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Cellulose Sulfate: A Fascinating Anticoagulant Material

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Abstract

Anticoagulants have been extensively employed in in vitro medical therapies and therapeutic procedures. In clinical applications, heparin is most frequently used, but regrettably, it has specific adverse effects, including bleeding and other drawbacks (heterogeneity and fluctuation of anticoagulant action). In addition, the isolation of heparin from animal sources increases the possibility of contamination by animal infections. Thus, the creation of heparin substitutes is a crucial area of study. One of the better options is a structurally well-defined, nonmammalian source of heparin derivative, polysaccharide sulfates being of particular interest. The most prevalent polysaccharide on Earth is cellulose. The discovery of novel uses for this adaptable material is facilitated by its inherent abundance. Cellulose can exist in different morphological forms: fiber, micro/nanocrystalline, and microfibril/nanofibril cellulose. This review extensively surveys sulfated cellulose preparation and its potential anticoagulation agent use.

Keywords: Heparin; Anticoagulant; Cellulose Sulfate; Acute Toxicity.

1. Introduction

Anticoagulants are commonly utilized in in vitro medical treatments and therapeutic processes. They are thought to be necessary for 2-4 people out of every 1000 who have pulmonary embolism or symptomatic deep vein thrombosis each year. Natural heparin is one of the most frequently utilized anticoagulants in clinical settings [1]. Heparin was the first anticoagulant found at the turn of the 20th century. It is sulfated glucosaminogly (linear) built of alternating uronic acid and α-D-glucosamine linked via α-(1-4) bonds and produced by the animal tissues mast cells (**Figure 1**). The anticoagulant mechanism revealed that heparin sped up the suppression of serine proteinases that are part of the blood clotting cycle. Due to its high efficiency, it is the most commonly used anticlotting drug in vascular surgery. Using heparin has adverse side effects, such as increased platelets functional activity, induced thrombocytopenia, osteoporosis, and hemorrhagic complications [2]. Furthermore, isolating heparin from animal sources increases the possibility of contamination by infections originating from animals, including BSE and AIV, among others [3].

Consequently, researchers focused on standardizing heparin preparations and searching for analogs in the 1980s. A low molecular weight heparin, which displayed lower heterogeneity and improved characteristics, started to be used, followed by fractionated heparin such as fraxiparin, dalteparin, etc. Another research focused on creating and detecting a practical anticoagulant free of undesirable side effects [4]. Heparin analogs, which are polyanions and display a heparin-like activity (anticoagulant), are referred to as heparinoids.

Heparin's anticoagulant effect is caused by Antithrombin III (ATIII)'s 1000-fold increased affinity for thrombin and factor Xa [5, 6]. Furthermore, heparin changes ATIII's conformation as needed and creates a catalytic surface to which thrombin and ATIII bind [7]. Positively charged amino acids in ATIII's heparin-binding pocket interact electrostatically with negatively charged sulfate residues to produce the particular interaction between ATIII and heparin [8, 9]. It has been demonstrated that the anticoagulant effect of modified heparin is increased when low-affinity heparin is chemically sulfated [10]. The mechanisms of the anticoagulant activity of heparinoids differ from that of heparin but give the same effect, which involves interaction with the anticoagulation proteinases. In particular, high plant sulfated polysaccharides like cellulose [11], amorphophallus glucomannan [12], oat xylans [13], and legume galactomannans [14]; chitin and chitosan [15]; pullulan [16], carrageenan [17], and curdlan [18] were synthesized. These sulfated polysaccharides show anticoagulant activity due to the pentasaccharide sequence with N- and O-sulfate groups forming the binding sites for antithrombin III (ATIII) [19, 20]. The most abundant sulfated polysaccharides is cellulose sulfate, a half-ester of cellulose that is water-soluble and has anticoagulant, antivirus, and antibacterial properties; accordingly, it can be used for several biotechnological and medical applications [21]. Also, it is a polyelectrolyte, so it can be used to encapsulate enzymes and cells [22].

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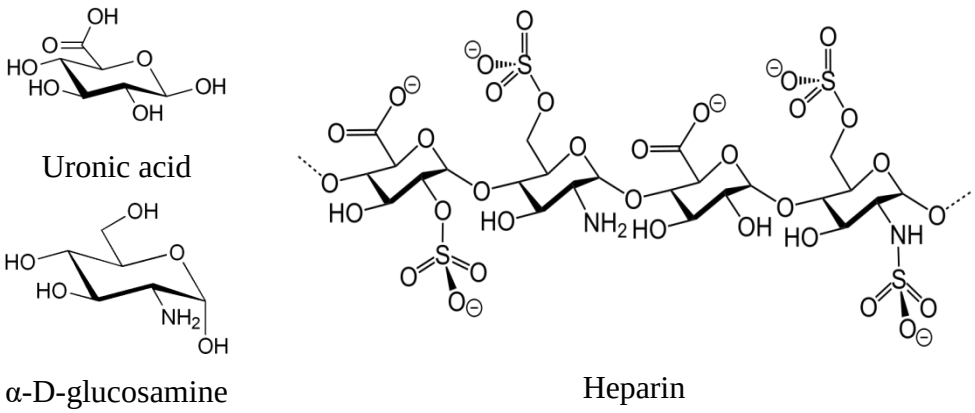


Figure 1. Uronic acid, α-D-glucosamine and heparin structures.

Groth et al. compare the anticoagulation activity of different cellulose derivatives by estimating the standard clotting assays, such as thrombin time and partial thromboplastin time, by introducing sulfate, quaternary ammonia, or phosphate groups in the C2, C3, and C6 of the anhydroglycose unit. By comparing the efficiency of these three derivatives, it was found that the anticoagulant activities mainly depend on the type of the substituent and the negative charge. The sulfated cellulose yielded superior activity than phosphated cellulose. Also, they discovered that anticoagulant activity was achieved with a sulfate degree of substitution above 1.0. The optimum activity was with a sulfate degree of substitution = 1.5 and decreased by increasing this degree. Furthermore, it was shown that sulfation in the C2-position of cellulose has a high anticoagulant effect [23].

Considering this survey, cellulose has been widely used in industry, with less early interest in the biomedical field, but it has considerable promise for use in many other fields. Thus, this review highlights recent advancements in the sulfonation of cellulose and its application as an anticoagulant agent.

2.Cellulose

The most prevalent polysaccharide on Earth is cellulose. Numerous things can provide it, including the cell walls of plants and wood, certain types of bacteria, algae, and tunicates, the only known mammal with cellulose. About 8 % of global cellulose production is based on agricultural wastes. 92 % of world production depends upon wood whether softwood or hardwood [24]. Accordingly, cellulose can be classified according to its source, which is wood-based, plant-based, bacterial, algae-based, and tunicate-based (**Figure 2**). Throughout the lengthy history of cellulose use, wood and plant-based cellulose as opposed to bacteria, tunicate, and algae-based cellulose have become the most well-known forms of cellulose due to their abundance and affordability.

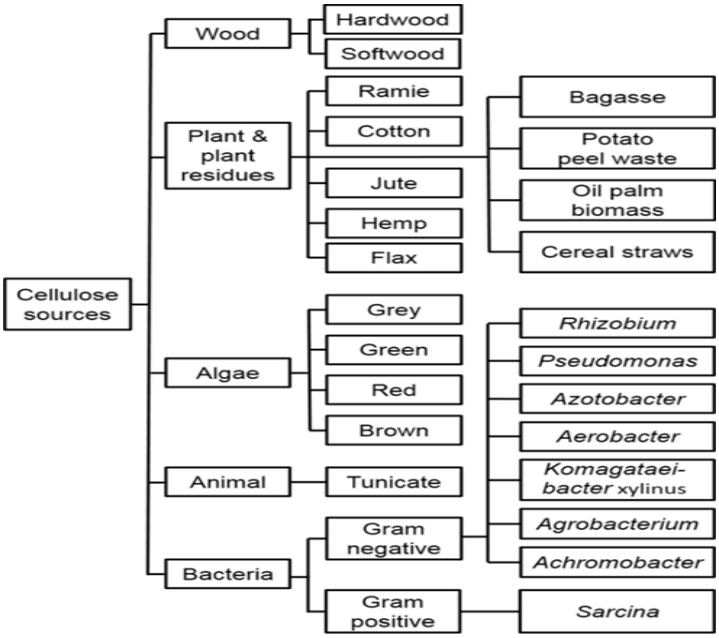


Figure 2. Sources of cellulose (Adapted from reference [25]).

The content and structure of wood and plant-derived fibers are similar, containing biodegradable carbohydrate polymers such as cellulose, hemicellulose, and lignin. The intricate, multi-level structure of cellulose molecules is found in wood and plants; they are connected to other biopolymers such as hemicellulose and lignin [25]. While cellulose derived from wood and plants has the same chemical composition as cellulose derived from different sources, its microstructural organization is distinct. Hemicellulose and the lignin matrix hold the cellulose chains in wood- and plant-based cellulose together as they are arranged in layers as nanofibrils. Cotton fiber cellulose is plant-based but lacks significant amounts of lignin and hemicellulose. Wood and plants generally have a cellulose content of 40–50 and 30–75 %, respectively. There are differences in chemical compositions between hardwood and softwood (**Table 1**) [26].

Table 1. Chemical composition (wt. %) of wood and plant fibers [25].

Source	Cellulose	Hemicellulose	Lignin
Plant fibers*	30–75	10–35	0–20
Softwood	45–50	18–35	23–35
Hardwood	40–50	24–40	18–25

(*except cotton)

Cellulosic materials span from nanoscale to macroscopic dimensions as nanoscale, nanocrystallite, fibrils, and fibril aggregates (**Figure 3**). Cellulose is built of bundles of superfine fibrils that contain several cellulose chains. Depending on the source, each fibril comprises repeated crystalline (large ordered) and amorphous (small disordered) domains. Cellulose units are linked by a strong β-(1→4) glycosidic bond with intra-hydrogen bonding between hydroxyl groups and oxygen of the adjoining ring. This bond stabilizes the linkage and leads to unbranched cellulose chain conformation [27]. Cellulose is a semi-crystalline with crystallinity degree ranging from 40 – 60 and 80 – 100 % for wood/plant and bacteria/tunicin cellulose, respectively [28]. According to origin and treatments, cellulose has a degree of polymerization based on glucose units, ranging from 1000 to 15,000. The hydroxyl groups and oxygen of the adjacent ring of cellulose molecules produce an intra-chain hydrogen bond that stabilizes the linkage and causes the cellulose chains to assume an unbranched shape [27]. Basic fibrils are formed when organized parallel layers of cellulose molecules come together. These fibrils, which have a lateral diameter of 3-5 nm, are closely aggregated together due to strong intra-and/or intermolecular hydrogen bonds and van der Waals forces. Every elementary filament consists of alternating disordered domains and cellulose crystals along the filament axis. Depending on where they originate, bundles of primary filaments can also be classified as cellulose microfibrils, which have a length of several micrometers and a cross-sectional width of 5–20 nm [29]. The intra-and/or intermolecular hydrogen bonds and hydrophobic and electrostatic interactions inside the integrated fibrils creating robust bundles lead to cellulose insolubility in water and regular solvents [30]. Also, due to the high crystallinity and intermolecular interactions between cellulose chains, cellulose has high strength and specific modulus, e.g., the elastic modulus of cellulose I has been reported to be 124–155 GPa [31]. Cellulose has a low wet strength due to its inherent hygroscopic characteristics forming hydrogen bonding with water, but water penetration is limited due to the disordered domains. Cellulose disposed to significant swelling due to absorption of liquid water or moist air along its hydrophilic surface, and due to the higher surface area of nanocellulose, it can take up more water and swell much more compared with cellulosic fiber [32].

Cellulose I, II, III, and IV are the allomorphs of crystalline cellulose; in the crystalline structure, cellulose I contains parallel chains and forming sheets stacked together by Van der Waals interactions and hydrogen bonds. Cellulose I can be converted into either II or III. Cellulose II is obtained from I by an alkali treatment; it is typically obtained by mercerization (aqueous NaOH treatment) or regeneration (dissolution and recrystallization) of native cellulose. During this treatment, the cellulose I parallel chain changes into an antiparallel chain of cellulose II. Cellulose I or II can be converted onto cellulose III by treatment with liquid ammonia. Cellulose IV can be obtained by thermal treatment of cellulose III [33].

Since materials toxicity is considered for biomedical applications and sources of cellulosic particles are low or no toxicity, the aggregation, dimension, hydrophobization, hydrophilization, and surface modification might influence their biocompatibility and cytotoxicity. However, particle nanoscale has been known as the main factor for generating material toxicity composed by this particle [34]. There are conflicting reports on geno-, cyto-, and immunotoxicity vitro studies for cellulosic nanoparticles. Also, after exposure to cellulosic nanoparticles, an inflammation occurs as a typical physiologic reaction to an alien substance, but it may disappear [35].

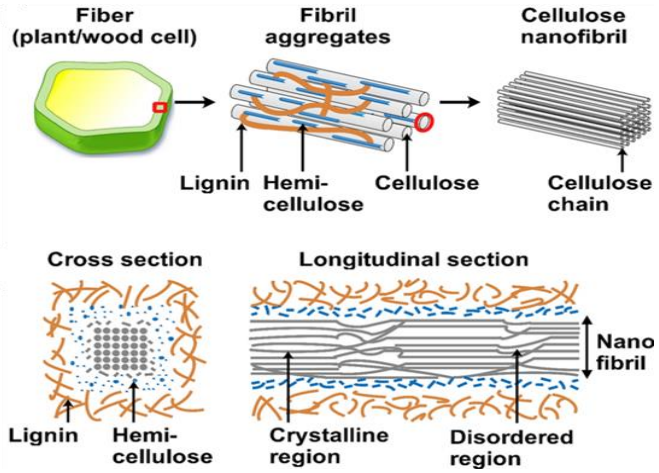


Figure 3. Schematic illustration of the microstructure of a cellulosic fiber (Adapted from reference [25]).

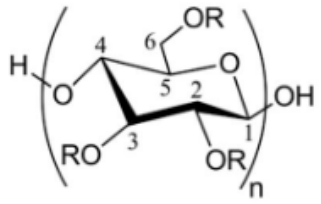
2.1. Cellulose Modifications

Functionalization or chemical treatment of cellulose has been developed to increase cellulose's worth or adaptability. The properties of modified or cellulose derivatives are determined by the degree and type of substitution, as well as the functionalization pattern along the cellulose chain. Cellulose has three hydroxyl groups in each glucose unit, depending on treatments and the cellulose origin. Cellulose can be modified chemically by substituting its hydroxyl groups with functional groups, such as chlorides, specific acids, and oxides, to develop new characteristics. The stiff and bulky main cellulose chain and its poor solubility in organic solvents pose a significant steric hindrance to the regioselective synthesis of cellulose derivatives. The hydroxyl group is the reactive component most often targeted on the cellulose chain. Taking advantage of the minor reactivity differences between the C2, C3, and C6-OH groups is complex since cellulose hydroxyl groups are not strongly nucleophiles and require strong reactions. Thus, regioselective replacement is one of the outstanding difficulties in synthesizing cellulose derivatives. The relationship between cellulose derivatives' regiochemical structure and characteristics must be understood to synthesize them under more general and realistic circumstances for commercial use.

Cellulose functionalization can be performed homogeneously or heterogeneously. To obtain cellulose derivatives with a high degree of substitution through heterogeneous functionalization, cellulose needs to be pretreated (activated) to decrease the interfiber bonding caused by hydrogen bonds. The most common activation procedure for cellulose for heterogeneous reactions is swelling in polar liquids such as ethanol, dimethyl sulfoxide, NH_3, or aqueous NaOH [36].

Etherification and esterification are two standard processes used in the chemical derivatization of cellulose based on the hydroxyl group. The derivatives may differ in essential properties such as chemical structure, water interaction, moisture sorption, solubility, and surface activity [37]. Acids and acid anhydrides are used as reagents in heterogeneous reactions to create commercial cellulose esters and ethers. The hydroxyl groups of cellulose can be totally or partially etherified by different reagents, e.g., halogenoalkanes, epoxides, and alpha-halogenated carboxylic acids. While cellulose esters are the main class of commercially thermoplastic biopolymers, they melt before decomposition and have good solubility in common solvents. Due to less complicated production processes, they can be utilized in biomedical applications. Esterification can be carried out using mineral, organic acids, or their anhydrides in the presence of a dehydrating agent so that esters can be either organic or inorganic [38]. In the case of the esterification of cellulose by organic acids, at a significant excess, the organic acid reactivity is insufficient for performing the esterification reaction. Therefore, acids chlorides or anhydrides are used as esterification reagents under acidic conditions; these conditions lead to backbone cleavage. The irreversible saponification of cellulose ester can take place under highly alkaline conditions. Accordingly, various cellulose esters have been synthesized like cellulose acetate, cellulose format, esters of high aliphatic acids, esters of mono-, di- and tricarboxylic aliphatic acids, esters of aromatic acids, esters of phosphonic or sulfonic acids [39]. The p-toluenesulfonyl cellulose (tosyl cellulose) is an imported ester that forms versatile intermediates in the synthesis of cellulose derivatives. Also, the tosyl group is considered a protective group, leaving the group for nucleophilic substitution reaction [40]. The homogenous or heterogeneous synthesis of tosyl cellulose introduces the tosyl group onto C6 [41]. The heterogeneous synthesis of tosyl cellulose is prepared by reacting suspended cellulose with excesses of p-toluenesulfonyl chloride (40 mol) to 1 mol of anhydroglucose in the presence of pyridine for a long time at 80 °C. So, the disadvantages of this method are the high reagent amounts, long reaction times, aminodesoxy group formation,

chlorination, and poor product solubilizes. All of these can be avoided by synthesis in a homogenous system. Rahn et al. prepared tosyl cellulose with a degree of substitution 0.4 - 2.3 by dissolving cellulose in dimethyl acetamide/LiCl and using 0.6-9.0 mol of tosyl chloride/mol of anhydroglucose unit in the presence of triethylamine as the base for 24 h at 8 °C [42]. The available inorganic cellulose esters are few compared with the large number of available inorganic acids. The oxygen-containing inorganic acids of the elements phosphorus, nitrogen, boron, and sulfur produce several cellulose esters such as cellulose phosphate, cellulose nitrate, cellulose borates, and cellulose sulfates [43].Since this review aims to highlight the application of cellulose sulfate as an anticoagulant agent, the following section will focus on the sulfonation of cellulose as a selected cellulose ester.



Cellulose Ethers (R = H or)	Cellulose Esters (R = H or)
Methyl Cellulose, CH_3	Cellulose Acetate, $(C=O)CH_3$
Ethyl Cellulose, CH_2CH_3	Cellulose Nitrate, NO_2
Hydroxyethyl Cellulose, CH_2CH_2OH	Cellulose Sulfate, SO_3H
Hydroxypropyl Cellulose, $CH_2CH(OH)CH_3$	
Carboxymethyl Cellulose, CH_2COOH	

Figure 4. Chemical structure of cellulose derivatives.

1.1Sulfonation of Cellulose

During the preparation of cellulose nanowhiskers by H_2SO_4, a negative charge is formed on the surface due to the esterification of OH groups by sulfate ions. The negative charge leads to a more stable suspended nanowhisker in an aqueous solution, and the esterification levels are highly dependent on acid concentrations and hydrolysis time [44]. Most inorganic and organic acids can be esterified with cellulose using techniques similar to simple alcohol use [45]. Cellulose sulfate refers to cellulose sulfuric acid half-ester, and it can be obtained by esterification of OH groups according to the following equations:

$$\text{Cell-OH} + SO_3 \rightarrow \text{Cell-OSO}_3\text{H}$$

$$\text{Cell-OH} + XSO_3H \rightarrow \text{Cell-OSO}_3\text{H} + XH \qquad (X = H_2N, HO, Cl)$$

The half-ester can be converted to its sodium salt, which is soluble in water with a sulfate degree of substitution > 0.2-0.3, depending on the ester moieties distribution. Also, it can be synthesized by using various sulfating agents to displace the nitrite group from its position in the anhydroglucose unit [46]. This allows cellulose to be directly sulfated without the need to isolate the cellulose trinitrite in a reaction system consisting of dissolved cellulose with an excess of N_2O_4 in dimethyl formamide and comprising an excess of N_2O_4 and HNO_3 as other components [46]. Another method for sulfating cellulose is combining sulfur trioxide and dimethyl sulfoxide to achieve a substitution degree up to 2 [47]. In general, sulfuric acid, sulfur trioxide, and chlorosulfonic acid are the most often utilized sulfating agents. They can be employed alone in reactions, with cellulose, or in inert mediums such as chlorinated hydrocarbons. An agent's molar ratio, temperature, and reaction time can all be adjusted to anhydroglucose, adjusting the sulfate group's degree of substitution range (0 – 3). The hydrolytic breakage of the glycosidic linkages during sulfation frequently results in the degradation of the cellulose chain [48]. Three methods can synthesize synthesize sulfate: homogeneously beginning with partially substituted cellulose derivatives, heterogeneously employing an activated cellulose solution, and by dsplacement reaction of an ether or ester group that already occurs on the macromolecule [49]. For the scaling up of the manufacture of cellulose sulfate, the sulfonation is carried out using a mixture of H_2SO_4 and *n*-propanol; the yield and conversion ratio is increased with increasing H_2SO_4 concentration in the reaction solution. The reaction mixture contains *n*-C_3H_7OH, H_2SO_4, H_2O and *n*-$C_3H_7OSO_3H$ as follows:

$$\alpha H_2SO_4 + \beta CH_3CH_2CH_2OH \xrightarrow{t<20^\circ C} \beta CH_3CH_2CH_2OSO_3H + (\alpha - \beta)H_2SO_4 + \beta H_2O$$

The presence of H_2O and H_2SO_4 leads to undesired reactions during the sulfonation process, such as hydrolysis or carbonization. The undesired reactions can be reduced by controlling the experimental conditions as T = –5°C, t<5 h, H_2SO_4 conc. 20–35% (w/w) and H_2O content <11% (w/w) according to Yao et al [50].

Cellulose sulfate with range of substitution degree (0.28–0.77) and (2%, w/v) viscosity (115–907) mPa s were produced by using anhydrous Na_2SO_4 as water absorbent, sulfuric acid/ethanol solution as sulfonating agent and changing the parameters such as temperature, the reaction time, Na_2SO_4 amount, liquid/solid ratio and sulfuric acid/ethanol ratio. The ^{13}C NMR analysis indicated that the sulfonation was produced at the C6 position. This process was eco-friendly and could be applied to scale production instead of traditional systems such as sulfuric acid/N-propanol [51].

Cellulose sulfate was homogenously prepared from partially functionalized cellulose ester such as tosyl cellulose (**Figure 5**). This sulfation is regioselective sulfation in which the tosyl group acts as a protecting group during the sulfation process, as it is stable under the sulfating process using a sulfur trioxide/ pyridine system. The tosylation occurs at C6 of the anhydroglucose unit and acts as a good leaving group for subsequent nucleophilic displacement reactions at C6 [52].

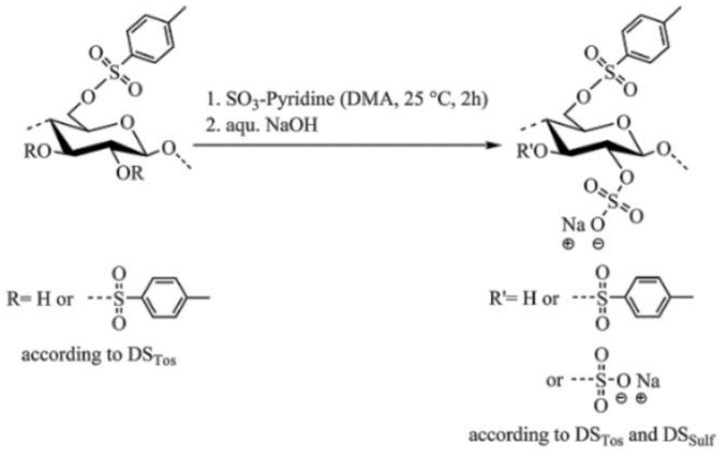


Figure 5. Schematic synthesis path of tosyl cellulose sulfate. (Adapted from reference [53]).

As mentioned before, the distribution and type of substituents in cellulose play an essential role in the anticoagulant activity of cellulose derivatives, cellulose phosphate, sulfate, and quaternary ammonia groups were prepared [23]. The preparation was performed with cellulose acetates with different degrees of substitution (1.9, 2.4, and 2.9). For regioselective deacetylation, cellulose triacetate was prepared in dimethylsulfoxide at 100°C; after cooling to 80°C hexamethylene diamine was added, giving cellulose acetate with a degree of substitution between 2.4 and 0.7. After neutralization, the product was precipitated in water, and in case of degree of substitution ≤1, ethanol was used to create the precipitation to prevent the precipitate from expanding too much. After this, sulfation of the partially substituted cellulose acetates, dissolved or suspended in dry dimethylformamide (**Figure 6a**). For cellulose phosphates, tri-n-butylamine was added to the dimethylformamide solution of partially substituted cellulose acetate, followed by the addition of tetrapolyphosforic, and the polymer was precipitated using ethanolic NaOH. Also, cellulose sulfate was prepared via trimethylsilyl cellulose as an intermediate (**Figure 6b**). In the case of quaternary ammonium groups, an aqueous solution of the half ester was reacted with (3-chloro-2-hydroxypropyl)-trimethylammonium chloride and the product was precipitated in methanol and neutralized with acetic acid (**Figure 6c**).

Cellulose sulfate is the one ester of cellulose produced by nucleophilic substituting one hydroxyl group in cellulose. The degree of sulfonation depends on the sulfonating agent, reaction components' molar ratio, medium, reaction temperature, and reaction time. According to organic chemistry rules, the primary hydroxyl groups are the most reactive species; thus, the substitution occurs first at position C6 in the cellulose. Prior research on cellulose sulfation has been done in homogeneous systems beginning with a partly substituted cellulose derivative in an aqueous solution or heterogeneous systems starting with an activated cellulose suspension [49, 54].

Two routes introduced carboxyl and sulfate groups regioselectively into anhydroglucose units of cellulose-given carboxyl cellulose sulfates. The first route was sulfonation of cellulose followed by 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO) oxidation, and the second was 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO) oxidation followed by sulfonation [55].

Synthesis of cellulose sulfate has been developed in the last three decades; one of these is the formation of nitrite ester groups by dissolving of cellulose in N_2O_4/N, N-dimethylformamide, the nitrite ester groups can be easily substituted by sulfate groups using chlorosulfonic acid or SO_3 as sulfating agents (**Figure 7**) [56].

Using LiCl/N, N-dimethylacetamide, or N–methylmorpholine-N-oxide as a homogeneous medium for sulfation of cellulose yields partially water soluble cellulose sulfate due to coagulation of the reaction system [57]. Although the heterogeneous synthesis, like a mixture of isopropyl alcohol and sulfuric acid of cellulose sulfate, is water soluble, the acidity of the reaction medium leads to chain degradation and uncontrolled substituent distribution [58]. However, water-soluble with homogeneous sulfation is associated only with low cellulose degradation [57]. Also, it can be prepared quasi-homogeneously through acetosulfation or direct sulfation, in which the cellulose suspension turns into a transparent solution during the reaction, and the cellulose is dissolved in the reaction mixture [59].

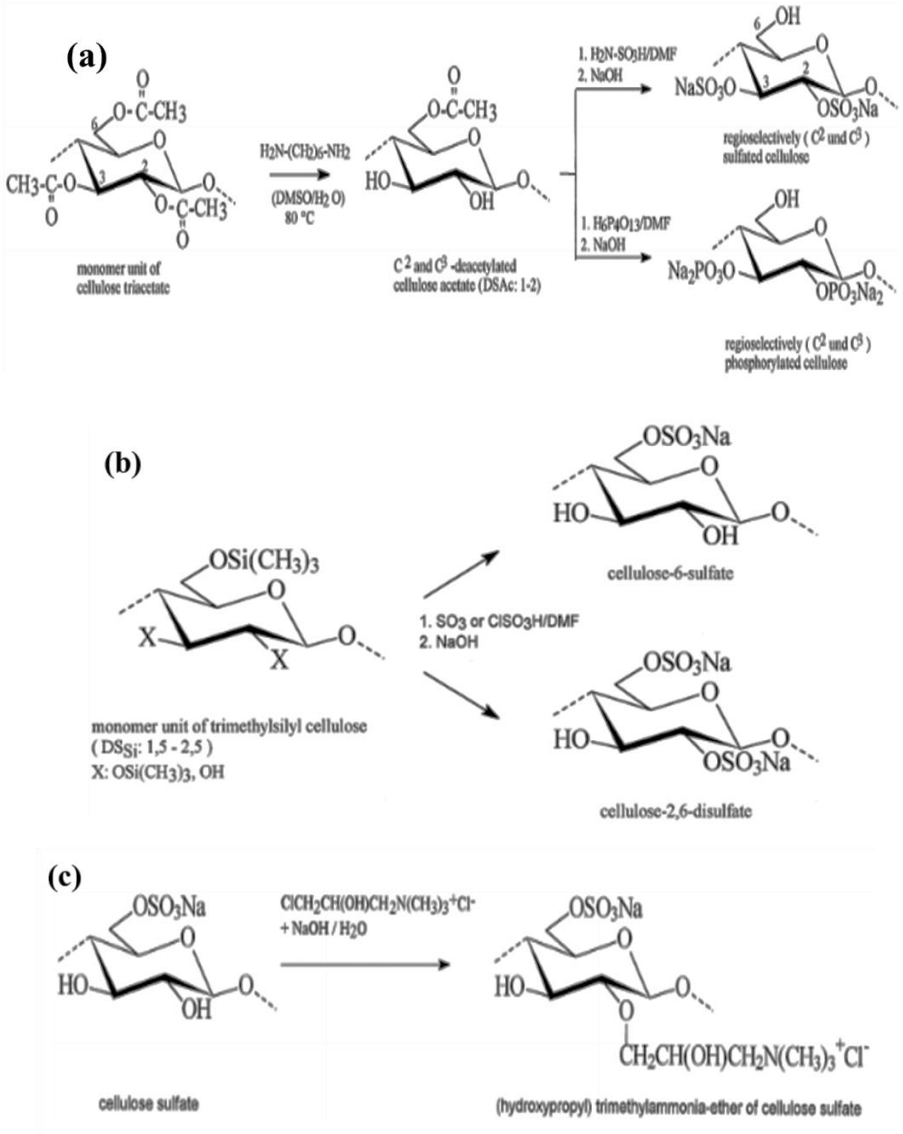


Figure 6. Schematic synthesis of (a) cellulose sulfates and phosphates via cellulose triacetate, (b) cellulose sulfates via trimethylsilyl cellulose, and (c) cellulose ampholytes by cationization of cellulose sulfates (Adapted from reference [23]).

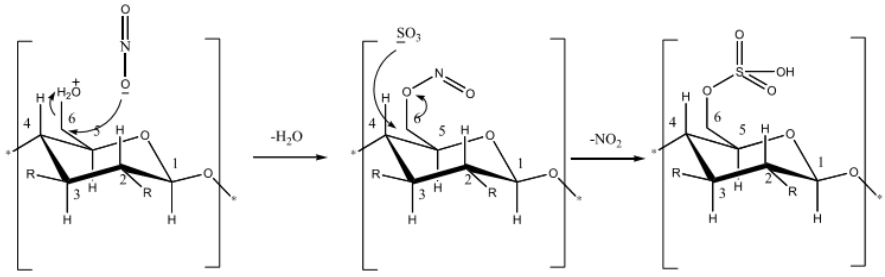


Figure 7. Mechanism of the cellulose sulfate formation via cellulose nitrite intermediate.

Bhatt et al. prepared water-soluble cellulose sulfate with a substitution degree of 0.392 by the sulfation of α-cellulose extracted from stems of Lantana camara in a heterogeneous medium using sulfuric acid [60]. The reaction was at 8 C for 60 min and 40 mL of an aqueous sulfuric acid (34.2N)/ 1 g of α-cellulose. The sulfating agent, hydrogen sulfate ion (HSO^-_3), is produced by dissociation of sulfuric acid in water (1). So, sufficient water is needed in the reaction mixture to produce the sulfate agent. The hydrogen sulfate ion reacts with the OH group of cellulose, forming cellulose sulfate, and by adding NaOH, the sodium salt of cellulose sulfate was produced

$$H_2SO_4 + H_2O \rightarrow HSO_4^- + H_3O^+ \quad (1)$$

$$(C_6H_7O_2)_x\,(OH)_{3x} \xrightarrow{HSO4-} (C_6H_7O_2)_x\,(OH)_{3x-a}\,(OSO_3H)_a \quad (2)$$

Cellulose — Cellulose sulfate

$$(C_6H_7O_2)_x\,(OH)_{3x-a}\,(OSO_3H)_a \xrightarrow{NaOH} (C_6H_7O_2)_x\,(OH)_{3x-a}\,(OSO_3Na^+)_a \quad (3)$$

Ragab et al. sulfated cellulose extracted from different sources, such as rice husk, rice straw, bagasse, and wheat straw, using sulfuric acid and 4-(Dimethylamino) pyridine as the sulfation agent and catalyst, respectively. The highest anticoagulation activities were observed in sulfated cellulose of bagasse, rice straw, rice husk, and wheat straw. They confirmed the formation of sulfated cellulose using FT-IR spectra by comparing the OH absorption intensity; due to the substitution of the sulfate group at OH, the OH intensity of sulfated cellulose should be less than that of cellulose (**Figure 8**) [61].

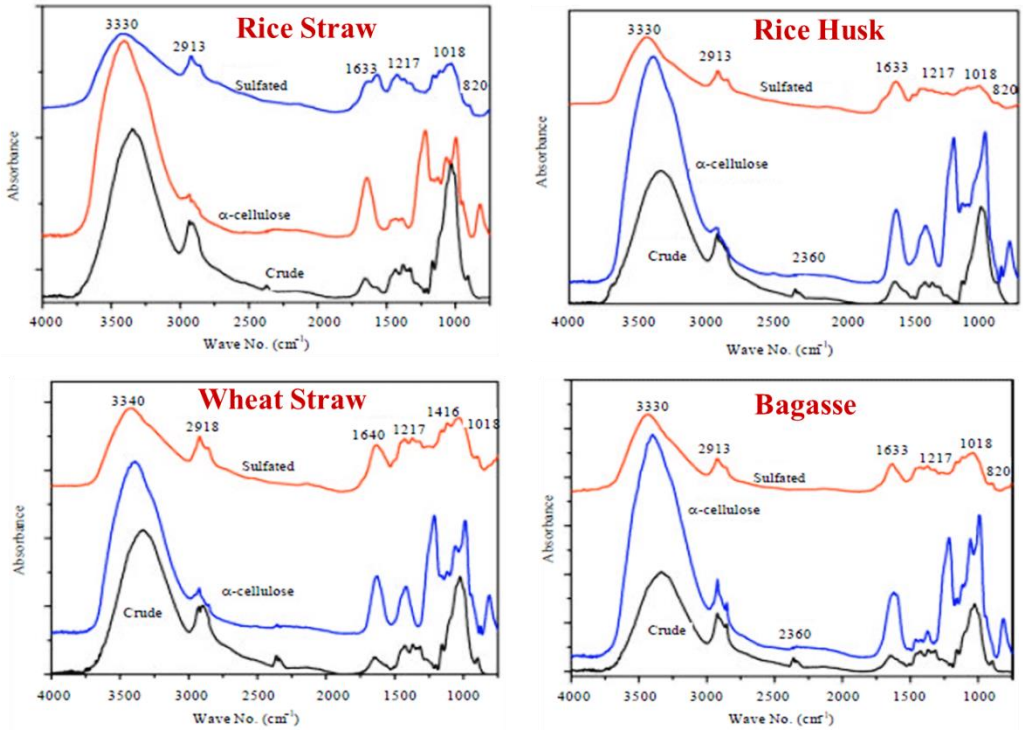


Figure 8. FT-IR spectra of crude cellulose, α-cellulose, and sulfated cellulose from rice straw, rice husk, wheat straw, and bagasse (Adapted from reference [60]).

Sodium cellulose sulfate with substitution degree ranged (from 1.1 to 1.7), and the average molecular weight (1.1 and 3.5 × 104 Da) was prepared by sulfation with chlorosulfonic acid/dimethylformamide as a sulfating agent. At room temperature, dimethylformamide (anhydrous) suspended microcrystalline cellulose was stirred overnight. The chlorosulfonic acid in dimethylformamide was added to the suspended solution with vigorous stirring. After 3 h, the mixture was poured into a saturated ethanolic solution of anhydrous sodium acetate. Using a centrifuge, the precipitate was collected and washed with anhydrous alcohol. It was water dissolved and neutralized with NaOH; the sodium salt was dialyzed against water and dried. The elemental analysis, FT-IR, and NMR spectroscopy analysis confirmed no substitution at C3, while sulfation mainly occurred at C6 and partially at C2 [62]. Cellulose sulfates with different degrees of substitution (0.21 and 2.59, attributed to sulfate groups)

and degrees of polymerization (59 and 232) were prepared by Zhang et al. It was found that the degree of polymerization had an inverse relationship between the amount of sulfur agent and the sulfur temperature [63].

Using ionic liquids as non-derivatizing cellulose solvents to prepare water-soluble cellulose sulfate leads to homogeneously direct sulfation of cellulose. The preparation of cellulose sulfate using 1-butyl-3-methylimidazolium chloride/dimethyl formamide led to a product with a degree of substitution ranging from 0.14 to 1.46. The NMR spectroscopy found that the sulfonation in ionic liquid leads to C-6-O-sulfated cellulose with minor polymer degradation. In addition, the used ionic liquids can be recycled and reused for sulfation of cellulose [57].

Zhang et al. prepared carboxyl- or carboxymethyl- and sulfated cellulose to study the effect of reaction conditions on the substitution degree of sodium cellulose sulfates through quasi-homogeneous direct sulfation or acetosulfation. Also, this study aimed to control the reaction conditions to regulate the substituents' distribution and molecular weights of the prepared cellulose sulfate. It was found that the sodium cellulose sulfate properties depend on starting materials and reaction parameters like reaction duration and temperature, e.g., with less acetic anhydride and high chlorosulfonic acid, a high substitution degree was obtained. Comparing direct Sulfation, the Sulfation at O-6- and O-2-positions was preferred, and no sulfation at O-3-position through acetosulfation was preferred. The -OH groups at C6 of cellulose sulfate could be converted into -COOH through 2,2,6,6-tetramethylpiperidine-1-oxyl radical (TEMPO) oxidation. Also, the -OH groups could be carboxymethylated with chloroacetic acid, but it was not selective, and the -COOH groups were found at all three positions (**Figure 9**) [59]. **Table** 2 summarizes recent studies on synthesizing cellulose sulfate from various materials and its applications in the medical field.

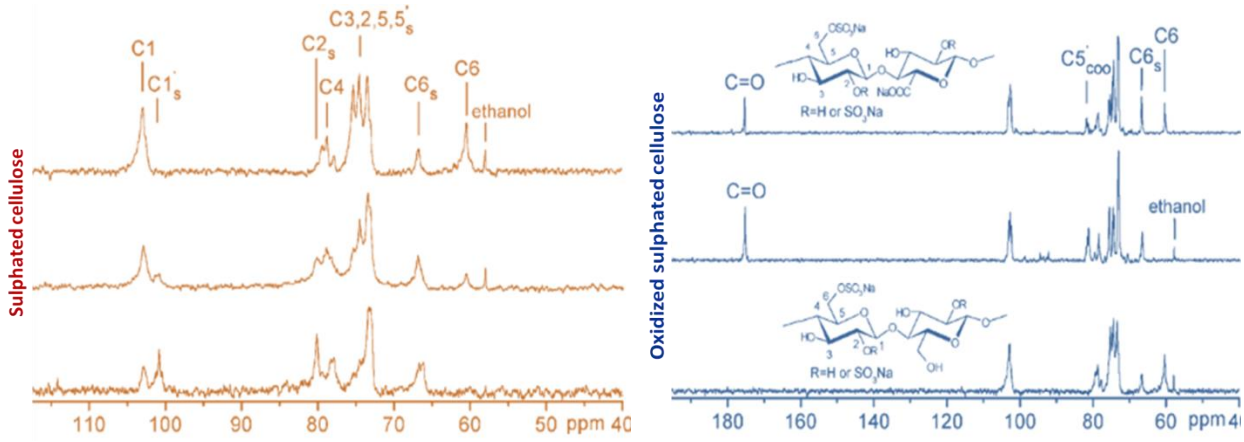


Figure 9. ^{13}C-NMR spectra of sulfated cellulose and oxidized sulfated cellulose in D_2O at room temperature (Adapted from reference [56]).

Table 2. Synthesis of cellulose sulfate from various materials and its applications in the medical field.

Cellulose source	Sulfonating agent	Application	Reference
Birch Cellulose	Sulfamic acid/ graphite-like catalyst	Anticoagulant and hypolipedemic substance	[64]
Cellulose sulfate	Sulphuric acid or sulphur trioxide or chlorosulfonic acid	Antiviral, antibacterial, anticoagulant, tissue engineering, and drug delivery	[65]
Bacterial cellulose	Cholorosulfonic acid	Tissue engineering	[66]
Cotton cellulose	Sulphuric acid	Antimicrobial	[67]
Tunicate cellulose	Sulphuric acid	Bone tissue engineering	[68]
Microcrystalline cellulose	Cholorosulfonic acid	Tissue engineering	[69]
Carboxymethyl cellulose	Chlorosulfonic acid/ *N*, *N*-Dimethylformamide	Osteoarthritis	[70]

2.Manifestation of Anticoagulant Activity

Cellulose sulfate is a water-soluble cellulose derivative with antibacterial, antiviral, and anticoagulant properties due to the sulfate groups' high degree of substitution. Due to the simplicity of its preparation, large-scale production and affordable cost, biocompatibility, biodegradability, and film-forming ability, cellulose sulfate is the frontrunner for potential biomedical applications like anticoagulant [25]. As was already noted, anticoagulants are widely used in many clinical applications. Certain anticoagulants are needed for specific medical disorders or application fields. For instance, an anticoagulant with a long-lasting impact is necessary to prevent venous thromboembolism, and an agent with the ability to prevent bleeding is needed for pre- and post-surgery administration [71]. The sulfate groups have a high negative charge, so sulfated cellulose nanocrystals have been

used as anticoagulant surfaces that mimic the heparin action [72]. By comparing the prepared sodium cellulose sulfate by Wang et al. from microcrystalline cellulose with heparin, it was found that heparin was discovered to be absent from the blood three hours later, sodium cellulose sulfate prolonged clotting time to three times that of the control until 12 h after subcutaneous treatment. Because of this, sodium cellulose sulfate has a longer half-life than heparin, meeting the need to prevent venous thromboembolism.

Furthermore, compared to heparin, sodium cellulose sulfate had less action in mice, as determined by the thrombin time assay and clotting time determination (**Figure 10)**. In the in vivo test, a slight dose-response activity was triggered by sodium cellulose sulfate rather than a dramatic effect caused by heparin. Therefore, sodium cellulose sulfate can replace heparin in clinical settings [62]. Cui et al. found that the important factors in determining the anticoagulation efficiency of polysaccharides are their monosaccharide composition, sulfate ester, and molecular weight [73]. The anticoagulant activity of the sulfated cellulose increases with an increasing degree of sulfation.

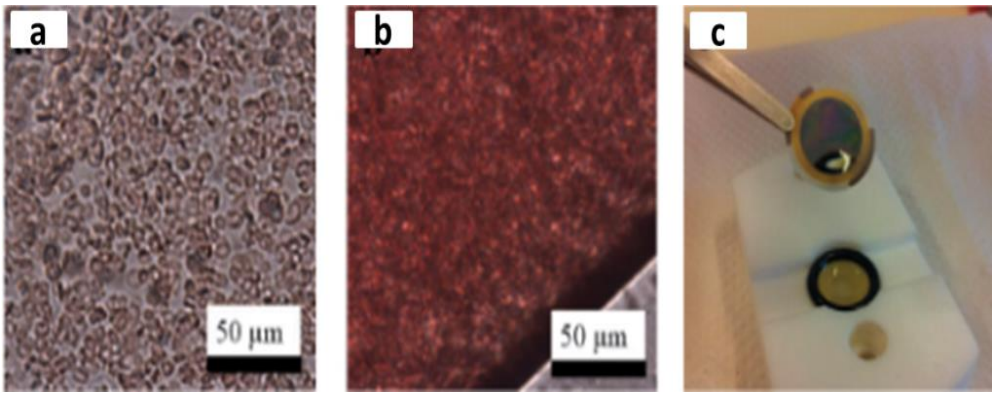


Figure 10. Optical microscopy images of blood contacted with branched polyethyleneimine (a), sulfated cellulose nanocrystal (b), and the Quartz Crystal Microbalance with Dissipation monitoring (c) (Adapted from reference [64]).

A study determined the relationships between sodium cellulose sulfate's chemical properties, blood anticoagulant action, and toxicity. Different samples of cellulose sulfate ranging in number-average molecular weights (800 - 36.8 x 104) and total degree of substitutions (0.5 - 2.75) were prepared. The way in which substituent groups are distributed on the C2, C3, and C6 in a glucopyranose unit was determined by the NMR method [74]. **Figure 11** shows the relation between anticoagulant activity and total cellulose sulfate substitution degree. Qualitatively, by increasing the degree of substitution, anticoagulant activity tends to approach the good equivalent or superior grade. In samples with a degree of substitution less than 1.94, the anticoagulant activity was of neither good equivalent nor superior grade. It is noted from the Figure that the degree of substitution is not the only controlling factor for anticoagulant activity because when the degree of substitution is 1.88-2.05, anticoagulant activity covers all grades. All cellulose sulfate samples having a degree of substitution >1.88 showed a degree of substitution of the superior or good equivalent grade, but samples with a degree of substitution of 1.97 showed anticoagulant activity of the non-active grade. Also, it is noted that the anticoagulant activity is related not only to but also to the average distribution of the substituent groups in C2, C3, and C6. **Figure 11** shows the anticoagulant activity of cellulose sulfate as a function of substitution in C2, C3, and C6. Substitution in C2, C3, and (C2 + C3) show good correlation with anticoagulant activity, but C6 does not. All cellulose sulfate samples with C2 < 0.75 or C3 < 0.48 have anticoagulant activity of the non-active grade, and (C2 + C3) has the best correlation with anticoagulant activity. It is also found that the average molecular weight significantly affects the anticoagulant activity, as the anticoagulant activity tends to increase with a decrease in the average molecular weight.

At the same average molecular weight, acute toxicity decreases as substitution in (C2 + C3) increases. So, it is challenging to obtain cellulose sulfate with suitable acute toxicity and high anticoagulant activity under these conditions. In particles, the cellulose sulfate is non-toxic, but its anticoagulant activity is of the non-active grade.

From the study also, it was found that cellulose sulfate has an acute toxicity more significant than heparin, which is found in the arterial walls of both humans and animals, so no cellulose sulfate sample with anticoagulant activity and acute toxicity comparable to heparin can be predicted. Except for coagulation factor VIII (antihemophilic globulin), the action of cellulose sulfate on all coagulation factors is the same as that of heparin, and it inhibits factor VIII much more effectively than heparin. Consequently, factor VIII appears to be the primary target of cellulose sulfate, with a minor effect on factor IX (plasma thromboplastin component), which heparin was discovered to inhibit.

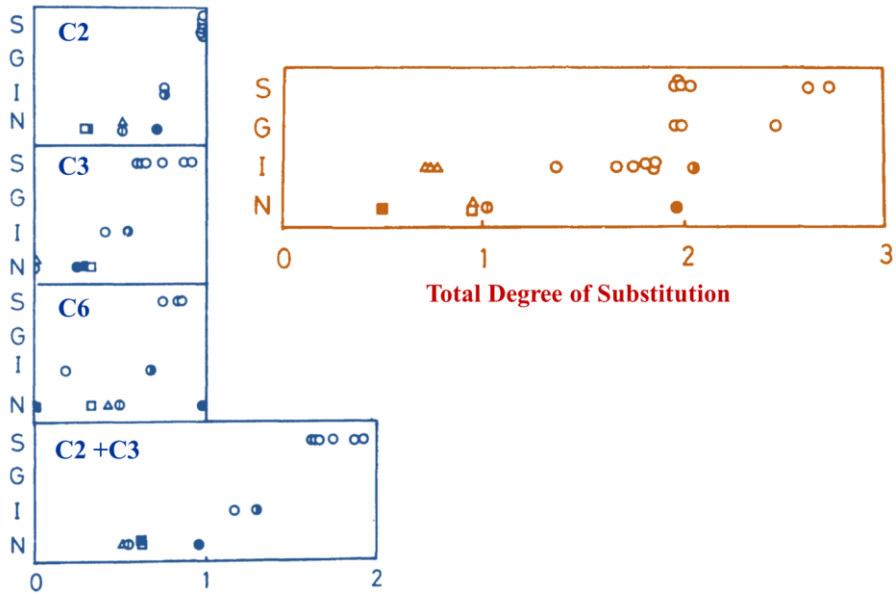


Figure 11. Effect of substitution in C2, C3, and C6 and the total degree of substitution of cellulose sulfate on anticoagulant activity (I = inferior, G = good equivalent, N = non-active, and S = superior grade) (Adapted from reference [66]).

In rabbit experiments, increased plasma clotting times in some coagulation tests and plasma anticoagulant activity were seen when cellulose sulfate prepared from wheat straw with a dynamic viscosity of 3.4 cP and sulfur content of 14.1% was given intravenously. The plasma clotting time in the presence of the anticoagulant (five minutes after delivery) was approximately three times longer after cellulose sulfate (1 mg/kg) than after saline administration [75].

Carboxymethyl cellulose was reacted with an aqueous sodium bisulfite/sodium nitrite solution, giving a sulfate derivative. Their anticoagulant activity was investigated by the thrombin time (TT), activated partial thromboplastin time (APTT), and prothrombin time (PT). The results indicated that the sulfate groups improved the anticoagulant activity, and the activity relates to molecular weight, degree of substitution, and concentration; the low molecular weight promotes anticoagulant activity, and the high degree of substitution and concentration prolongs the activated partial thromboplastin time (APTT) (**Figure 12**) [76]. In another study, ethanolic solution of chitosan nanoparticles with ethylcellulose was electrospined onto a membrane of bacterial cellulose sulfate. The resulting membrane had good blood compatibility with non-adhere platelets; the anticoagulation times were the same as those of human plasma, and the inflammation was safe for application in the bioenvironment [77].

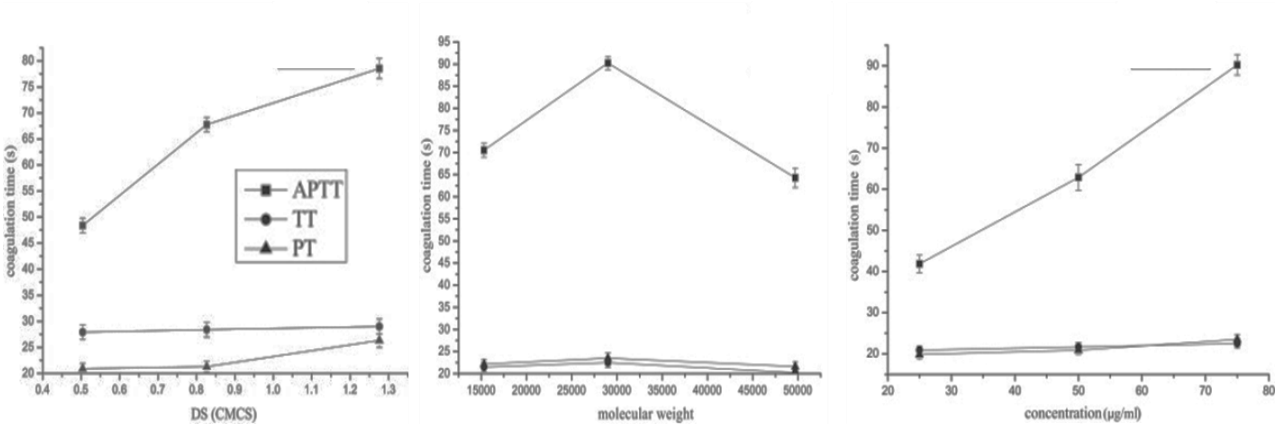


Figure 12. Effect of substitution degree (DS), concentration, and molecular weights on anticoagulant activity of carboxymethyl cellulose sulfates (Adapted from reference [68]).

3. Conclusions, Future Prospects and Outlook

- Numerous studies have been reported on modifying and using cellulosic materials over the past few decades. Because of their superior physical and biological qualities, biocompatibility, biodegradability, and low cytotoxicity, cellulosic materials

unquestionably hold great promise for applications in biological implants. This review summarizes the preparation of cellulose derivatives, focusing on cellulose sulfate. It also highlights their adaptability for use as an anticoagulant.

- Even though cellulose sulfate is non-toxic, further investigation is required to assess its cytotoxicity profile and possible pharmacological side effects.
- Because cellulose hydroxyl groups are not highly nucleophiles and hence require strong reactions, it is challenging to capitalize on the slight reactivity differences between the C2, C3, and C6-OH groups.
- The main issue in scaling up the manufacture of cellulose sulfate is recycling the reaction solution and other byproducts. Consequently, the regeneration approach is a fruitful step in achieving this objective.
- From the perspective of anticoagulant action, it is highly desirable to prepare cellulose sulfate with higher C2 + C3 and lower average molecular weight. This suggests that the mobility of cellulose sulfate in solution is a minor factor in its anticoagulant activity.
- By studying the relationships between sodium cellulose sulfate's chemical properties, blood anticoagulant action, and acute toxicity. It was discovered that cellulose sulfate had a far more potent inhibitory effect on coagulation factor VIII than heparin.

Conflict of Interests

The authors declare that they have no conflict with interests.

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References

[1] P.A. Mourão, M.S. Pereira, Searching for alternatives to heparin: sulfated fucans from marine invertebrates, Trends in Cardiovascular Medicine 9(8) (1999) 225-232.

[2] N. Mestechkina, V. Shcherbukhin, Sulfated polysaccharides and their anticoagulant activity: A review, Applied Biochemistry and Microbiology 46 (2010) 267-273.

[3] W. Mao, X. Zang, Y. Li, H. Zhang, Sulfated polysaccharides from marine green algae Ulva conglobata and their anticoagulant activity, Journal of Applied phycology 18 (2006) 9-14.

[4] T. Maruyama, T. Toida, T. Imanari, G. Yu, R.J. Linhardt, Conformational changes and anticoagulant activity of chondroitin sulfate following its O-sulfonation, Carbohydrate Research 306(1-2) (1998) 35-43.

[5] J. Choay, M. Petitou, J.-C. Lormeau, P. Sinay, B. Casu, G. Gatti, Structure-activity relationship in heparin: a synthetic pentasaccharide with high affinity for antithrombin III and eliciting high anti-factor Xa activity, Biochemical and biophysical research communications 116(2) (1983) 492-499.

[6] D.H. Atha, J.C. Lormeau, M. Petitou, R.D. Rosenberg, J. Choay, Contribution of 3-O-and 6-O-sulfated glucosamine residues in the heparin-induced conformational change in antithrombin III, Biochemistry 26(20) (1987) 6454-6461.

[7] A. Danielsson, E. Raub, U. Lindahl, I. Björk, Role of ternary complexes, in which heparin binds both antithrombin and proteinase, in the acceleration of the reactions between antithrombin and thrombin or factor Xa, Journal of Biological chemistry 261(33) (1986) 15467-15473.

[8] R. Carrell, P. Christey, D. Boswell, Serpins: antithrombin and other inhibitors of coagulation and fibrinolysis. Evidence from amino acid sequences, Thrombosis and haemostasis 58(05) (1987) 0097.

[9] R.E. Hurst, J.M. Menter, S.S. West, J.M. Settine, E.H. Coyne, Structural basis for the anticoagulant activity of heparin. 1. Relationship to the number of charged groups, Biochemistry 18(20) (1979) 4283-4287.

[10] P. Schoen, S. Wielders, M. Petitou, T. Lindhout, The effect of sulfation on the anticoagulant and antithrombin III-binding properties of a heparin fraction with low affinity for antithrombin III, Thrombosis research 57(3) (1990) 415-423.

[11] T. Groth, C. Willems, K. Zhang, S. Fischer, Development of bioactive cellulose sulfates for biomedical applications, Acta medica Medianae 59(3) (2020).

[12] Y. Chen, X. Sun, J. Shan, C. Tang, R. Hu, T. Shen, H. Qiao, M. Li, W. Zhuang, C. Zhu, Flow synthesis, characterization, anticoagulant activity of xylan sulfate from sugarcane bagasse, International journal of biological macromolecules 155 (2020) 1460-1467.

[13] L. Fras Zemljič, N. Dimitrušev, R. Zaplotnik, S. Strnad, Insights into adsorption characterization of sulfated xylans onto poly (ethylene terephthalate), Polymers 12(4) (2020) 825.

[14] M.S. Geronço, I.F. da Silveira Ramos, E.C. da Silva Filho, M. dos Santos Rizzo, A.B. Ribeiro, M.P. da Costa, Are structurally modified galactomannan derivatives biologically active?, Polysaccharides 2(1) (2021) 1-15.

[15] P. Ramasamy, S. Sekar, S. Paramasivam, P. Suri, U. Chinnaiyan, R. Singh, B.P. Tanguturi Raghavaiah, V.D. Seshadri, Sulfation of chitosan from Sepia kobiensis as potential anticoagulant and antibacterial molecule, Natural Product Research 36(12) (2022) 3216-3222.

[16] F.D. da Silva Chagas, G.C. Lima, V.I.N. Dos Santos, L.E.C. Costa, W.M. de Sousa, V.G. Sombra, D.F. de Araújo, F.C.N. Barros, E. Marinho-Soriano, J.P. de Andrade Feitosa, Sulfated polysaccharide from the red algae Gelidiella acerosa: Anticoagulant, antiplatelet and antithrombotic effects, International Journal of Biological Macromolecules 159 (2020) 415-421.

[17] K. Saluri, R. Tuvikene, Anticoagulant and antioxidant activity of lambda-and theta-carrageenans of different molecular weights, Bioactive Carbohydrates and Dietary Fibre 24 (2020) 100243.

[18] Y. Jin, Y. Mu, S. Zhang, P. Li, F. Wang, Preparation and evaluation of the adjuvant effect of curdlan sulfate in improving the efficacy of dendritic cell-based vaccine for antitumor immunotherapy, International journal of biological macromolecules 146 (2020) 273-284.

[19] U. Lindahl, L. Kjellén, Heparin or heparan sulfate-what is the difference?, Thrombosis and haemostasis 66(07) (1991) 044-048.

[20] J. Choay, Chemically Synthesized Heparin-Derived Oligosaccharides, Annals of the New York Academy of Sciences 556(1) (1989) 61-74.

[21] R. Schwartz-Albiez, Y. Adams, C.-W. von der Lieth, P. Mischnick, K.T. Andrews, M. Kirschfink, Regioselectively modified sulfated cellulose as prospective drug for treatment of malaria tropica, Glycoconjugate Journal 24 (2007) 57-65.

[22] V. Stadlbauer, P. Stiegler, S. Schaffellner, O. Hauser, G. Halwachs, F. Iberer, K. Tscheliessnigg, C. Lackner, Morphological and functional characterization of a pancreatic β-cell line microencapsulated in sodium cellulose sulfate/poly (diallyldimethylammonium chloride), Xenotransplantation 13(4) (2006) 337-344.

[23] T. Groth, W. Wagenknecht, Anticoagulant potential of regioselective derivatized cellulose, Biomaterials 22(20) (2001) 2719-2729.

[24] Y. Fahmy, T.Y. Fahmy, F. Mobarak, M. El-Sakhawy, M. Fadl, Agricultural residues (wastes) for manufacture of paper, board, and miscellaneous products: Background overview and future prospects, Fahmy Y, Fahmy TYA, Mobarak F, El-Sakhawy M, Fadl MH (2017) Agricultural Residues (Wastes) for Manufacture of Paper, Board, and Miscellaneous Products: Background Overview and Future Prospects. International Journal of ChemTech Research 10(2) (2017) 424-448.

[25] H. Seddiqi, E. Oliaei, H. Honarkar, J. Jin, L.C. Geonzon, R.G. Bacabac, J. Klein-Nulend, Cellulose and its derivatives: Towards biomedical applications, Cellulose 28(4) (2021) 1893-1931.

[26] T.S. Tanzila Shahzadi, S.M. Sajid Mehmood, M.I. Muhammad Irshad, Z.A. Zahid Anwar, A.A. Amber Afroz, N.Z. Nadia Zeeshan, U.R. Umer Rashid, K.S. Kalsoom Sughra, Advances in lignocellulosic biotechnology: a brief review on lignocellulosic biomass and cellulases, (2014).

[27] G. Agoda-Tandjawa, S. Durand, S. Berot, C. Blassel, C. Gaillard, C. Garnier, J.-L. Doublier, Rheological characterization of microfibrillated cellulose suspensions after freezing, Carbohydrate Polymers 80(3) (2010) 677-686.

[28] A. Müller, M. Zink, N. Hessler, F. Wesarg, F.A. Müller, D. Kralisch, D. Fischer, Bacterial nanocellulose with a shape-memory effect as potential drug delivery system, Rsc Advances 4(100) (2014) 57173-57184.

[29] D. Klemm, E.D. Cranston, D. Fischer, M. Gama, S.A. Kedzior, D. Kralisch, F. Kramer, T. Kondo, T. Lindström, S. Nietzsche, Nanocellulose as a natural source for groundbreaking applications in materials science: Today's state, Materials Today 21(7) (2018) 720-748.

[30] B. Lindman, B. Medronho, L. Alves, C. Costa, H. Edlund, M. Norgren, The relevance of structural features of cellulose and its interactions to dissolution, regeneration, gelation and plasticization phenomena, Physical Chemistry Chemical Physics 19(35) (2017) 23704-23718.

[31] A. Benítez, A. Walther, Cellulose nanofibril nanopapers and bioinspired nanocomposites: a review to understand the mechanical property space, Journal of Materials Chemistry A 5(31) (2017) 16003-16024.

[32] A.J. Benítez, J. Torres-Rendon, M. Poutanen, A. Walther, Humidity and multiscale structure govern mechanical properties and deformation modes in films of native cellulose nanofibrils, Biomacromolecules 14(12) (2013) 4497-4506.

[33] S. Kamel, N. Ali, K. Jahangir, S. Shah, A. El-Gendy, Pharmaceutical significance of cellulose: A review, Express Polym Lett 2(11) (2008) 758-778.

[34] J. Catalán, M. Ilves, H. Järventaus, K.S. Hannukainen, E. Kontturi, E. Vanhala, H. Alenius, K.M. Savolainen, H. Norppa, Genotoxic and immunotoxic effects of cellulose nanocrystals in vitro, Environmental and molecular mutagenesis 56(2) (2015) 171-182.

[35] C.C. Coelho, M. Michelin, M.A. Cerqueira, C. Gonçalves, R.V. Tonon, L.M. Pastrana, O. Freitas-Silva, A.A. Vicente, L.M. Cabral, J.A. Teixeira, Cellulose nanocrystals from grape pomace: Production, properties and cytotoxicity assessment, Carbohydrate polymers 192 (2018) 327-336.

[36] T. Heinze, K. Petzold-Welcke, J.E. van Dam, Polysaccharides: Molecular and supramolecular structures. Terminology, The European Polysaccharide Network of Excellence (EPNOE) Research Initiatives and Results (2012) 23-64.

[37] X. Zheng, R.D. Gandour, K.J. Edgar, Remarkably regioselective deacylation of cellulose esters using tetraalkylammonium salts of the strongly basic hydroxide ion, Carbohydrate polymers 111 (2014) 25-32.

[38] M.C. Shelton, Cellulose Esters, Inorganic Esters, Kirk-Othmer Encyclopedia of Chemical Technology (2000).
[39] T. Heinze, New ionic polymers by cellulose functionalization, Macromolecular Chemistry and Physics 199(11) (1998) 2341-2364.
[40] K. Petzold-Welcke, N. Michaelis, T. Heinze, Unconventional cellulose products through nucleophilic displacement reactions, Macromolecular symposia, Wiley Online Library, 2009, pp. 72-85.
[41] S.I. Takahashi, T. Fujimoto, B.M. Barua, T. Miyamoto, H. Inagaki, 13C-NMR spectral studies on the distribution of substituents in some cellulose derivatives, Journal of Polymer Science Part A: Polymer Chemistry 24(11) (1986) 2981-2993.
[42] K. Rahn, M. Diamantoglou, D. Klemm, H. Berghmans, T. Heinze, Homogeneous synthesis of cellulose p-toluenesulfonates in N, N-dimethylacetamide/LiCl solvent system, Die Angewandte Makromolekulare Chemie: Applied Macromolecular Chemistry and Physics 238(1) (1996) 143-163.
[43] T. Heinze, T. Liebert, A. Koschella, Esterification of polysaccharides, Springer Science & Business Media2006.
[44] A.H.A. Kader, S. Dacrory, T.A. Khattab, S. Kamel, H. Abou-Yousef, Hydrophobic and flame-retardant foam based on cellulose, Journal of Polymers and the Environment 30(6) (2022) 2366-2377.
[45] X. Huang, W.-D. Zhang, Preparation of cellulose sulphate and evaluation of its properties, Journal of fiber bioengineering and informatics 3(1) (2010) 32-39.
[46] T. Genco, Amino cellulose sulfate: synthesis, characterization and physicochemical behavior, 2013.
[47] R.L. Whistler, A.H. King, G. Ruffini, F. Lucas, Sulfation of cellulose with sulfur-trioxide-dimethyl sulfoxide, Archives of Biochemistry and Biophysics 121(2) (1967) 358-363.
[48] D. Klemm, B. Philpp, T. Heinze, U. Heinze, W. Wagenknecht, Comprehensive cellulose chemistry. Volume 1: Fundamentals and analytical methods, Wiley-VCH Verlag GmbH1998.
[49] M. Gohdes, P. Mischnick, Determination of the substitution pattern in the polymer chain of cellulose sulfates, Carbohydrate research 309(1) (1998) 109-115.
[50] S. Yao, An improved process for the preparation of sodium cellulose sulphate, Chemical Engineering Journal 78(2-3) (2000) 199-204.
[51] G. Chen, B. Zhang, J. Zhao, H. Chen, Improved process for the production of cellulose sulfate using sulfuric acid/ethanol solution, Carbohydrate polymers 95(1) (2013) 332-337.
[52] A. Koschella, D. Fenn, N. Illy, T. Heinze, Regioselectively functionalized cellulose derivatives: a mini review, Macromolecular Symposia, Wiley Online Library, 2006, pp. 59-73.
[53] T. Heinze, K. Rahn, The first report on a convenient synthesis of novel reactive amphiphilic polysaccharides, Macromolecular rapid communications 17(10) (1996) 675-681.
[54] W. Wagenknecht, I. Nehls, B. Philipp, Studies on the regioselectivity of cellulose sulfation in an N2O4-N, N-dimethylformamide-cellulose system, Carbohydrate research 240 (1993) 245-252.
[55] K. Zhang, D. Peschel, T. Klinger, K. Gebauer, T. Groth, S. Fischer, Synthesis of carboxyl cellulose sulfate with various contents of regioselectively introduced sulfate and carboxyl groups, Carbohydrate polymers 82(1) (2010) 92-99.
[56] M. Granström, Cellulose derivatives: synthesis, properties and applications, (2009).
[57] M. Gericke, T. Liebert, T. Heinze, Interaction of ionic liquids with polysaccharides, 8–synthesis of cellulose sulfates suitable for polyelectrolyte complex formation, Macromolecular Bioscience 9(4) (2009) 343-353.
[58] B. Lukanoff, H. Dautzenberg, Natriumcellulosesulfat als Komponente für die Erzeugung von Mikrokapseln durch Polyelektrolytkomplexbildung. I: Heterogene Sulfatierung von Cellulose unter Verwendung von Schwefelsäure/Propanol als Reaktionsmedium und Sulfatiermittel, Das Papier (Darmstadt) 48(6) (1994) 287-298.
[59] K. Zhang, D. Peschel, E. Brendler, T. Groth, S. Fischer, Synthesis and bioactivity of cellulose derivatives, Macromolecular symposia, Wiley Online Library, 2009, pp. 28-35.
[60] N. Bhatt, P. Gupta, S. Naithani, Preparation of cellulose sulfate from α-cellulose isolated from Lantana camara by the direct esterification method, Journal of applied polymer science 108(5) (2008) 2895-2901.
[61] T.I. Ragab, H. Amer, A. Wasfy, M.A. Hady, A.T.H. Mossa, F. Liebner, Sulfated cellulose from agriculture wastes, anticoagulant, fibrinolytic and toxicological studies, (2014).
[62] Z.M. Wang, L. Li, B.S. Zheng, N. Normakhamatov, S.Y. Guo, Preparation and anticoagulation activity of sodium cellulose sulfate, International Journal of Biological Macromolecules 41(4) (2007) 376-382.
[63] K. Zhang, D. Peschel, E. Bäucker, T. Groth, S. Fischer, Synthesis and characterisation of cellulose sulfates regarding the degrees of substitution, degrees of polymerisation and morphology, Carbohydrate polymers 83(4) (2011) 1659-1664.
[64] A. Kazachenko, Y. Berezhnaya, V. Borovkova, V. Sychev, O. Fetisova, Y. Malyar, N. Issaoui, Sulfation of Birch Cellulose with Sulfamic Acid on a Graphite-Like Catalyst, International Scientific Conference Fundamental and Applied Scientific Research in the Development of Agriculture in the Far East, Springer, 2023, pp. 606-614.
[65] N. Chandel, K. Jain, A. Jain, T. Raj, A.K. Patel, Y.-H. Yang, S.K. Bhatia, The versatile world of cellulose-based materials in healthcare: From production to applications, Industrial Crops and Products 201 (2023) 116929.

[66] V. Palaninathan, S. Raveendran, A.K. Rochani, N. Chauhan, Y. Sakamoto, T. Ukai, T. Maekawa, D.S. Kumar, Bioactive bacterial cellulose sulfate electrospun nanofibers for tissue engineering applications, Journal of tissue engineering and regenerative medicine 12(7) (2018) 1634-1645.

[67] N. Normakhamatov, P. Mischnick, B. Muhitdinov, I. Mukhamedov, A. Turaev, Sodium cellulose sulfate and its antimicrobial activity, Reactive and Functional Polymers 191 (2023) 105672.

[68] X. Lv, H. Yu, J. Han, Y. Hou, Y. Sun, K. Liu, W. Zhou, J. Chen, Tunicate cellulose nanocrystals reinforced modified calcium sulfate bone cement with enhanced mechanical properties for bone repair, Carbohydrate Polymers 323 (2024) 121380.

[69] N. Aggarwal, N. Altgärde, S. Svedhem, K. Zhang, S. Fischer, T. Groth, Study on multilayer structures prepared from heparin and semi-synthetic cellulose sulfates as polyanions and their influence on cellular response, Colloids and Surfaces B: Biointerfaces 116 (2014) 93-103.

[70] A. Bhattacharjee, D.S. Katti, Sulfated carboxymethylcellulose-based scaffold mediated delivery of Timp3 alleviates osteoarthritis, International Journal of Biological Macromolecules 212 (2022) 54-66.

[71] R.H. White, J.S. Ginsberg, Low-molecular-weight heparins: are they all the same?, British journal of haematology 121(1) (2003) 12-20.

[72] H.M. Ehmann, T. Mohan, M. Koshanskaya, S. Scheicher, D. Breitwieser, V. Ribitsch, K. Stana-Kleinschek, S. Spirk, Design of anticoagulant surfaces based on cellulose nanocrystals, Chemical Communications 50(86) (2014) 13070-13072.

[73] J. Cui, Y. Li, S. Wang, Y. Chi, H. Hwang, P. Wang, Directional preparation of anticoagulant-active sulfated polysaccharides from Enteromorpha prolifera using artificial neural networks, Scientific reports 8(1) (2018) 3062.

[74] K. Kamide, K. Okajima, T. Matsui, M. Ohnishi, H. Kobayashi, Roles of molecular characteristics in blood anticoagulant activity and acute toxicity of sodium cellulose sulfate, Polymer Journal 15(4) (1983) 309-321.

[75] N. Drozd, S. Kuznetsova, T. Kalinina, N.Y. Vasilieva, Dose dependence of the anticoagulant effect of intravenously administered cellulose sulfate, Bulletin of experimental biology and medicine 160(6) (2016) 767-771.

[76] L. Fan, X. Zhou, P. Wu, W. Xie, H. Zheng, W. Tan, S. Liu, Q. Li, Preparation of carboxymethyl cellulose sulfates and its application as anticoagulant and wound dressing, International journal of biological macromolecules 66 (2014) 245-253.

[77] Z. Li, J. Ma, R. Li, X. Yin, W. Dong, C. Pan, Fabrication of a blood compatible composite membrane from chitosan nanoparticles, ethyl cellulose and bacterial cellulose sulfate, RSC advances 8(55) (2018) 31322-31330.