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Review

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Sulfoethyl Chitosan: From Synthesis and Physicochemical Characterization to Functional Applications and Patent Perspectives

Sulfoethyl chitosan (SEC) is an anionic chitosan derivative obtained through N-, O-, or mixed O, N-substitution with sulfoethyl groups, which significantly enhances water solubility across a wide pH range, imparts strong polyelectrolyte character, and confers heparin-mimicking biological properties. This review provides a comprehensive overview of SEC synthesis approaches, chemoselective routes thereof, and the influence of the degree of substitution (DS) and substitution pattern on solubility, swelling, thermal stability, and metal-ion sorption selectivity. Characterization techniques including ^{1}H NMR, elemental analysis, FT-IR, X-ray diffraction and molecular weight determination approaches are discussed with their specific challenges for polyelectrolyte derivatives, including sample purity and aggregation. The impact of sulfoethylation on biological properties including anticoagulant activity, hemocompatibility, cytotoxicity, antimicrobial effect, DNA protective activity was evaluated for sulfoethylated chitosan and its precursors, chitin and chitin-glucan complex, revealing distinct structure-activity trends governed by the degree of acetylation and substitution pattern. Representative applications include selective solid-phase extraction of copper from environmental waters, anticoagulant coatings, and macroporous cryogels for long-term bacterial stabilization at ambient temperatures over 12 months. Despite its versatility, SEC remains under-patented compared to other chitosan derivatives. Critical knowledge gaps persist, including the absence of systematic DS-bioactivity and regioselectivity-bioactivity relationships and long-term stability studies, which raises doubts on the industrial demand of the biopolymer. Future perspectives emphasize standardized protocols for characterization and for biological activity assessment, pilot manufacturing with reduced toxic and environmental impact, and interdisciplinary efforts to develop advanced functional materials. With focused attention on these priorities, SEC has potential to transition from a laboratory derivative to a commercially viable biopolymer.

Keywords: sulfoethyl chitosan, biopolymer, polysaccharide, sulfoethylation, synthesis, characterization, chitosan derivatives, patent landscape.

Contents

List of abbreviations

AUC: analytical ultracentrifugation
DLS: dynamic light scattering
DS: degree of substitution
FT-IR: Fourier transform Infrared spectroscopy
GA: glutaraldehyde
GlcN: D-glucosamine
GlcNAc: N-acetyl-D-glucosamine
Gp: genipin
IPA: isopropyl alcohol
MKH: Mark-Kuhn-Houwink
MW: molecular weight
NaBES: sodium 2-bromoethylsulfonate
NaCES: sodium 2-chloroethylsulfonate
NaVS: sodium vinylsulfonate
N-SEC: N-sulfoethyl chitosan
O, N-SEC: O, N-sulfoethyl chitosan
O-SEC: O-sulfoethyl chitosan
SEC: sulfoethyl chitosan
SE-C: sulfoethylated chitin
SE-CG: sulfoethylated chitin-glucan complex
SLS: static light scattering

1 Review Plan

This critical review employed a structured literature search to identify all relevant publications on SEC. The search was conducted across major scientific databases including Scopus, Web of Science, PubMed, and Google Scholar, using the following search string: ("sulfoethyl chitosan" OR "N-sulfoethyl chitosan" OR "O-sulfoethyl chitosan" OR "N, O-sulfoethyl chitosan") AND ("synthesis" OR "characterization" OR "application" OR "properties"). No date restrictions were applied to ensure comprehensive coverage of the historical development of SEC chemistry. The search was supplemented by manual screening of reference lists from retrieved articles and review papers to identify additional relevant studies. Inclusion criteria comprised original research articles, review papers, and patent documents reporting on SEC synthesis, physicochemical characterization, or functional applications. Studies were excluded if they (i) focused exclusively on other chitosan derivatives without comparative SEC data, or (ii) lacked sufficient experimental detail for critical evaluation. The retrieved literature was organized thematically according to the three main axes of the review: synthesis methodology, structural characterization, and application domains. This combined approach ensured comprehensive coverage while maintaining focus on the specific chemistry and applications of SEC.

2 Introduction

Chitosan is a naturally derived, cationic biopolymer obtained through the chemical deacetylation of chitin, the second most abundant natural biopolymer after cellulose occurring as a major fraction of the exoskeletal matrices of crustaceans, including either shrimps or crabs, but also derived from insects, as well as the cell walls of certain fungi. As a derivative of chitin, chitosan retains its precursor's structural backbone composed of β-(1→4)-linked D-glucosamine (GlcN) and N-acetyl-D-glucosamine (GlcNAc) units [1, 2].

The presence of free amino groups along its polymeric chain highlights distinguishes chitosan among numerous naturally-derived polymers, providing its advantageous properties, including biodegradability, biocompatibility, mucoadhesivity, and intrinsic antimicrobial activity. In this regard chitosan has been established as a sustainable and environmentally friendly alternative to synthetic polymers in biomedical, pharmaceutical, agricultural, and environmental applications [3-5].

However, the same properties that enable its functionality also impose limitations. Thus, chitosan is only soluble in dilute acidic media with a pH below 6.5, exhibits poor mechanical stability in physiological environments, and offers limited control over its bioactivity profile [6, 7].

These shortcomings have motivated a rich field of chemical modification aimed at tailoring chitosan's physicochemical and biological behavior for specific applications [8, 9]. The resulting wide arsenal of chemical modification strategies has been developed to introduce functional groups into the chitosan backbone. Among the primary targets are the C-2 amino group and the C-3 and C-6 hydroxyl groups, each offering distinct reactivity patterns (Figure 1) [10, 11].

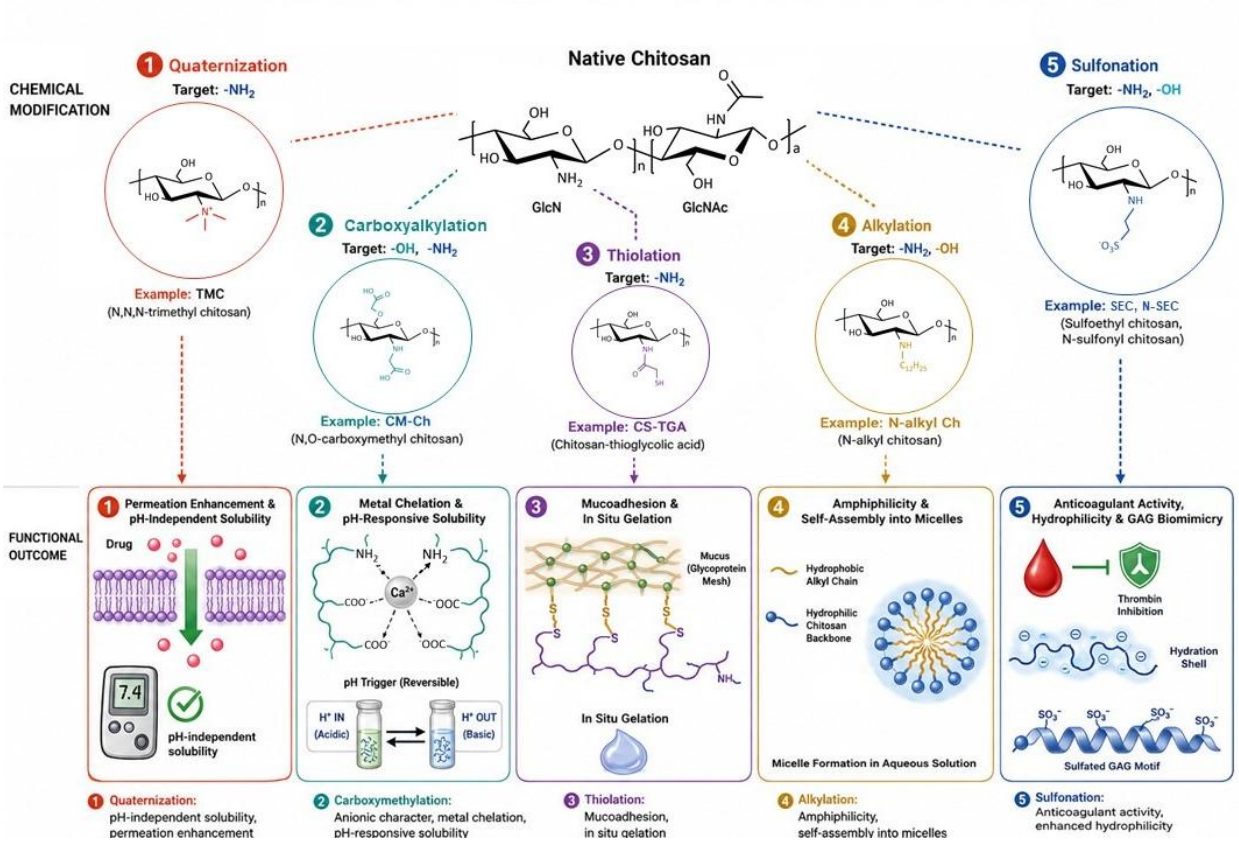

Figure 1. Key targets for chemical modification of chitosan.

Note: The conceptual graphical layout and illustrative elements were assisted using ChatGPT-4o. Chemical structures and reaction schemes were manually verified and curated by the authors for scientific accuracy

For instance, quaternization of the amino group yields N,N,N-trimethyl chitosan, which exhibits enhanced water solubility and permeation-enhancing effects irrespective of pH [12, 13], which allows chitosan's application in drug delivery, while carboxyalkylation, typically at O-6 or N-2 positions, introduces anionic character, enabling pH-responsive solubility and metal chelation [14-17]. Thiolation improves mucoadhesion and in situ gelling capacity via disulfide bond formation with cysteine-rich mucus glycoproteins [18]. And either alkylation or acylation allows modulation of the amphiphilicity, promoting self-assembly into nanoparticles for hydrophobic drug delivery [19].

Each modification, however, involves trade-offs: increased solubility may come at the cost of biodegradability as demonstrated by carboxyalkylation, while enhanced bioactivity may reduce mechanical integrity. Recognizing these patterns is essential before focusing on a particularly promising but underexplored class of modifications — the introduction of sulfo groups.

Sulfo groups (-SO_3H or their anionic form -SO_3^-) take a special place in biopolymer chemistry. Naturally sulfated polysaccharides such as carrageenan obtained from red algae, fucoidan derived from brown algae, or heparin extracted from animal tissues mediate critical biological processes including anticoagulation, antiviral defense, inflammation modulation, and growth factor sequestration [20, 21].

The biological activity of these molecules is strongly correlated with their degree and pattern of sulfation. For example, κ-carrageenan, with one sulfate per disaccharide, primarily formulates gels, while λ-

carrageenan, with three sulfate groups per disaccharide, remains water soluble and exhibits stronger immunomodulatory effects [22-24].

Synthetic introduction of sulfo groups into biopolymers thus represents a rational strategy to mimic nature's sulfated glycosaminoglycans while overcoming the heterogeneity and supply limitations of natural extracts, such as heparin [25, 26].

Compared to carboxyl or amino modifications, sulfo groups confer strong negative charge even at low pH, high hydrophilicity, and specific interactions with positively charged protein domains — properties exploited in heparin-like anticoagulant materials and in scaffolds for growth factor delivery [27-29].

Notably, carrageenan itself has been extensively studied, but its rigid backbone and fixed sulfation pattern limit tunability [30-32]. Chitosan, by contrast, offers a flexible platform where the number and position of sulfo groups can be precisely controlled via synthetic chemistry [33, 34].

Against this backdrop, SEC — a derivative bearing sulfoethyl groups typically introduced via reaction of chitosan with sodium 2-chloroethylsulfonate (NaCES), sodium vinylsulfonate (NaVS) or sodium 2-bromoethylsulfonate (NaBES) — has emerged as a particularly interesting candidate. Early studies suggested that introducing sulfoethyl moieties preserves chitosan's film-forming ability while dramatically improving water solubility and imparting anticoagulant activity reminiscent of heparin [35-39].

However, a comprehensive and critical synthesis of the literature on SEC has been lacking. Individual reports focus either on synthetic optimization, on structural characterization (e.g., degree of substitution, patterns of O- vs N-substitution), on functional properties (solubility, viscosity, thermal stability), or on biological assays. Moreover, contradictions exist regarding the relationship between the degree of substitution (DS) and biological efficacy, and no systematic comparison of different sulfoethylation methods has been published, thus, the present review aims to address this gap.

3 Synthesis and Characterization

3.1 Sulfoethyl Chitosan Synthesis

The first documented synthesis of SEC is attributed to the pioneering work of Nud'ga et al. [40] who produced SEC with a DS of 0.11-0.35 and a sulfur content of 1.39-5.32 % by employing NaCES in the presence of alkali in isopropyl alcohol (IPA) and in mixtures of o-xylene with propyl and IPA. Both O-6- and N-sulfoethylation were confirmed by amino-nitrogen analysis and conductimetric titration. In another study, selective O-substitution was achieved by protecting the amino groups of chitosan with an aromatic aldehyde, resulting in the formation of a Schiff base. The final product had a highest DS of 0.31 [41-43].

Later recorded synthesis was made by Muzzarelli in 1992, who suspended water-insoluble N-carboxymethyl chitosan in IPA, followed by treatment with NaOH and NaCES [41].

A further contribution to understanding the reactivity and optimization of chitosan’s sulfoethylation came from the Nud'ga et al. research group in their 2001 comparative study [44]. Using sodium NaCES in IPA at 80 °C under alkaline conditions within a portionwise addition mechanism, they achieved the DS of 0.35 for chitosan. Under alkaline conditions the authors proposed that NaCES undergoes in situ dehydrohalogenation to form NaVS, after which the reaction proceeds via a Michael-type addition mechanism. The reaction with NaVS in similar conditions had been found significantly more efficient for chitosan sulfoethylation, yielding products with DS values of 0.76-0.98 that were completely soluble in water. Despite requiring an additional step (the preparation of NaVS from NaCES) along with the removal of NaCl, this approach offers substantial advantages, including lower NaOH consumption and minimal deacetylation of the chitin fraction [44].

A significant methodological advancement in the synthesis of SEC was reported by Pestov et al. in 2013, introducing a "synthesis in a gel" technique that has since become a foundational protocol for achieving highly regioselective N-substitution. In contrast to earlier developed heterogeneous methods using NaCES in alkaline organic media, which produced non‑selective N, O‑substitution with low DS ($\leq$ 0.35), the authors suggested a homogeneous protocol: chitosan finely dispersed in 0.8M HCl was left to swell for 10 min formulating a gel, afterwards NaBES was added, and the mixture was stirred at 70 °C heating for 24 h. The resulting reaction media was then neutralized with NaOH, SEC was precipitated with acetone, extracted with hot ethanol, and dried at 50 °C to constant weight. This reaction proceeds via an S_N2 mechanism, primarily resulting in N-substituted chitosan [45].

By performing the reaction in this acidic gel state, the authors suppressed the reactivity of the hydroxyl groups, directing the nucleophilic substitution almost exclusively to the primary amino groups on the chi-

tosan backbone. This strategy enabled the production of N-SEC with a substantially higher DS of up to 0.5. The high N-selectivity observed in neutral or mildly acidic conditions arises from the distinct nucleophilic properties of the functional groups on the chitosan backbone. Although a significant fraction of the primary amino groups is protonated under these conditions, a reactive equilibrium population of free $–NH_2$ remains available. These unprotonated amino groups are strongly nucleophilic and therefore the preferred site of attack. In contrast, the hydroxyl groups stay protonated and, in the absence of their conversion into alkoxide ions, possess only low intrinsic nucleophilicity, effectively suppressing O-substitution. As a result, the reaction proceeds with high selectivity towards nitrogen [46, 47]. In strongly alkaline conditions, however, the situation changes dramatically. The hydroxyl groups are partially deprotonated to form alkoxide ions ($–O^-$), which are much stronger nucleophiles. This leads to competing O-alkylation alongside N-substitution, resulting in reduced selectivity and often a mixture of N- and O-modified products [48-50]. By performing the reaction in a neutral or weakly acidic medium, O-substitution is minimized, enabling clean and highly selective N-functionalization of chitosan.

Another work of the same research group appeared in a study published by Petrova et al. in 2014. Herein the authors had refined the technique by successfully conducting the biopolymer-analogous transformation without the addition of any acid or base. In this optimized procedure, chitosan was simply mixed with sodium NaBES in water. During heating at 70 °C, the reaction mixture self-assembled into a physical gel as the progressively modified polymer became more hydrophilic. This novel approach in a neutral aqueous medium totally prevents O-substitution, resulting in N-SEC with a moderate DS of up to 0.55 and high yields about 81 % [51].

The suggested method represented a crucial step towards a simpler route for producing well-defined SEC. In a later study, varying the reaction parameters Petrova et al. reported the synthesis of N-SEC with a DS of up to 0.78 and up to 1.00, following a similar reaction pathway used in their previous work [52, 53]. Collectively, all reported methods for the preparation of N-SEC across different reaction conditions are presented in Table 1.

Table 1

SEC parameters depending on the reaction conditions

Molar ratio Chitosan: NaBES:HCl	Concentration of chitosan in reaction media, %	T, ˚C	Sulfur content, %	DS calculated by ^{1}H NMR data	Reference
1:1:0.25	21	70	5.06	0.31	[45]
1:1:0.5	23		4.17	0.24	
1:1:1	20		0.20	0	
1:2:0.25	23	50	5.24	0.30	
		70	6.55	0.44	
		90	6.23	0.46	
1:2:0.5	23	50	3.44	0.16	
	10	70	4.64	0.24	
	20		7.12	0.50	
	30		4.12	0.24	
1:2:0.5 (acetic acid)	20	70	6.45	0.40	
1:3:0.25	23	50	6.87	0.33	
1:3:0.5	23	50	3.28	0.16	
		70	4.86	0.30	
1:2:0	21	50	6.46	0.45	[51]
		70	7.48	0.55	
1:1:0	12	70	8.94	0.71	[52]
1:2:0	12	70	9.22	0.72	
	21	50	6.46	0.45	
		70	7.48	0.55	
1:3:0	12	70	9.66	0.78 (the DS was unchanged after 48 h)	
		90	9.32	0.70	
1:4:0	12	70	8.92	0.68	
1:5:0	8	70	9.01	0.70	
1:2:0	12	70	ns	1.0 (reaction was carried out for 12 h)	[53]

The data presented in Table 1 demonstrates that DS in the N-2-sulfoethylation of chitosan with NaBES in a gel phase was significantly influenced by the reaction parameters. The molar ratio of chitosan to NaBES and the amount of acid used for gel formation proved to be the most critical factors. An increase in the NaBES excess up to a 1:2-1:3 ratio enhanced the DS, whereas a further increase in the molar ratio did not result in higher DS. Notably, the presence of hydrochloric acid was essential for physical gel formation but exerted a pronounced negative effect on substitution efficiency; excess HCl (e.g., 1:1:1 ratio) completely suppressed the reaction (DS = 0), whereas reactions performed with minimal or no HCl afforded the highest DS values. Replacement of HCl with acetic acid had negligible impact on the DS, offering a safer alternative for gel preparation while maintaining comparable efficiency. The optimal chitosan concentration in the reaction medium was found to be approximately 12-20 %, consistent with a pronounced gel effect that maximizes local reagent concentration and minimizes diffusion limitations. The reaction temperature of 70 °C provided the highest DS, whereas lower temperatures (50 °C) halved the DS and higher temperatures (90 °C) yielded only slight or no additional benefit. This behavior is most readily explained by the onset of competing side-reactions of the alkylating agent NaBES that becomes kinetically significant above 70 °C. In aqueous media NaBES can undergo both hydrolysis to the inactive 2-hydroxyethanesulfonate and elimination of HBr to vinylsulfonate. Both conversions reduce the effective concentration of the S_N2-active reagent intended to interact with chitosan amino groups. Under the mildly acidic conditions used in several of the entries, a secondary contribution from limited acid-catalyzed scission of the polysaccharide backbone cannot be excluded, although the thermal sensitivity of the reagent itself turns out the primary limitation. Consequently, 70 °C represents the practical optimum that maximizes the rate of the desired N-alkylation while minimizing reagent loss.

The reaction time had limited influence on the outcome: most experiments were conducted for 24 h, a DS of 1.0 was achieved in one case after only 12 h, and prolongation of the reaction up to 48 h did not lead to any further increase in DS. These findings demonstrate that careful control of reaction conditions is crucial for achieving high degrees of N-substitution in SEC.

In contrast to the regioselective gel-phase protocols proposed by Pestov's group, earlier established N, O-sulfoethylation protocols considered heterogeneous conditions with NaCES in alkaline organic media. Owing to the high crystallinity and limited reactivity of chitosan, a pretreatment step was essential to disrupt intermolecular hydrogen bonds and expose the reactive sites.

In another study by Petrova et al., the biopolymer was pretreated by swelling in water for 5 days or through ultrasonication, followed by reaction of the swollen chitosan with excess NaCES in 85 % 2-propanol in the presence of NaOH (typical molar ratios chitosan:NaCES:NaOH = 1:6:6 or 1:6:9) under a nitrogen atmosphere at 80 °C for 3 h. Products were purified by dialysis and freeze-dried. The pretreatment increased the DS, improved water solubility at higher DS, and reduced intrinsic viscosity due to bulky substituents and partial degradation. This less selective route still enabled practical access to water-soluble SEC with tunable DS [37].

Gabriel and Heinze conducted a comparative study on the synthesis of SEC using two distinct reagents: NaVS in an alkaline IPA slurry (heterogeneous) and NaBES under acidic aqueous conditions (homogeneous). For the NaVS route, the authors modified the reagent stoichiometry (chitosan:NaVS:NaOH = 1:4:4) and reaction conditions (stirring for 1 h at room temperature followed by 5 h at 80 °C) relative to earlier protocols (e.g., a 1:6:6 ratio and a single 3 h step at 80 °C) [37]. A pivotal finding of this work was the exclusive formation of O-substituted chitosan at the C-3 and C-6 positions. Thus, it was demonstrated that quantitative conversion of NaCES to NaVS does not occur fully under the alkaline conditions. Residual NaCES therefore coexists with NaVS and reacts with chitosan predominantly via an S_N2 (Williamson-type) pathway, which favors N-substitution at the more nucleophilic amino groups. In contrast, pure NaVS under heterogeneous alkaline conditions (isopropanol/NaOH) promotes chemoselective O-substitution at the C-3 and C-6 hydroxyl groups. This distinction accounts for the mixed N, O-substitution patterns reported in earlier NaCES-based protocols and for the exclusively O-substituted products obtained when NaVS itself is used. In the same study, the NaBES protocol was also modified; chitosan was suspended in water (3 % gel concentration) under nitrogen, acidified with concentrated HCl for 10 minutes, and then reacted with NaBES at 70 °C for 24 hours [39].

Subsequent investigations generally aimed to refine the existing methodologies.

Another study was directed towards process purification facilitation when Bolshakov et al. developed a streamlined protocol employing a 1:2 molar ratio of chitosan to sodium NaBES. The reagents were mixed in their dry state, hydrated, and heated at 70 °C for 24 hours with periodic agitation. Following the addition of a

NaOH solution, the reaction mass was cooled, stirred intensively for 10 hours, and then shaken for 15 hours. The product was isolated by precipitation in acetone and dried at 40-50 °C. The principal advancement of this method was the simplification of the purification stage, effectively eliminating the laborious Soxhlet extraction required in earlier procedures [54].

More recently, Heise et al. revised the dual-reagent strategy, investigating the sulfoethylation of chitosan with both NaVS and NaBES under alkaline pH. Diverging from prior protocols, the NaVS reaction was conducted without IPA or a nitrogen atmosphere, which resulted in a comparatively lower DS of 0.69. Conversely, the NaBES reaction, performed in IPA under alkaline conditions, achieved a higher DS of 1.05. Notably, both synthetic pathways predominantly yielded O-substitution at the C-6 position [35].

In summary, the collective work of Pestov and Petrova established foundational protocols for synthesizing N-SEC with DS varying from 0.2 to 1. Subsequently, iterative modifications by other researchers, building upon both these methods and the earlier work of Nud'ga for O, N-substituted derivatives, have progressively steered the field towards the selective synthesis of O-SEC.

Overall, the synthetic routes for chitosan sulfoethylation can be summarized as presented in Figure 2.

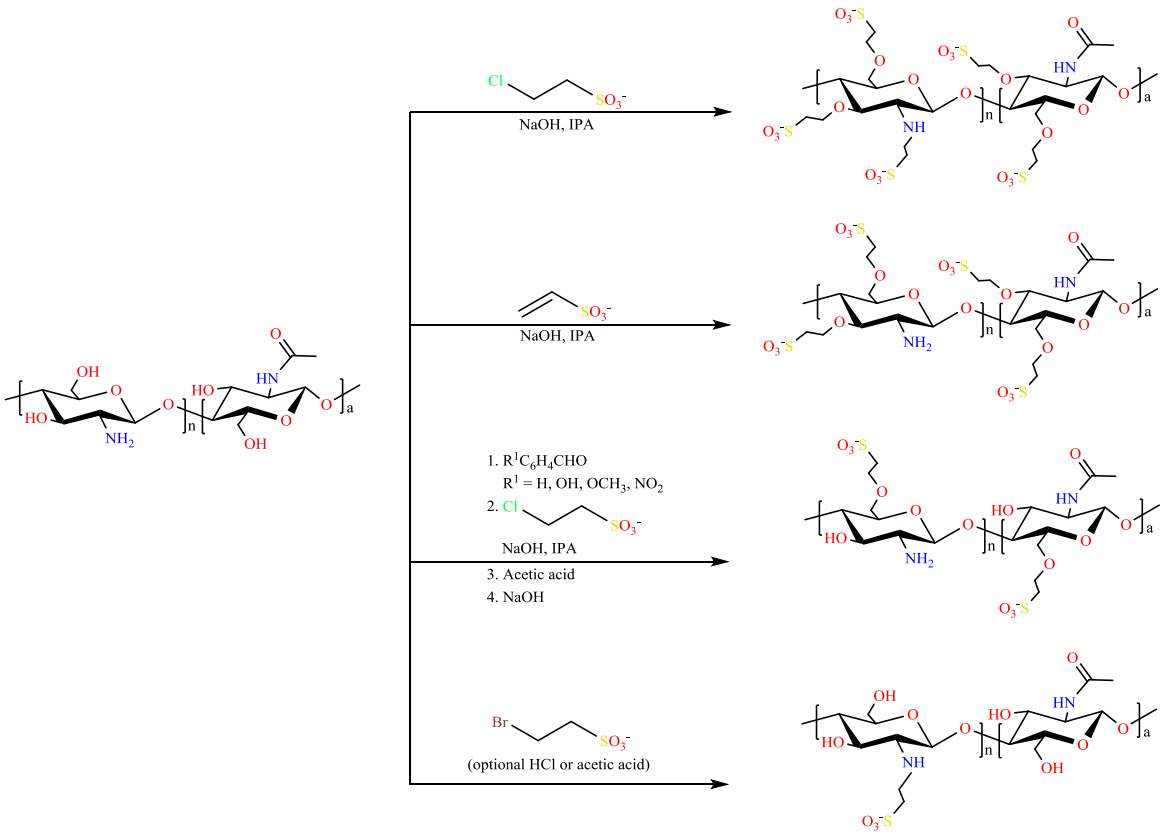

Figure 2. Synthetic routes of sulfoethyl chitosan

The DS of SEC is governed by several reaction parameters, including reagent, reagent ratio, reaction medium, temperature, reaction time, and pretreatment of the starting polymer. Early studies demonstrated that increasing the molar ratio of sulfoethylation reagent generally increases sulfur incorporation and, consequently, DS. However, excessively high reagent ratios may adversely affect the physicochemical properties of the final product.

So far, chemical approaches to synthesize SEC are various and well-studied. But still sulfoethylation of chitosan remains a laboratory-scale technology without market distribution.

Considering the reasons for that one would find that the synthesis of SEC traditionally relies on high-cost sulfoethylating agents. From an economic perspective, sodium NaCES is substantially more cost-effective than NaBES, but its lower reactivity and tendency to form NaVS in situ under alkaline conditions may limit the achievable DS [39]. This trade-off between reagent cost, reactivity, and substitution selectivity must be carefully considered when designing a scalable and economically viable synthesis route. Also, purification procedure typically involves precipitation with large volumes of organic solvents either ethanol or

acetone, followed by exhaustive dialysis, which contributes to a high E-factor [39]. Overall, the precursor costs, synthesis yield, and the amounts of chitosan and reagents turn out to be the key challenges for both economic and environmental sustainability [55]. This means that future research should prioritize the development and adaptation of greener methodologies for SEC production with systematic evaluation, to enable truly scalable and environmentally responsible manufacturing.

3.2 Characterization

Comprehensive physicochemical characterization of any biopolymer is a prerequisite for establishing reliable structure-property correlations and ensuring batch-to-batch reproducibility in both synthetic and applicative contexts. Analytical protocols must consider multiple hierarchical levels of structural organization, including primary structure, molar mass characteristics, and conformational behavior in solution, all of which govern macroscopic properties such as rheology, complexation ability, and bioactivity. In the case of SEC, the introduction of anionic sulfoethyl groups further complicates the analytical landscape: the presence of strong acid sulfonate moieties not only enhances polyelectrolyte character and modulates the overall charge density but also introduces additional variables, such as the position of substitution (O-, N- or mixed substitution), the relative distribution along the polymer chain, and the resulting changes in the hydrophile–lipophile balance. Moreover, the charged nature of the derivative makes its solution properties highly sensitive to ionic strength, pH, and counterion type, necessitating analytical strategies capable of distinguishing between substitution-derived effects and those arising from purely conformational or associative phenomena. Consequently, a rigorously designed analytical framework for SEC must integrate complementary orthogonal techniques that address both covalent structural features and non-covalent dynamic behavior, thereby enabling unambiguous interpretation of its functional performance.

3.2.1 Sulfoethylation Confirmation and Degree of Substitution

The proton magnetic resonance analysis is a typical procedure for structure investigation but one of the key methods to determine the DS. Its accurate determination is essential for rationalizing SEC data, since DS directly affects the polymer's hydrodynamic radius and its interaction with the stationary phase. Petrova et al. suggested the formula for calculating the DS from 1H NMR spectra represented by equation (1) [51]

$$DS = \frac{m + 2d}{m + d + a}, \quad (1).$$

However, we suggest that the degree of acetylation (DA) of chitosan shall be considered, so the formula version of this expression that additionally accounts for this will be described by the equation (2):

$$DS = \left(\frac{m + 2d}{m + d + a} \right) \times (1 - DA), \quad (2)$$

where *m* — the mole fraction of monosulfoethylated glucosamine units, *d* — the mole fraction of disulfoethylated glucosamine units, *a* — the mole fraction of glucosamine units, *DA* — degree of acetylation. *m*, *d*, and *a* values were calculated using integral intensities of the corresponding signals in ^{1}H NMR spectrum.

The spectrum also reveals the structure of the biopolymer reflecting its modification type. For N-SEC the typical ^{1}H NMR spectrum can be described as (D_2O/DCl), δ, ppm: 2.08 (CH_3), 3.23 (H 2 $GlcNH_2$), 3.35 ($NHCH_2CH_2$), 3.44–4.10 (H 2,3,4,5,6 $NHCH_2CH_2$), 4.62 (H 1 GlcNHAc), 4.94 (H 1 $GlcNH_2$), 5.06 (H 1 $GlcNHCH_2CH_2$), 5.12 (H 1 $GlcN(CH_2CH_2SO_3H)2$) [51], while O-SEC shows the overlapping NMR pattern in the H-1 region as indicating mainly free amino groups with a small amount of N-monosubstituted SEC [37].

Another independent approach to investigate and cross-validate the DS values obtained from ^{1}H NMR is C, H, N, S elemental analysis. It is typically used to characterize the DS using the equation represented by equation (3):

$$DS = \frac{\omega(S) \times M(N)}{\omega(N) \times M(S)} \times 100\%, \quad (3)$$

where ω*(S)* and ω*(N)* are the mass fractions and *M(S)* and *M(N)* are the atomic weights of sulfur and nitrogen, respectively [37].

This method is particularly advantageous for SEC analysis due to the fixed nitrogen content per repeating polysaccharide unit (one N atom per glucosamine ring), while the sulfur content scales linearly with the number of introduced sulfoethyl groups.

However, the elemental analysis critically depends on the sample's purity. The often underestimated concern is that any nitrogen- or sulfur-containing impurities including residual unreacted reagents, inorganic salts, solvents, or by-products will distort the elemental composition data and lead to an incorrect DS value. This problem is particularly acute in polysaccharide chemistry, where the high molecular weight and macromolecular nature of the polymer make it hard to remove low-molecular-weight contaminants completely [56]. The removal of low-molecular-weight impurities from SEC samples is typically achieved by exhaustive dialysis against deionized water followed by lyophilization. This approach was employed by Gabriel and Heinze [39] in their chemoselective sulfoethylation study, where the reaction product was purified by dialysis to remove residual NaVS and other by-products prior to characterization. Similarly, Petrova et al. [53] utilized ethanol precipitation and extensive washing to purify N-SEC after synthesis, ensuring removal of unreacted NaBES before analysis for DS calculation.

Consequently, following a proper sample preparation the calculated DS reflects the average number of substituents per monomer unit, providing a bulk compositional parameter that complements the site-specific information available from NMR.

Covalently bound sulfonates can be successfully detected by Fourier transform Infrared spectroscopy (FT-IR). While investigating the FT-IR spectrum one should consider the background of the characteristic chitosan bands — broad N-H and O-H stretching (≈3376 cm^{-1}), C-H stretching (≈2881 cm^{-1}), amide I (≈1652 cm^{-1}), and C-O/C-C vibrations (≈1067 cm^{-1}) while for SEC new intense absorption bands appear in the regions of 1159-1164 cm^{-1} and 1040-1043 cm^{-1}, corresponding to the asymmetric and symmetric S=O stretching vibrations of the sulfonate group. Additional characteristic bands at approximately 740-744 cm^{-1} and 594 cm^{-1} are attributed to C-S and C-SO_3 stretching vibrations, respectively [45, 51, 54].

The position and shape of these bands, together with changes in the amino/hydroxyl regions, allow differentiation of the substitution patterns. In O-substituted chitosan, the band with a maximum at 1530 cm^{-1} is particularly prominent as a complex band arising from the overlap of amide II (1550-1560 cm^{-1}) and protonated amino group (1560-1520 cm^{-1}) contributions [37, 39].

Complementary Raman spectroscopy provides further structural insight, revealing new bands at 745 cm^{-1} (S–C stretching) and 1044 cm^{-1} (symmetric SO_3^- stretching), along with enhanced CH_2-related vibrations (2940 cm^{-1} ν_{sym}/a_{sym}, 1410 cm^{-1} δ, 806 cm^{-1} ρ) due to the introduced ethylene spacer. Importantly, the intensity of these sulfoethyl-specific bands increases proportionally with the DS, offering a convenient qualitative tool for monitoring the extent of modification [35]. These combined vibrational data corroborate the substitution patterns established by NMR and elemental analysis.

Overall, key analysis of SEC can be structured as represented in Figure 3.

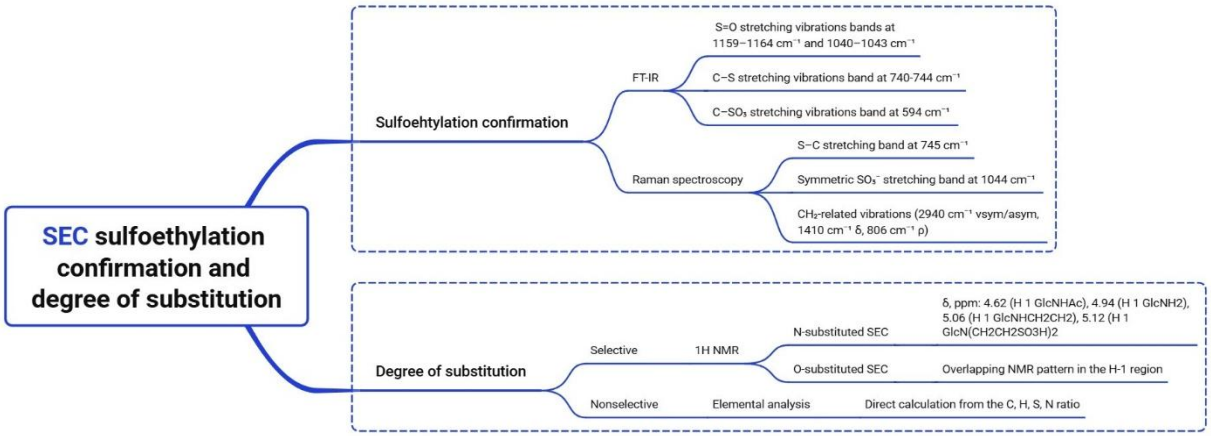

Figure 3. Sulfoethylation confirmation and degree of substitution of SEC

3.2.2 Molecular Structure

X-ray diffraction analysis of SEC films cast from 2 % acetic acid solutions, which resulted in protonated amino groups and sulfonate groups predominantly in the anionic form, initially balanced by acetate and

residual sodium counter-ions, revealed significant changes in the supramolecular structure compared to unmodified chitosan. The native chitosan film exhibited a characteristic reflection at 2θ = 20-22°, typical of its semi-crystalline structure. On the contrary, introduction of sulfoethyl groups led to progressive amorphization of the polymer, manifested as weakening or broadening of the main reflection and the appearance of a diffuse halo at 20-23°. This effect became more pronounced with increasing DS. Additionally, new reflections appeared (e.g., at 13° for hydrated chitosan polymorph in SEC with DS = 0.45 and at 16.5° for anhydrous polymorph in aged SEC with DS = 0.94), indicating the presence of different crystalline modifications. Notably, SEC films gradually lost solubility in 2 % acetic acid and water upon storage (partially after 3 months, completely after 6 months), in contrast to much higher stability of chitosan films. The observed structuration during aging is attributed to the formation of intra- and intermolecular ionic crosslinks involving the sulfonate and amino groups, which enhances the ordering of the polymer matrix over time [37]. It should be noted that the crystalline/amorphous character of SEC is expected to differ substantially if the polymer is examined in the pure sodium-salt form, the fully protonated (acid) form, or after exchange with multivalent cations (e.g., Ca^{2+}), because ionic cross-linking by such cations can further rigidify or reorganize the network.

Increasing DS substantially modifies the supramolecular organization of N-SEC in solution. The intrinsic viscosity decreases continuously with increasing substitution, reflecting a reduction in hydrodynamic volume and molecular chain expansion. For example, intrinsic viscosity decreased from approximately 7.7 dL/g for native chitosan to about 1.5 dL/g for highly substituted derivatives. Simultaneously, the average aggregate size decreased markedly as DS increased.

At the same time dynamic light scattering measurements showed that the hydrodynamic radius of polymer aggregates decreased from approximately 242 nm at DS ≈ 0.94 to about 82 nm at DS ≈ 1.30 in alkaline solution. In addition to size reduction, aggregate morphology changes from predominantly spherical structures to more elongated aggregates depending on both DS and solvent composition. These observations indicate that sulfoethyl substitution weakens intermolecular associations while promoting more compact and solvent-dependent molecular assemblies.

The increasing sulfoethylation also alters the optical anisotropy of the polymer. Molecular hydrodynamics and optical methods in shear flow have been employed to study the conformational and supramolecular behavior of SEC in dilute solutions. Measurements of reduced viscosity showed linear concentration dependences, allowing reliable determination of intrinsic viscosity values using the Huggins equation and confirming the absence of noticeable polyelectrolyte effects under the studied conditions. Flow birefringence (Maxwell effect) was directly proportional to the velocity gradient, indicating that the solutions were molecularly dispersed. Significant differences were observed in the optical shear coefficient ([n]/[η]) between chitosan and its sulfoethylated derivatives. While unmodified chitosan exhibited positive birefringence, SEC displayed strong negative birefringence. This sign inversion suggests that in SEC aggregates the axis of highest polarizability is oriented perpendicular to the longest geometrical axis of the particle, pointing to a highly ordered supramolecular organization driven by short-range intermolecular interactions (van der Waals, hydrophobic, and hydrogen bonding). These findings highlight that sulfoethylation induces substantial changes in the orientational and conformational characteristics of chitosan macromolecules in solution [37].

3.2.3 Molecular Weight Determination

The introduction of bulky sulfoethyl groups as well as the reaction conditions such as temperature, acidity of the reaction media and process duration, can lead to depolymerization of the polymer chain, which substantially affects the physicochemical and biological activity of SEC discussed further. Nevertheless, molecular-weight analysis is required for proper interpretation of the physicochemical and biological properties of the synthesized biopolymer. Several methods have been developed and readapted for SEC.

3.2.3.1 Capillary Viscometry

For native chitosan, capillary viscometry has historically been the most widely employed indirect method for molecular weight (MW) estimation, relying on the Mark-Kuhn-Houwink (MKH) relationship [57]. For chitosan derivatives, however, this approach becomes fundamentally invalid without prior determination of new MKH constants, because chemical modification drastically alters chain conformation and hydrodynamic volume. Studies on sulfated chitosan derivatives have demonstrated that viscosimetry yields MW values that are not comparable with results from absolute methods [58]. The determination of molecular weight for sulfated chitosan based on constants established for heparin has been recognized as incorrect, while ap-

plying the Kasaai equation, originally derived for native chitosan, to its derivatives introduces substantial errors. The fundamental principle articulated for native chitosan applies equally to SEC: the MKH equation relies on constants (K and α) specific to a given polymer-solvent-temperature system, reflecting the unique hydrodynamic volume and chain conformation of the unmodified polymer in that solvent. Introduction of sulfoethyl groups inevitably changes chain stiffness, solvation, excluded volume and polyelectrolyte behavior, rendering literature constants for native chitosan inapplicable.

3.2.3.2 Size-Exclusion Chromatography

Aqueous size-exclusion chromatography is one of the key methods for MW determination of SEC [35]. However, several specific challenges emerge when analyzing chitosan derivatives. Firstly, the refractive index increment $\frac{dn}{dc}$ for derivatives can differ substantially from that of native chitosan. While expected $\frac{dn}{dc}$ values for related polysaccharides fall within 0.136-0.176 mL/g, sulfated derivatives require experimental determination of this parameter on a case-by-case basis [59]. The dependence of $\frac{dn}{dc}$ on DS, ionic strength, and pH must be carefully characterized. Failure to use the correct $\frac{dn}{dc}$ value introduces large errors in the calculated MW. This is because the optical constant of the light-scattering detector (often denoted K_{LS}) contains the term $\left(\frac{dn}{dc}\right)^2$, consequently, the light-scattering signal itself scales with $\left(\frac{dn}{dc}\right)^2$. An inaccurate $\frac{dn}{dc}$ therefore propagates directly and quadratically into the molecular-weight result.

Secondly, column calibration using polymer standards (e.g., dextrans or pullulans) introduces uncertainties comparable to those encountered for native chitosan. The conformational disparity between SEC and neutral polysaccharide standards remains a fundamental limitation. Even when using universal calibration procedures, the scatter in reported MKH constants for chitosan derivatives poses a significant challenge [60]. Next, the polyelectrolyte nature of SEC demands careful optimization of mobile phase conditions. Sufficient ionic strength is required to suppress electrostatic chain expansion and obtain stable, well-defined hydrodynamic volumes. However, the presence of both cationic (amino) and anionic (sulfonate) groups may create complex charge-shielding requirements that are not fully addressed by simple salt addition.

3.2.3.3 Light Scattering Techniques

Static light scattering (SLS) can provide absolute weight-average molecular-weight information, whereas dynamic light scattering (DLS) provides hydrodynamic size and aggregation information through measurement of translational diffusion. Similarly to native chitosan, these two techniques offer significant advantages for SEC analysis. For N-SEC with DS ≈ 0.5-0.7, light scattering has been applied to determine MW and radius of gyration in solution, enabling conformational characterization [37]. However, being recognized as a critical limitation for native chitosan, the extreme sensitivity of light scattering to aggregates is equally problematic for SEC [61]. Rigorous sample preparation, including filtration and use of DLS to deconvolute contributions from aggregates and individual macromolecules, remains imperative. The presence of aggregates in SLS is revealed by concave Zimm (or Berry) plots; however, linear dependences may still be observed for samples with bimodal distributions, requiring more advanced or combined analysis with size-exclusion chromatography. The dn/dc for SEC must be measured under the same solvent and temperature conditions used for scattering experiments since it strongly depends on DS, pH, ionic strength, and potentially on the presence of heavy metal impurities.

3.2.3.4 Analytical Ultracentrifugation

The matrix-free method that requires no calibration against polymer standards, analytical ultracentrifugation (AUC) remains the "gold standard" for absolute MW determination of chitosan and its derivatives [62]. For sulfoethylated chitosan, AUC offers particular value in cases where other methods yield inconsistent results due to aggregation or polyelectrolyte effects. Implementation of this method allows the characterization of complex formation including polyelectrolyte complexes with other charged polymers, which is especially relevant for SEC, given its application in drug delivery and biomedical contexts. Sedimentation equilibrium provides absolute MW independent of molecular shape, while sedimentation velocity offers insights into conformation through the sedimentation coefficient [63]. For SEC, sedimentation coefficients and intrinsic viscosities can be combined to construct hydrodynamic parameter-MW relationships,

similarly to the MKH equation, as demonstrated for other chitosan derivatives. Recent advances in multi-wavelength detection add an orthogonal spectral dimension to conventional hydrodynamic characterization. However, as for native chitosan, AUC of SEC is labor-intensive, requires expensive instrumentation and highly trained operators, and is generally limited to specialized applications or as a confirmatory reference method.

3.2.3.5 Gel Electrophoresis

An emerging and promising approach for rapid analysis of chitosan derivatives is agarose gel electrophoresis [64]. The electrophoretic mobility of charged macromolecules in agarose gels is governed by three principal factors: molecular weight, degree of substitution (charge density), and degree of deacetylation. For SEC, electrophoretic mobility differs from native chitosan, making this method suitable for monitoring modification reactions and estimating DS. A key advantage of electrophoresis is the ability to screen large numbers of samples rapidly with minimal material consumption and without expensive instrumentation [65]. However, the method remains semi-quantitative and requires calibration using well-characterized samples, as inherent polydispersity produces diffuse bands rather than sharp zones. As with native chitosan, the lack of narrow-disperse SEC standards necessitates calibration with heterologous polymers, introducing uncertainty due to differences in charge density and hydrodynamic properties. Nevertheless, for comparative analyses within a single experimental run, electrophoresis offers significant practical utility, which is useful when the MW changes during storage or hydrolytic degradation.

Summarizing the MW estimation approaches, it becomes clear that the aggregation remains a persistent challenge for SEC analysis and may be even more complex compared to native chitosan [37]. The introduction of sulfoethyl groups introduces additional anionic centers which can promote the formation of zwitterionic structures or intramolecular ion pairs. This significantly complicates the solution behavior of the biopolymer and consequently, the analytical determination of its MW [66]. The balance of electrostatic repulsions, hydrophobic interactions (from residual acetyl groups), and hydrogen bonding in SEC differs markedly from that of native chitosan, affecting chain conformation, hydrodynamic volume, and aggregation propensity.

3.2.4 Thermal Stability

Thermogravimetric analysis demonstrates that SEC has only a minor influence on the thermal degradation profile of N-chitosan. All derivatives exhibit similar decomposition behavior regardless of DS, indicating that the DS has little effect on the degradation mechanism. The first weight loss observed between approximately 50 and 110 °C corresponds to evaporation of physically adsorbed and bound water. The principal degradation stage occurs between 210 and 300 °C and involves decomposition of the polysaccharide backbone together with cleavage of sulfoethyl substituents, releasing water, sulfur dioxide, carbon dioxide, and small amounts of acetic acid originating from residual acetyl groups. At higher temperatures, up to approximately 500 °C, further degradation produces ethylene and ammonia. Although increasing DS does not significantly change the thermal degradation pattern, SEC generally exhibits lower thermal stability than native chitosan, with decomposition beginning at approximately 200 °C due to the presence of thermally less stable sulfoethyl groups [51, 52].

Taken together, these analytical parameters provide a basis for relating SEC structure to its physicochemical properties and potential applications.

4 Sulfoethyl Chitosan Applications

The unique combination of water solubility, negative surface charge, and biomimicry of natural sulfated polysaccharides has opened diverse application domains for SEC. In drug delivery, nanoparticles based on SEC have been explored as carriers for positively charged therapeutic agents, exploiting electrostatic interactions for sustained release. While several studies have demonstrated the utility of chitosan-based nanoparticles and nanogels in this context, dedicated investigations specifically employing sulfoethyl chitosan remain limited and warrant further exploration [20, 67-69]. SEC has also been examined for its anticoagulant properties. In particular, sulfoethyl chitosan and SECS-capped silver nanoparticles have been shown to inhibit factor Xa and exhibit heparin-like activity [35]. Anticoagulant activity has likewise been reported for sulfated chitosan [70].

A thorough assessment of any novel biomaterial requires systematic evaluation of its interactions with biological systems. The available data for SEC allows several clear trends to be identified.

Most reported studies on sulfoethyl- or closely related sulfonated chitosan derivatives and its N-acetylated precursor chitin indicate low-to-moderate cytotoxicity. Work on O, N-SEC implanted in animal models noted the absence of significant local toxicity [54]. At the same time latest findings of Samokhin et al. revealed the potential of SEC-based gels for bacterial delivery in the gastrointestinal tract with no toxic effect on fibroblast cell cultures in vitro. Moreover, when administered to mice, no adverse reactions, neither behavioral nor allergic were observed in the animals [71]. Moreover, sulfoethylated chitin and chitin-glucan complex (SE-C and SE-CG respectively) both protect plasmid DNA and mammalian cells against oxidative damage. In the DNA-topology assay, SE-CG suppresses Fe^{2+}-induced single-strand breaks by chelating ferrous ions, yet it can enhance H_2O_2-mediated nicking under certain conditions [72]. In V79 hamster lung cells, 24 h pre-incubation with SE-G (150-750 μg mL^{-1}) significantly reduces both H_2O_2-generated strand breaks and FPG-sensitive oxidized purines produced by visible-light-excited methylene blue [73]. The same SE-CG preparation is antimutagenic in the Ames assay against 9-aminoacridine, sodium azide and MNNG [74]. Remarkably, the revealed protective effects are observed at lower DS values than those typically required for carboxymethyl derivatives, underscoring the advantage of the strongly acidic sulfoethyl group.

SEC exhibits good hemocompatibility according to Heise et al. The permanent negative charge of the sulfonate groups electrostatically repels the negatively charged erythrocyte membrane. In plasma coagulation assays the polymer prolongs activated partial thromboplastin time. Low-molecular-weight SEC and its silver-core nanoparticles have been shown to inhibit factor Xa and to prolong both activated partial thromboplastin time and prothrombin time [35]. Anticoagulant activity of SEC is the most thoroughly documented biological property of the biopolymer and is driven by its structural analogy to heparin. Although SEC lacks the precise pentasaccharide sequence of heparin, its sulfonate groups enable electrostatic interactions with coagulation factors. In vitro assays consistently show DS- and concentration-dependent prolongation of aPTT (and often PT). Later study demonstrated that both neat low-molecular-weight sulfoethyl chitosan and the corresponding silver-core nanoparticles inhibit factor Xa and markedly prolong clotting times [35]. However, unlike heparin, the structure-activity relationship for SEC appears in a simpler manner: higher DS generally correlates with stronger activity.

Unmodified chitosan shows broad-spectrum antimicrobial activity mainly through its protonated amino groups, which disrupt microbial membranes [75], and is readily degraded by lysozyme via recognition of N-acetylglucosamine sequences [76]. Sulfated chitosan derivatives often display reduced or even absent antibacterial activity compared with the parent biopolymer [77] and show altered (frequently slower or pattern-dependent) lysozyme-mediated degradation [32]. Although it requires a systemic investigation, SEC is expected to behave similarly: diminished antimicrobial activity and slower or modified biodegradation relative to unmodified chitosan. Once confirmed, the reduced degradability may be useful for long-term implants but less suitable for rapidly clearing systems. Direct experimental data on both properties for sulfoethyl chitosan remain limited.

The influence of the pattern of deacetylation on the functional properties of chitosan has been well established [78, 79]. For SEC a structural complexity arises from the pattern of sulfoethylation, which can similarly modulate the polymer's physicochemical and biological behavior.

Furthermore, the properties of SEC are not solely determined by the overall degree of substitution but also by the regio-selectivity of the modification. Substitution at the amino groups (N-SEC) versus hydroxyl groups (O-SEC) yields derivatives with fundamentally different characteristics.

Monosubstituted N-SEC units retain a secondary amino group that remains protonatable, while N, N-disulfoethylated units contain a tertiary nitrogen. In both cases a strongly acidic sulfoethyl moiety is introduced in close proximity to the nitrogen. This architecture creates a chelating, amphoteric ligand environment that is particularly effective for selective metal-ion binding. Cross-linked N-SEC resins exhibit high affinity and selectivity toward Ag^{+} and Cu^{2+} in multicomponent solutions containing other transition and alkaline-earth metals, making them attractive as sorbents for the recovery or pre-concentration of these ions from industrial or analytical matrices [53].

O-sulfoethyl chitosan derivative preserves the primary amino groups of the original chitosan while placing the anionic sulfoethyl substituents exclusively on the hydroxyl positions. The retained primary amines maintain the classical cationic character of chitosan under acidic conditions and enable classical amine-mediated interactions (electrostatic complexation, Schiff-base chemistry, or coordination). At the same time the permanently anionic sulfoethyl groups improve water solubility and introduce polyampholytic behavior [39]. Consequently, O-SEC is well suited for the formation of polyelectrolyte complexes with polycations,

the fabrication of multilayer membranes, and applications that benefit from a high density of free primary amines (e.g., enzyme immobilization or mucoadhesive formulations).

Mixed O, N-SEC combines both substitution patterns and typically reaches higher overall degrees of substitution. The resulting materials display enhanced water solubility across a broader pH range, pronounced ampholytic character, and the ability to form both intra- and intermolecular ionic cross-links upon drying. These features have been exploited in several biomedical contexts: as templates and stabilizing agents for silver nanoparticles that exhibit anticoagulant activity (particularly inhibition of factor Xa), and as components of polyelectrolyte-complex membranes and films whose aggregation behavior and hydrodynamic properties can be tuned by the degree of substitution [35, 37]. The simultaneous presence of primary and secondary amines together with sulfonate groups also makes mixed O, N-SEC a versatile platform for subsequent dual functionalization or for constructing hierarchical materials with spatially differentiated charge densities.

In summary, the choice among O-, N-, and O, N-regioisomers of sulfoethyl chitosan is not merely a synthetic detail but a rational design parameter. N-SEC is preferred when selective metal chelation is required; O-SEC is advantageous when free primary amines and controlled polyelectrolyte complexation are needed; and mixed O, N-SEC offers the broadest solubility window and the most versatile platform for biomedical and nanoparticle applications. Precise control of regioselectivity therefore enables the tailoring of sulfoethyl chitosan derivatives to specific end-use requirements.

Beyond these biological properties, several practical applications of SEC have been disclosed in patent. The suggested approach was aimed to focus on the direct use of the biopolymer in the inventions and resulted only in several patent applications worldwide, which strongly correlate with the functional properties of SEC mainly governed by its molecular architecture, which combines the rigid, hydrophilic backbone of chitosan with bulky anionic sulfoethyl substituents.

Such a unique structure imparts two major characteristics: a pronounced polyelectrolyte behavior arising from the presence of strong acid sulfonate groups, and excellent water solubility across a wide pH range, overcoming the main limitation of the native chitosan insolubility in neutral and alkaline media. However, these two features also strongly depend on the DS of the biopolymer. Sulfoethyl substituent is strongly hydrophilic, which affects even chitin making it fully water-soluble at DS ≈ 0.3 [80]. Thus, the presence of N-acetyl groups does not preclude solubility once a modest density of $–SO_3^-$ groups is introduced.

Similarly, for chitosan, increasing DS progressively decreases the basicity of the amino groups in SEC. The electron-withdrawing sulfoethyl substituents exert a negative inductive effect, reducing the electron density on the amino nitrogen and consequently lowering its proton affinity. Potentiometric titration studies consistently demonstrated a decrease in the dissociation constants of amino groups along with DS growth. Sulfoethylation has important consequences for both ion exchange and coordination properties because the reduced Lewis basicity weakens the stability of metal complexes formed through amino nitrogen atoms while simultaneously altering the balance between electrostatic and coordination interactions, which allows considering SEC as a promising biosorbent with tunable properties [52, 53, 81].

However, the influence of DS on sorption behavior is more complex than a simple increase or decrease in adsorption capacity. In general, increasing substitution of the amino groups hydrogen atoms by sulfoethyl residues reduces the overall sorption capacity towards numerous transition metal ions due to the lower amino-group basicity that weakens possible coordination interactions. Consequently, dynamic exchange capacities for Cu(II) and Ag(I) decreased with increasing DS, while stability constants of their complexes with the biopolymer became progressively smaller [52, 81].

Despite this anticipating a negative effect, higher DS markedly improved sorption selectivity. Increasing sulfoethyl content shifts the optimum sorption pH toward more acidic values and enhances discrimination between the metal ions, which are chemically similar. For example, derivatives with the DS around 0.5 exhibit significantly greater selectivity towards Ag(I) over Cu(II) than less substituted SEC samples. This behavior had been attributed to several complementary factors, including decreased amino-group basicity, participation of sulfonate oxygen atoms in mixed-ligand coordination, formation of chelate structures involving both amino and sulfonate groups, and the appearance of disulfoethylated glucosamine units at higher substitution levels. It has been proposed that since Ag(I) is a soft Lewis acid, reduction of amino-group basicity favors the formation of relatively more stable silver complexes, resulting in higher Ag/Cu selectivity [51, 81].

This result underlines the importance of the acetylation degree of chitosan. Previously it has been reported that electron-beam cross-linked SE-CG resins adsorb Cu(II), Ni(II), Co(II) and Zn(II) from mixed

solutions at pH 6.5 with a total capacity of 0.319 mmol g^{-1} — higher than the parent chitin-glucan (0.205 mmol g^{-1}) [82]. Selectivity follows the order Co(II) < Ni(II)<Cu(II)>Zn(II). The sulfonate groups act primarily by ion exchange rather than by strong five-membered chelate formation, explaining the modest capacity relative to carboxymethyl analogues. In contrast, N-SEC of comparable DS exhibits higher Cu(II)/Ag(I) selectivity because the free amino groups that remain after partial N-substitution participate in coordination [52, 81, 83].

Similar trends have been reported for SEC biosorption towards noble metal ions. Increasing DS enhanced the sorption selectivity of SEC towards Pd(II) relative to Pt(IV), while simultaneously shifting the optimum recovery conditions toward less acidic solutions. In contrast, sorption of perrhenate ions decreases with increasing DS because of steric hindrance and reduced amino-group basicity lower the number of effective anion-exchange sites. Therefore, the effect of sulfoethyl substitution strongly influenced the adsorption mechanism of a particular metal species. Overall, increasing DS decreases the absolute adsorption capacity for many coordination-controlled systems but substantially improves metal-ion selectivity, making highly substituted SEC particularly attractive for selective separation and analytical applications [83, 84].

The foregoing trends were successfully used as a key feature for the representative analytical application: the use of crosslinked N‑SEC with a DS of 0.5 as a solid-phase extraction sorbent for copper preconcentration in environmental water analysis, which has been patented by Ural Federal University named after the first President of Russia B.N. Yeltsin under the IPC classification G01N 21/63, which refers to systems or processes for investigating or analyzing materials using light, specifically where the material being investigated is optically excited [85]. In this method, the SEC-based material was packed into a cartridge, through which the water sample is percolated at a flow rate of 1.0-2.0 mL/min while copper ions were quantitatively retained on biosorbent. The coexisting metal ions were extracted by no more than 10 % reaching 15 % for Iron ions. Subsequent elution with 0.1 mol/L nitric acid yielded a copper-enriched eluate almost free of interfering matrix components, enabling sensitive and interference-free determination by atomic spectroscopy. The remarkable selectivity of N‑SEC observed in the work along with the natural origin of the biosorbent ensures the additional advantage in terms of environmental safety, distinguishing this approach from conventional methods that employ synthetic resins or hazardous organic reagents

The DS also governs the solubility of N-SEC. Introduction of sulfoethyl groups progressively converts chitosan from a weakly acidic polymer into a polyampholytic material with enhanced hydration and reduced intermolecular interactions. At relatively low substitution levels with DS below 0.45, N-SEC remains soluble only in dilute acidic media, similarly to unmodified chitosan. However, when the DS reaches 0.94 or higher, the biopolymer becomes readily soluble in water as well as in both acids (2 % acetic acid) and alkalies (2 % NaOH). This behavior originates from the increasing contribution of negatively charged sulfonate groups, which compensate the protonated amino groups and suppress extensive interchain hydrogen bonding. Nevertheless, the substitution pattern also plays an important role. While O-SEC dissolves only under strongly acidic (pH < 5) or strongly alkaline (pH > 13) conditions, the N-SEC remains soluble throughout the entire pH range because of its polyampholytic character [37, 39].

Logically the water-soluble compositions would require the use of N-substituted chitosan derivative.

The application scope of SEC further encompasses the textile industry under the classification C11D3/001 referring to the softening compositions in terms of textile, where it has been developed as an effective soil-release additive for washing agents. Herein Henkel AG and Co KGaA suggested the innovation addressed to cleaning cotton and cotton–polyester blends, which dominate modern textiles. By introducing N‑SEC with the DS = 0.52 into model detergent formulations containing surfactants (alkylbenzene sulfonate, sodium lauryl ether sulfate, fatty alcohol ethoxylate, fatty acid salt), builders, chelating agent, and auxiliary components afforded a substantial improvement in fatty stain removal on cotton and cotton‑polyester blend fabrics, whereas similarly introduced N, O‑carboxymethyl chitosan additive provided no significant benefit over the base composition. This finding highlights that exclusive N‑substitution is advantageous for soil-release performance and further illustrates the technological versatility of sulfoethyl chitosan [86].

At the same time, it has been demonstrated that highly substituted chitosan may undergo structural evolution during storage. Although freshly prepared films from SEC with DS = 0.94 were soluble over a broad pH range, prolonged storage results in complete loss of solubility while maintaining substantial swelling ability. This behavior has been attributed to the gradual formation of ionic crosslinks between oppositely charged amino and sulfonate groups during dehydration, producing physically crosslinked networks with

reduced solubility but preserved water uptake that should be considered within the studies and product development and storage [37, 83].

Another important property of SEC stipulating its functional performance in diverse applications that has been mentioned is its swelling behavior. Swelling itself reflects the complex interplay between the biopolymer chains caused by electrostatic repulsion among charged sulfonate groups, counterion distribution, and the osmotic pressure generated within the biopolymer network.

In this regard it also should be considered that sulfoethylation progressively disrupts the ordered crystalline structure of chitosan. The bulky sulfoethyl substituents interfere with intermolecular hydrogen bonding, resulting in partial amorphization of the polymer matrix. The reduced crystallinity enhances water penetration into the polymer network and consequently increases its swelling capacity. Even relatively low-substituted samples with the DS about 0.45 demonstrate significantly greater swelling than unmodified chitosan, consistent with the increased amorphous character of the material [37, 83].

Consequently, a thorough understanding of the structure-swelling relationship is essential for rational design and predictive use of this derivative in hydrated environments

The swelling behavior of SEC films in physiological saline solution was significantly influenced by the DS and storage time. Films were cast from 2 % acetic acid and subsequently treated with ammonia in ethanol to deprotonate the amino groups. Introduction of sulfoethyl groups markedly increased the swelling degree even at a relatively low DS = 0.45, which is attributed to the amorphization of the polymer matrix caused by the bulky substituents [37].

Further increase in swelling ratio can be reached by a widely introduced cross-linking approach. However, compared with the extensive literature addressing the influence of the DS, the effect of the degree of cross-linking on the physicochemical and sorption properties of SEC has received very limited attention. Up-to-date, only one systematic study has evaluated the influence of cross-linking density on SEC using glutaraldehyde cross-linked N-SEC with a fixed DS of 0.5. Increasing the degree of cross-linking resulted in a progressive decrease in both the moisture-content coefficient and the static exchange capacity toward hydroxide ions, reflecting reduced swelling and the consumption of amino groups during cross-link formation. Despite the lower number of accessible amino groups, the sorption selectivity toward Ag(I) over Cu(II) increased with increasing cross-linking density, particularly at pH 6.0-7.0 which had been attributed this behavior to the reduced availability of primary amino groups for coordination and the preferential stabilization of Ag(I) complexes through cooperative interactions involving the remaining amino groups and neighboring sulfonate functionalities. Consequently, a higher degree of cross-linking improved sorbent selectivity at the expense of exchange capacity and water uptake, highlighting cross-linking as an additional parameter for tuning SEC performance in selective metal-ion separation [51, 87].

Following the described strategy another solution in the field of preserving or maintaining viable microorganisms under the classification C12N 1/04 has been proposed by Novosibirsk State Technical University of Russia.

The cross-linking approach of SEC was involved in cryogel formation and further applied for bacterial stabilization. In this context, cross-linking with glutaraldehyde (GA) was conducted under subzero conditions resulting into an interconnected 15-50 μm macroporous structure that exhibited a swelling degree of 500-700 wt.% and porosity of 10-50 wt.%, which was found structurally optimal for bacterial entrapment. The cross-linking reaction consumed a significant part of free amino groups, further reducing the antimicrobial activity of SEC thus creating a bacteria-friendly environment. The cryogel effectively stabilized E. faecium L3, E. faecalis, B. subtilis, and E. coli over 12 months at both 4 °C and 22 °C, maintaining high bacterial titers (10^6-10^9 CFU/mL) without additional cooling making it also economically beneficial [88].

Considering the latest patent, it is important to acknowledge that GA is proven to exhibit cytotoxicity due to the residual unreacted aldehyde groups that can potentially leach from the cross-linked matrix. Moreover, the cross-linking reaction with GA may yield products containing oligomeric GA chains, further complicating toxicity assessment [89, 90]. Nevertheless, several factors mitigate this concern in the context of cryogel fabrication for bacterial stabilization and biomedical applications. Firstly, cryotechnological approach and high reactivity of GA, result in stable cryogels which can be obtained at remarkably low cross-linker concentrations, thereby reducing the cytotoxic burden [91]. Secondly, the application is primarily intended for ex vivo microbial preservation rather than direct implantation or systemic administration, which substantially lowers the toxicological risk. In this regard, the use of GA cross-linked biopolymers are well-established and legally accepted in various industrial biotechnological applications.

Nonetheless, for biomedical or food-contact applications where long-term biocompatibility is emergent, the alternative cross-linking strategies should be involved. The versatility and tunability of chitosan hydrogels through various cross-linking mechanisms has been represented by numerous publications. As a direct substituent for GA, a naturally occurring genipin (Gp) exhibits low cytotoxicity and high biocompatibility [92]. Besides that, the natural origin and low toxicity make Gp increasingly preferred for biomedical applications, the structure of chitosan cross-linked products turn out to be unpredictable due to competing cross-linking steps and the polymerization of Gp into grafted oligomeric chains [93]. Similarly, diepoxy compounds, such as ethylene glycol diglycidyl ether, present an advantage: although they are cytotoxic, the unreacted epoxy rings undergo hydrolysis upon contact with water, yielding non-toxic products, thereby eliminating the need for rigorous removal of residual cross-linker [94]. Another naturally occurring cross-linker, citric acid, is a generally recognized as safe compound, that has been widely explored as a non-toxic cross-linking agent for chitosan-based materials, forming ester linkages between carboxyl groups and hydroxyl groups of the polymer backbone under mild conditions [95].

Beyond covalent cross-linking, physical cross-linking strategies offer a route to materials that entirely avoid potentially toxic chemical reagents. Physical cross-linking relies on reversible non-covalent interactions such as ionic complexation, hydrogen bonding, hydrophobic interactions, and electrostatic associations to form stable hydrogel networks [96]. In these cases, the structure and interactions in both covalently and ionically cross-linked chitosan hydrogels are fundamentally different. The ionic cross-linking offers gentler conditions and greater biocompatibility, while covalent cross-linking provides superior mechanical strength. Recent advances have enabled the fabrication of injectable self-healing hydrogels that combine the reversibility of physically cross-linked networks with the mechanical robustness of covalently cross-linked materials [97]. Furthermore, stimuli-responsive chitosan hydrogels capable of responding to pH, temperature, and other physiological triggers have been extensively explored, with physical cross-linking playing a key role in enabling such responsiveness [98].

In summary, while GA remains a cost-effective and widely accepted cross-linker for ex vivo applications such as bacterial stabilization, the choice of cross-linking strategy should be carefully tailored to the intended implementation route. The greener alternatives, such as Gp, diepoxy compounds, citric acid or physical cross-linking are particularly attractive for applications requiring stringent biocompatibility, such as drug delivery, wound healing, and tissue engineering [99].

Despite its remarkable history covering more than fifty years of research, the patent portfolio for SEC appeared remarkably modest. These isolated examples, however, stand in sharp contrast to the thousands of patents granted for other chitosan derivatives, which highlights that the translation of this fundamental knowledge into intellectual property has been conspicuously slow in this area. Several factors may contribute to this patent gap including a more complicated synthesis route of SEC, which may limit its adoption by industrial players, and the market trajectories tending to application mainly of the native chitosan or its carboxymethylated derivative.

However, the current gap can be evaluated not as an abandoned frontier but a green field requiring a proactive patent strategy, encouraging researchers and innovators for new attempts and efforts for SEC implementation in everyday life.

5 Conclusions and Future Perspectives

This critical review provides a comprehensive overview of sulfoethyl chitosan (SEC), covering its synthesis, characterization, physicochemical, biological properties, current applications and future perspectives. Regioselective synthetic routes enable N-, O-, and mixed O, N-substituted derivatives with controlled degrees of substitution. The choice of reagent and reaction conditions, whether NaBES in acidic gel-phase for N-selectivity or NaVS under alkaline conditions for O-substitution, determines the regioselectivity and, consequently, the physicochemical and biological behavior of the resulting product. Characterization of SEC presents specific challenges especially for elemental analysis and molecular weight determination. Elemental analysis provides a bulk estimate of DS, provided that the sample is rigorously purified, whereas ^{1}H NMR provides site-specific structural information and is more suitable for distinguishing N- and O-substitution patterns. The presence of residual reagents, inorganic salts, or by-products can distort elemental analysis data, leading to incorrect DS values, thus requiring purification for reliable data. The purification affects analytical accuracy and influences biological properties since the residual reagents may affect cytotoxicity, hemocompatibility, and antimicrobial activity. The biological properties of SEC include anticoagulant activi-

ty, hemocompatibility, cytotoxicity, slight antimicrobial effects and bacterial stabilization capacity and are governed by the degree of substitution and substitution pattern. Comparative analysis with SEC precursors, such as chitin and chitin‑glucan complexes, reveals distinct structure–activity trends.

Despite its demonstrated versatility, SEC has been translated into only a handful of patented applications. These include selective solid‑phase extraction of copper from environmental waters, anticoagulant coatings, and macroporous cryogels for bacterial stabilization. The limited patent portfolio indicates that SEC remains an underexploited biopolymer with substantial space for intellectual property development. To systematically guide future research and industrial translation, Table 2 presents a structured gap analysis. For each identified gap, we propose specific future research directions and outline the potential impact of their achievement, providing an actionable framework for researchers, industrial partners, and funding agencies.

Table 2

SEC research gap analysis

	Knowledge Gap	Future Direction	Potential Impact
Synthesis and characterization	Lack of standardized purification procedures for SEC compromises DS determination by elemental analysis	Develop and mandate rigorous purification prior to analysis, and cross-validate the elemental analysis data with ^{1}H NMR spectra corrected for the DA.	Accurate and reliable DS determination
	Structural characterization often remains incomplete, no O- vs. N-substitution ratio is determined	Mandate combined NMR and FT-IR analysis for routine characterization, report DS, DA, substitution pattern, and MW as minimal dataset	Improved understanding of structure–property relationships and rational design of SEC for specific applications
	Lack of validated analytical methods for residual reagent quantification in SEC samples	Develop and validate liquid, gas or ion chromatography methods for quantitative determination of residual sulfoethylating agents and by-products	Improved quality control most important for biomedical applications and regulatory acceptance
	No systematic study on the stability of SEC solutions and solid-state SEC under different storage conditions	Conduct accelerated stability studies, monitor DS, MW, and solubility over time, establish shelf-life and storage recommendations	Reliable product quality over time and regulatory compliance
Biomedical and biological evaluation	SEC's potential immunomodulatory activity performed by other sulfated polysaccharides, has not been explored	Evaluate SEC's effect on macrophage polarization, cytokine secretion, and complement activation	Identification of immunomodulatory applications and possible synergy
	Antimicrobial activity of SEC against clinically relevant pathogens, including antibiotic-resistant strains, has not been systematically studied	Perform standardized antimicrobial susceptibility testing (MIC, MBC) against Gram-positive, Gram-negative, and fungal pathogens; evaluate potential synergy with conventional antibiotics and biopolymers	Identification of potential antimicrobial applications
	No data on SEC's interaction with cell membranes	Perform cellular uptake studies using fluorescently labeled SEC, investigate endocytosis pathways, evaluate intracellular trafficking and localization	Understanding of SEC's cellular interactions, safety assessment
Application-specific gaps	Limited systematic comparison with established commercial sulphated materials	Benchmark SEC against heparin (anticoagulant) and commercial biosorbents (metal recovery) under standardized conditions	Identification of competitive advantages and niches
	Biomedical applications of SEC remain underexplored: lack systematic evaluation on drug delivery systems and pharmaceutical excipient	Systematically develop SEC-based drug delivery platforms including nanoparticles, hydrogels, polyelectrolyte complexes also as mucoadhesive formulations	Expansion of SEC application portfolio, high-value biomedical products

	Potential of SEC for environmental remediation beyond metal sorption (e.g., dye removal, oil spill cleanup, PFAS adsorption) remains unexplored	Evaluate SEC-based biosorbents for removal of various pollutants and compare with commercial solutions	Expansion into the environmental market, sustainable remediation solutions, circular economy applications
Scaling-up and industrialization	SEC remains significantly underpatented compared to other chitosan derivatives	Prioritize patent gaps and fulfill those providing a competitive media in this field	Established IP position attractive for industry and future commercialization
	No established cost structure for SEC production	Perform comprehensive techno-economic analysis across the reported synthetic routes, identify cost drivers among raw materials and reagents, purification, energy and compare with competing chitosan derivatives and commercial alternatives.	Attraction of industrial investment by the evidence-based selection of the most economically viable synthesis route
	Lack of standardized quality control protocols for industrial-grade SEC	Develop and validate quality control protocols for DS, MW, purity, residual reagents, and endotoxin levels; establish specifications for different application grades (pharmaceutical, food, environmental, industrial)	Regulatory compliance and customer confidence
	SEC remains a laboratory-scale technology without market distribution, no pilot-scale data available	Conduct pilot-scale studies (10-100 L reactors), assess batch consistency and scalability different synthetic approaches, propose the optimized process parameters for large-scale production considering personnel and environmental safety	Lowering the scaling-up risks, demonstration of manufacturing feasibility
	No defined manufacturing process for SEC and SEC-based products	Establish good manufacturing practice (GMP) guidelines for SEC and SEC-based products with protocols optimized for end-use applications	Regulatory acceptance for biomedical and pharmaceutical products

Addressing these gaps will accelerate the transition of SEC from a laboratory derivative to a commercially viable biopolymer for medical, food, and environmental applications. With focused attention on these priorities, SEC has potential to establish itself as a sustainable and functional material in the global biopolymer market.

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Declaration of Generative AI and AI-Assisted Technologies in the Manuscript Preparation Process

During the preparation of this work, the author(s) used ChatGPT-4o to assist in creating the graphical layout and illustrative concepts for the chemical modification of chitosan and its functional outcomes in Figure 1. After using this tool, the authors thoroughly reviewed and edited the content. All chemical structures were manually cross-checked and verified for scientific accuracy. The authors take full responsibility for the ultimate content of the publication.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. CRediT: **Ksenia S. Bedritskikh** data curation, investigation, writing-original draft, writing-review & editing; **Arsalan Saeidi** data curation, investigation, visualization, writing-original draft, writing-review & editing; **Anna E. Ivanova** investigation, formal analysis; **Alexandr V. Pestov** writing-review & editing; **Ekaterina A. Litvinova** funding acquisition, writing-original draft; **Aleksandr A. Drannikov** conceptualization, supervision, visualization, writing-original draft, writing-review & editing.

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