect. The anticoagulant activity of SHG and sulfated modification samples was evaluated by the classic coagulant assays of prothrombin time (PT), activated partial thrombin time (APTT) live enzymes, and plasma thrombin time (TT) [8].

Similar anticoagulant effects have been reported for other sulfated polymers such as gum obtained from partially ripe fruits of Aegle marmelos (BFG). In this paper, nine sulfated BFG (sBFG), with various degrees of sulfation (DS), were prepared by using CSA-Pyr method. The data summarized in paper suggested that moderate temperature of 70°C and prolonged reaction time of 2 h were optimum time for obtaining highest DS in sBFG-2 sample. The incorporation of sulfate groups into the gum structure appeared to be responsible for the anticoagulant activity since native gum hardly exhibited anticoagulant effect. sBFG-2 exhibited more than 4 min for APTT (activated partial thromboplastin time), when used in the concentration range of 50-100 g/mL [9].

In addition to DS, the molecular weight (Mw) of polysaccharide is another important parameter influencing biology activity. In this study, three kinds of sulfated derivatives of polysaccharides from persimmon fruits (PFP) were obtained, and named as PFP-SI, PFP-SII and PFP-SIII. PFP-SI, PFP-SII and PFP-SIII had almost identical Mw, their DS were 0.8, 1.7 and 2.5, respectively. PFP-SIII had a higher APTT and TT activity than PFP-SI and PFP-SII. The anticoagulant activity improved with increasing DS, indicating that sulfate esters played a major role in the anticoagulation activity. With respect to molecular weight, the results suggested that the anticoagulant activity improved with decreasing the molecular weights. In this paper, the sulfated polysaccharide with the DS of 1.7 and the lowest Mw of $1.0 \times 10^4$ was best in the prolongation of APTT and TT. It was speculated that higher

Mw of PFP sulfates could prevent them from interacting with coagulation cofactors and their target proteases [10].

Xiaoyun showed that anticoagulant properties of sulfated polysaccharide (PFP-S) obtained from persimmon (*Diospyros kaki* L.) fruits (PFP) strongly depend on their DS and MW, but sulfated derivative of highest degree did not show the highest anticoagulant activities. This indicated that though the sulfate group was essential, the certain degree was also necessary. In their study, they presumed that degree of sulfation must be above one certain value for anticoagulant activity. In this paper, Lu also studied the changes in structure of polysaccharides depended on volume ratio of Chlorosulfonic acid pyridine, volume ratio of SO3Pyr to polysaccharide and reaction time in sulfation process. For example, they found that high levels of molecular weight of sulfated polysaccharide were obtained at low volume ratio of SO3Pyr to PFP short reaction time, as well as, a higher DS was obtained at a high ratio of SO3Pyr to PFP and long reaction time. However, the DS of PFP-S decreased with the extension of reaction time when a high volume ratio of Chlorosulfonic acid-pyridine and volume ratio of SO3Pyr to polysaccharide was used, which was possibly due to the degradation of polysaccharides by sulfating reagent [10].

Studies have demonstrated that chemical sulfation of pectins, including citrus pectin (CP), give products with anticoagulant properties, with the activity depending on the quantity of sulfate groups. Citrus pectin (CP) and its carboxy- reduced derivatives (CP-CR1 and CP-CR4) were chemically sulfated, resulting in samples CP-S, CP-CR1S and CP-CR4S, according to the method described by O'Neill modified. In this study was demonstrated that the anticoagulant activity was dependent on the sulfation pattern of the polysaccharides. The sulfation patterns of the polysaccharides were determined by methylation analysis, where O-methyl substitutes the free hydroxyl groups. Sulfated hydroxyl groups are not methylated, allow in the determination of their position. As observed, chemical sulfation occurred preferably at 6-O-position over 3-O- or 2-O- positions, probably due to stereochemistry of CP-CR1 and CP-CR4. In this work, the proportion and/or distribution of 6-O-sulfated units may be crucial for the anticoagulant and antithrombotic effects of CP-CR1S and CP-CR4S. Moreover, sulfated citrus pectin (CP-S), which is devoid of 6-O- sulfated units, had low anticoagulant activity and has not antithrombotic effect [11].

## Conclusion

Studies and applications of natural and sulfated polysaccharides are expanding in the pharmaceutical field, receiving remarkable attention as anticoagulants. The studies addressing the use of chemical sulphation to obtain molecules with anticoagulant effect are promising since, adapted and controlled by O'Neil methodology, it is possible to achieve the optimum degree of substitution compatible with the desired biological activity. It is observed that the sulfated polysaccharides search field as anticoagulants is large, since there is a wide variety of polysaccharides that have not yet been chemically sulfated and the possibility of obtaining new materials is endless. In addition, the number of articles found concerning this theme highlights the importance of this material in scientific and technological advancement in the area of drugs and materials.

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## References

1. Dorea CMPG, Alvesa MGCF, Willa LSEP, Costa TG, Sabryb DA, et al. (2012) A sulfated polysaccharide, fucans, isolated from brown algae Sargassum vulgare with anticoagulant, antithrombotic, antioxidant and anti-inflammatory effects. Carbohydrate Polymers 91: 467-475.
2. Gracher AHP, Santana AG, Cipriani TR, Lacomini M (2015) A procoagulant chemically sulfated mannan. Carbohydrate Polymers 136: 177-186.
3. Kuzhim AA, Drozd NN, Torlopov MA, Il'ina AV (2013) Relationship between the anticoagulant activity of sulfated plant polysaccharides and the area of their precipitation with polycations during biospecific electrophoresis. Eksp Klin Farmakol 76: 20-24.
4. Muschina T, Budragcha D, Kanamotoc T, Nakashimac H, Ichiyamad K, et al. (2016) Chemically sulfated natural galactomannans with specific antiviraland anticoagulant activities. International Journal of Biological Macromolecules 89: 415-420.
5. Bhatia S, Ratheea P, Sharmab K, Chaugulec BB, Kard N, et al. (2013) Immuno-modulation effect of sulphated polysaccharide (porphyran) from Porphyra vietnamensis. International Journal of Biological Macromolecules 57: 50-56.
6. Bae IY, Joe YN, Rha HJ, Lee S, Yoo SH, et al. (2009) Effect of sulfation on the physicochemical and biological properties of citrus pectins. Food Hydrocolloids 23: 1980-1983.
7. Drozd NN, Torlopov MA, Kuzhim AA, Makarov VA (2012) Dependence of the anticoagulant activity of starch and inulin on their degree of sulfonation. Eksp Klin Farmakol 75: 31-35.
8. Guo L, Yang YL, Yang T, Liu ZH, Feng SL (2013) Sulfated modification and anticoagulant activity in vitro of sulfated glucan isolated from the aqueous extract of Hedysarum polybotrys. Acta Pharmaceutica Sinica 48: 1665-1670.
9. Jindal M, Rana V, Kumar V, Singh RS, Kennedy JF, et al. (2012) Sulfation of Aegle marmelos gum: synthesis, physico-chemical and functional characterization. Carbohydr Polym 92: 1660-1668.
10. Lu X, Mo X, Guo H, Zhang Y (2012) Sulfation modification and anticoagulant activity of the polysaccharides obtained from persimmon (Diospyros kaki L.) fruits. Int J Biol Macromol 51: 1189-1195.
11. Maas NC, Gracher AH, Sassaki GL, Gorin PA, Iacomini M, et al. (2012) Sulfation pattern of citrus pectin and its carboxy-reduced derivatives: influence on anticoagulant and antithrombotic effects. Carbohydr Polym 89: 1081-1087.