









Polina I. Dubovskaia^{1*}, Saeidi Arsalan^{1,2}, Anna A. Pronchenko¹,
Anastasiia I. Drannikova¹, Ivan A. Lukoyanov^{1,3}, Farida K. Aripova^{1,4},
Mariia E. Savenko¹, Elizaveta A. Veretennikova⁵, Alexander V. Pestov⁵,
Ekaterina A. Litvinova¹, Aleksandr A. Drannikov¹

¹*Novosibirsk State Technical University, Novosibirsk, Russia;*

²*Novosibirsk State University, Novosibirsk, Russia;*

³*Borisev Institute of Catalysis, Siberian Branch of the Russian Academy of Sciences, Novosibirsk, Russia;*

⁴*Novosibirsk State Agrarian University, Novosibirsk, Russia;*

⁵*I. Ya. Postovsky Institute of Organic Synthesis, Ural Branch of the Russian Academy of Sciences, Yekaterinburg, Russia*

(*Corresponding author's e-mail: dubovskaiapolina@gmail.com)

Gel-Phase Synthesis and pH-Sensitive Swelling-Structure Relationships of N-Carboxyethylchitosan

The applicability of native chitosan-based compositions is constrained by their limited solubility in weakly alkaline and neutral media, a consequence of inherent structural features. To overcome this limitation, carboxyalkylation strategies such as the gel-phase Michael synthesis of N-carboxyethylchitosan (N-CEC) were investigated with a focus on optimizing reaction parameters to enhance yield and tailor biopolymer properties. Structural confirmation of the synthesized polymers was performed via FT-IR and SEM, while elemental analysis quantified the degree of substitution (DS), which correlated with temperature in the following way: DS = 0.96–1.10 at 50 °C, 1.07–1.12 at 60 °C and 1.16–1.32 at 70 °C. Porosity measurements indicated pore sizes ranging from 50 to 200 µm in all samples; however, total porosity varied significantly, reaching a maximum of 15 % at 70 °C and decreasing to 4–10 % at lower temperatures. N-CEC exhibited pH-dependent swelling, with minimal expansion (100–150 %) at low pH and a 2–3-fold increase at pH > 7, which was attributed to COO[−] group formation. These findings position N-CEC as a promising material for pH-responsive applications.

Keywords: carboxyethylchitosan, biopolymer, gel-phase synthesis, derivatization, Michael reaction, pH-sensitive swelling, porosity, green chemistry

1. Introduction

Chitosan is a natural polymer that has garnered significant attention due to its versatile applications in various fields. This aminopolysaccharide is typically derived through the deacetylation of chitin, which is predominantly obtained from crustacean exoskeletons, including those of shrimp and crabs [1–3]. The unique properties of chitosan, such as its biodegradability, low toxicity, and antimicrobial activity, make it an important material for various industries, particularly in biotechnology, medicine, water treatment and agriculture [4–8]. One of chitosan's most notable features is its excellent biocompatibility, enabling its use in various biomedical applications without provoking adverse reactions [4, 9]. This makes chitosan an ideal candidate for drug delivery systems, wound healing, and tissue engineering [10–15]. Its polyelectrolyte properties provide the interaction with negatively charged molecules, such as DNA and RNA, making it a useful material for gene delivery applications [16, 17]. In addition to its primary applications, chitosan exhibits a wide range of other valuable properties, including anti-inflammatory, antioxidant, antimicrobial, antifungal, antihyperglycemic, and antitumor activities. These characteristics make it a highly versatile compound with great potential in the development of novel therapeutic agents and in improving overall quality of life [6, 18–26].

Chitosan-based injectable hydrogels possess high potential for biomedical applications; however, their pH sensitivity limits their application in alkaline and neutral media [27]. To address this, chitosan polymers intended for practical use in vaccine delivery often require chemical modifications to improve their stability and solubility [28]. One of the primary strategies involves structural modification of the chitosan molecule to overcome its poor solubility under neutral and basic conditions [29, 30].

The solubility of chitosan can be improved by introducing various functional groups, such as carboxymethyl, sulfonic, or quaternary ammonium groups [28–34]. In addition to better solubility, chitosan derivatives often demonstrate superior biological activity compared to the native polymer [35]. Chitosan derivatives with substituted functional groups at both the –OH and –NH₂ reactive centers exhibit higher bactericidal activity against both Gram-positive and Gram-negative bacteria [36, 37]. Furthermore, quaternized chitosan derivatives have demonstrated higher antifungal activity than unmodified chitosan [38]. These modifications are particularly significant in applications such as wound dressings, drug delivery systems, and tissue engineering, where robust antimicrobial performance is essential. Additionally, derivatization has been employed to improve the antioxidant capacity of chitosan [39, 40]. The introduction of phenolic groups, for example, has been reported to boost both antioxidant and antitumor activities [41], opening new prospects for their use in cosmetics and food preservation [39, 42].

Another major objective of chitosan derivatization is to improve its drug delivery potential. Under acidic conditions (pH < 6.5), protonated chitosan enhances the paracellular transport of peptide drugs across mucosal epithelia. However, at neutral pH levels — such as those in the intestinal tract — native chitosan loses this absorption-enhancing ability. To overcome this limitation, a variety of chitosan derivatives have been developed and evaluated for performance in such physiological conditions [43, 44]. Moreover, the derivatization of chitosan can introduce stimuli-responsive properties, including pH and temperature sensitivity. These features are especially useful in designing smart drug delivery systems that release therapeutics in response to specific physiological conditions [45–47]. Chitosan is also chemically modified to enhance its biocompatibility and reduce its immunogenicity, making it more suitable for biomedical applications such as tissue scaffolds [30]. Overall, chitosan derivatization significantly expands the polymer's functional versatility and performance across medicine, biotechnology, and environmental science. Figure 1 illustrates the correlation between types of derivatization and the resulting improvements in physicochemical or biological parameters.

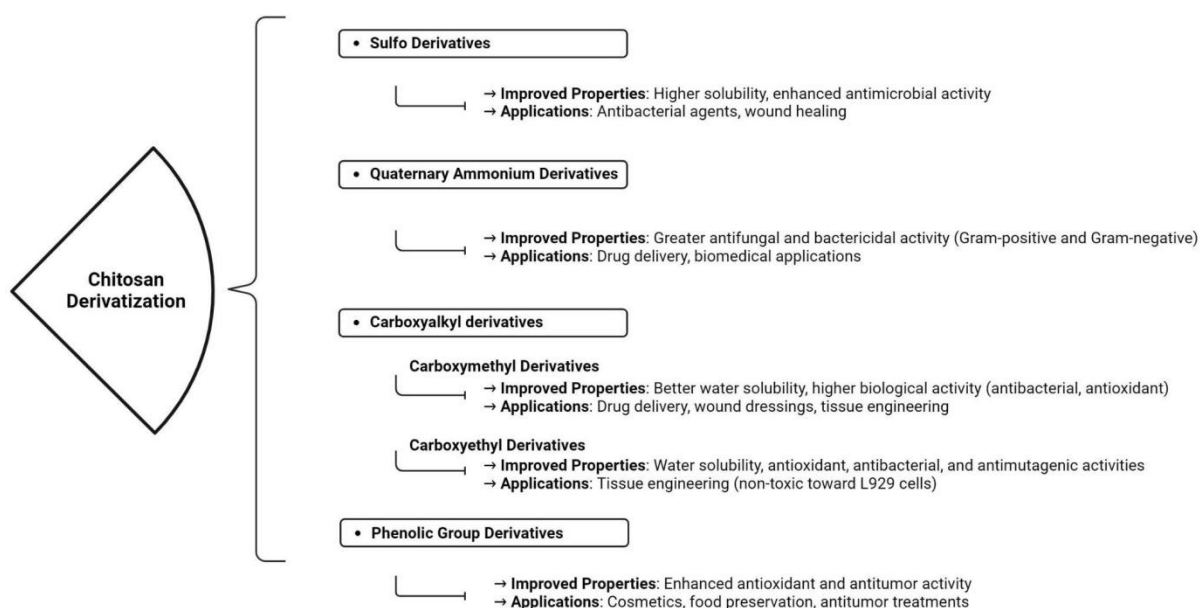


Figure 1. Influence of chitosan derivatization on its properties

Among the carboxyalkyl derivatives carboxyethyl chitosan (CEC) has attracted increasing attention due to its improved characteristics, including enhanced biocompatibility and water solubility [48, 49]. Recent studies have further confirmed the antioxidant and antimutagenic activities of N-(2-carboxyethyl)chitosan (N-CEC) [50], along with its improved antibacterial properties [51]. Additionally, CEC-based fibers have shown great potential as scaffolds for tissue engineering, demonstrating no cytotoxicity toward L929 fibroblast cells [52]. CEC is synthesized through the carboxyethylation of chitosan, in which the hydroxyl and amino groups of chitosan are substituted with carboxyethyl moieties. Based on the substitution sites of the 2-carboxyethyl group, CECs are generally classified into three types: O-CEC, N-CEC, and N,O-CEC [53]. Carboxyalkylation is typically carried out under either heterogeneous or homogeneous conditions through

nucleophilic substitution, addition, and addition-elimination reactions, often followed by reduction. In these reactions, chitosan serves as a nucleophilic polymer substrate [54, 55]. Figure 2 illustrates the synthetic pathways of carboxyethyl chitosan derivatives.

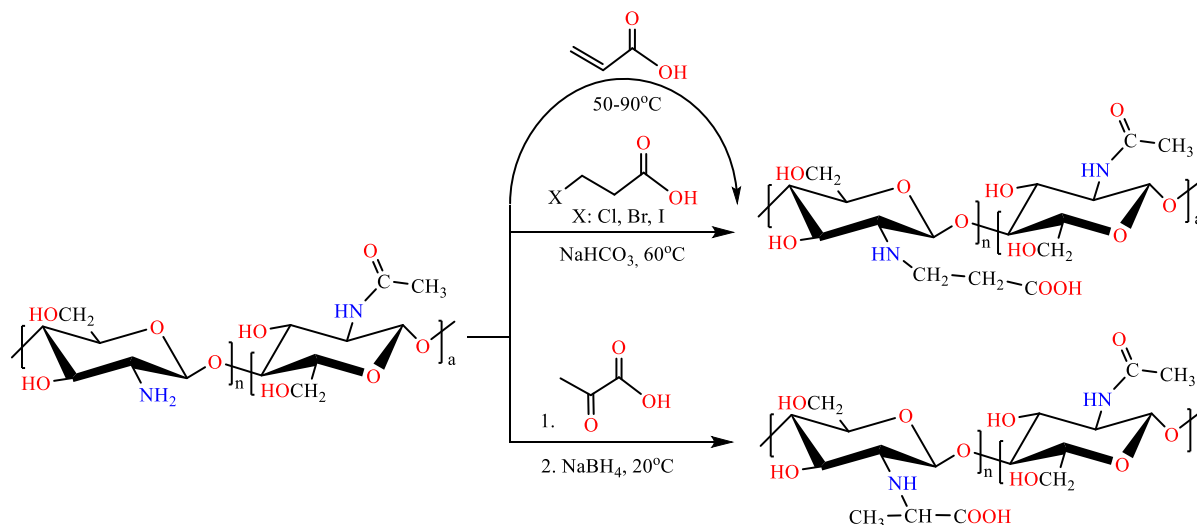


Figure 2. Synthetic routes of carboxyethyl chitosan

Herein, we investigated how gel-phase synthesis of N-CEC, as previously described in [56], influences the biopolymer properties and their relationship with structural features. This approach represents a highly efficient, cost-effective, and environmentally friendly process due to the minimal use of water and the complete absence of organic solvents. Previous studies have demonstrated that temperature and reaction time are the key factors affecting the degree of substitution (DS) of N-CEC [56]. In the present work, we focused on evaluating how variations in DS influence both the yield of N-CEC and its behavior in aqueous environments, particularly in terms of porosity and pH-dependent swelling.

2. Experimental

Chitosan (degree of deacetylation: 85.5 %) was purchased from Orisone Chemicals Limited (China) and used for the gel-phase synthesis of N-CEC without further purification. Acrylic acid (type P) was supplied by SIBUR LLC and was distilled prior to use. The resulting purity of acrylic acid was determined to be 99.4 % by titration.

2.1. Gel-phase synthesis of N-CEC

The synthesis of N-CEC was carried out following the previously reported procedure [56], with modifications to several experimental conditions. Briefly, 1.8 mol of acrylic acid was dissolved in 160 mL of distilled water under stirring using a Scilogex OS20-S Overhead LED Digital Stirrer. Subsequently, 0.9 mol of chitosan was gradually added to the solution to prevent aggregation. After complete dissolution of chitosan, the reaction mixture was stirred for an additional 60 minutes, then transferred to a Kitfort KT-1910 Professional Series oven and incubated under various conditions to complete the carboxyethylation process. The specific parameters used for carboxyethylation are summarized in Table 1.

Table 1

Sample codes

Time, h	Temperature, °C and code		
	50	60	70
24	N-CEC 1	N-CEC 2	N-CEC 3
48	N-CEC 4	N-CEC 5	N-CEC 6
72	N-CEC 7	N-CEC 8	N-CEC 9

After drying, the N-CEC samples were ground into particles approximately 6 mm in size and washed with distilled water until acrylic acid content remained constant at the level of 0.1–0.2 %. The washed

N-CEC was then dried further until the moisture content reached 1-2 %, as measured using a PMB Weighing Moisture Analyzer (Adam Equipment Company, UK). The process yield was calculated according to Equation (1):

$$\eta = \frac{m_{exp}}{m_{calc}} * 100 \%,$$

where m_{exp} is the mass of the product obtained experimentally, g; m_{calc} is the theoretically calculated mass of the product, g.

2.2. Polymer characterization

2.2.1. Fourier-transform infrared spectroscopy

The Fourier-transform infrared (FT-IR) spectroscopy was carried out in triplicate using a Spectrum One spectrometer (PerkinElmer, USA).

2.2.2. Degree of substitution

The degree of substitution (DS) of N-carboxyethylchitosan was determined using a C, H, N, S automated analyzer (PE 2400, Perkin Elmer, US). The resulting DS was calculated according to Equation (2):

$$DS = \frac{\left[\left(\frac{nC}{nN} \right)_{\text{product}} - \left(\frac{nC}{nN} \right)_{\text{chitosan}} \right]}{3},$$

where nC/nN is the atomic ratio of elements in the product and in the initial chitosan, respectively.

2.3. Functional properties evaluation

2.3.1. Swelling studies

The swelling capacity of the samples was measured using a widely accepted gravimetric method, as previously described [57]. Aqueous buffer solutions of varying pH were used as swelling media, including: phosphate buffer solution pH = 3.0, buffered copper sulfate solution pH = 4.0, acetate buffer solution pH = 6.0, phosphate buffer solution pH = 7.0, buffer (phosphate) solution pH = 9.0 and borate buffer solution pH = 10. Prior to testing, the hydrogels were dried to constant weight; the initial dry mass was designated as W_d . The sample was then immersed in 10 mL of distilled water at a temperature of 25 °C. After a specified time, the sample was removed, the excess water on the surface was blotted with tissue paper, and the weight (W_w) was recorded. The swelling percentage (S) was then calculated according to Equation (3):

$$S(\%) = \frac{W_w - W_d}{W_d} * 100.$$

2.3.2. Porosity

The pore volume was determined using the liquid displacement method described in [8]. Ethanol was used as the displacement fluid due to its ability to infiltrate the samples without inducing swelling or structural damage. In summary, the dry sample (initial weight designated as W_d) was immersed in ethanol under vacuum for 30 minutes, and the weight of the sample in ethanol was recorded as W_1 . The sample was then removed, and the surface liquid was blotted with filter paper. The weight of the wetted sample was recorded as W_w . The porosity was calculated according to Equation (4):

$$\varepsilon(\%) = \frac{(W_w - W_d)}{(W_w - W_1)} * 100 \, \%.$$

Pore size analysis was carried out using scanning electron microscopy (SEM) with an EVO 50 microscope (Zeiss, Germany). For sample preparation, the N-CEC specimens were sputter-coated with a 20 nm layer of copper using a JEOL JFC-1600 sputter coater (Japan) under a pressure not exceeding 8 Pa for 40 seconds.

2.4. Statistics

Data of yield, porosity and swelling are presented as mean \pm standard deviation. The data are visualized using histograms and a scatter plot. Statistical analysis was performed using Past 4.15 statistical software [58]. For data not following a normal distribution, nonparametric statistical methods, including

PERMANOVA, were applied. Comparison between three groups of non-normally distributed samples was performed using the Kruskal-Wallis test.

3. Results and Discussion

Each chitosan carboxyalkylation reaction mechanism strongly depends on reaction conditions, such as pH, temperature, and interaction time. Among these, pH has been shown to be the most critical factor for achieving total conversion. Typically, the reaction is carried out under acidic conditions, as the total reactivity of acrylic acid with the ammonium salt of chitosan exceeds that of acrylate with the free amino groups of chitosan. For instance, a pH of approximately 4.5 promotes the formation of O-carboxymethyl chitosan through a pathway favorable for imine formation; however, lower pH values require higher temperatures — specifically, temperatures sufficient for gel formation [59]. However, under low alkaline conditions (pH = 8), the reaction shifts towards the formation of N-substituted derivatives [60]. The pH parameter is particularly important and more easily controlled when the synthesis is conducted in the liquid phase. However, due to the large volumes of water required for liquid-phase reactions, we opted for the gel-phase synthesis of N-CEC, as previously described in [56]. This method has been reported as a green and efficient approach, with temperature and reaction time identified as the key parameters influencing the quality of the resulting N-CEC. Temperature is a limiting factor for the reaction rate in carboxyethyl chitosan synthesis. It plays a crucial role, with effective reaction temperatures reported at 50 °C [61–64], 60 °C [65–68], and up to 90 °C [56]. The effect of reaction time on the carboxyethylation process remains a matter of debate. Earlier studies showed that extending the reaction duration from 48 h to 240 h increases the DS but significantly reduces the yield by approximately twofold [69]. Other results indicated that 40 h is sufficient to complete the reaction, with only a marginal increase in DS observed beyond this point [70]. To date, it is widely accepted that 48 h represents the optimal reaction time for carboxyethylation of chitosan [71]. In our study, N-CEC samples were synthesized via gel-phase reaction between chitosan and acrylic acid in water under heating. The reaction yield was calculated according to Equation (1), and the obtained results are presented in Figure 3.

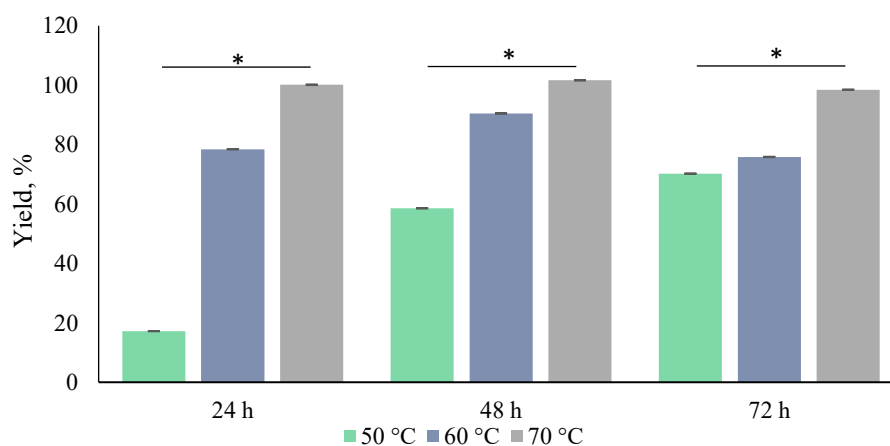


Figure 3. N-CEC yield depending on temperature and reaction time. * $p < 0.05$ Kruskal-Wallis test

A significant effect of both temperature and reaction time on N-CEC yield was observed (two-way PERMANOVA, $F(2, 26) = 866,590$, $p < 0.01$ for temperature; $F(2, 26) = 128,440$, $p < 0.01$ for reaction time). As shown in Figure 3, the yield reaches nearly 100 % at 70 °C after both 48 and 72 hours, with no statistically significant difference between these time points. Nevertheless, we hypothesized that the quality of the resulting N-CEC polymers may also depend on reaction conditions, potentially affecting the structural and functional properties of the final product. For all synthesized samples (coded NCEC-1 to NCEC-9), the FT-IR and SEM analyses yielded consistent results, confirming the successful modification of chitosan.

3.1. Fourier-Transform Infrared Spectroscopy

The FT-IR spectra of chitosan and N-CEC are presented in Figure 4. In the spectrum of N-CEC, absorption bands are observed at 2927, 1775, 1734, 1658, 1561, and 1419 cm^{-1} , which are indicative of successful functionalization of chitosan with 2-carboxyethyl groups.

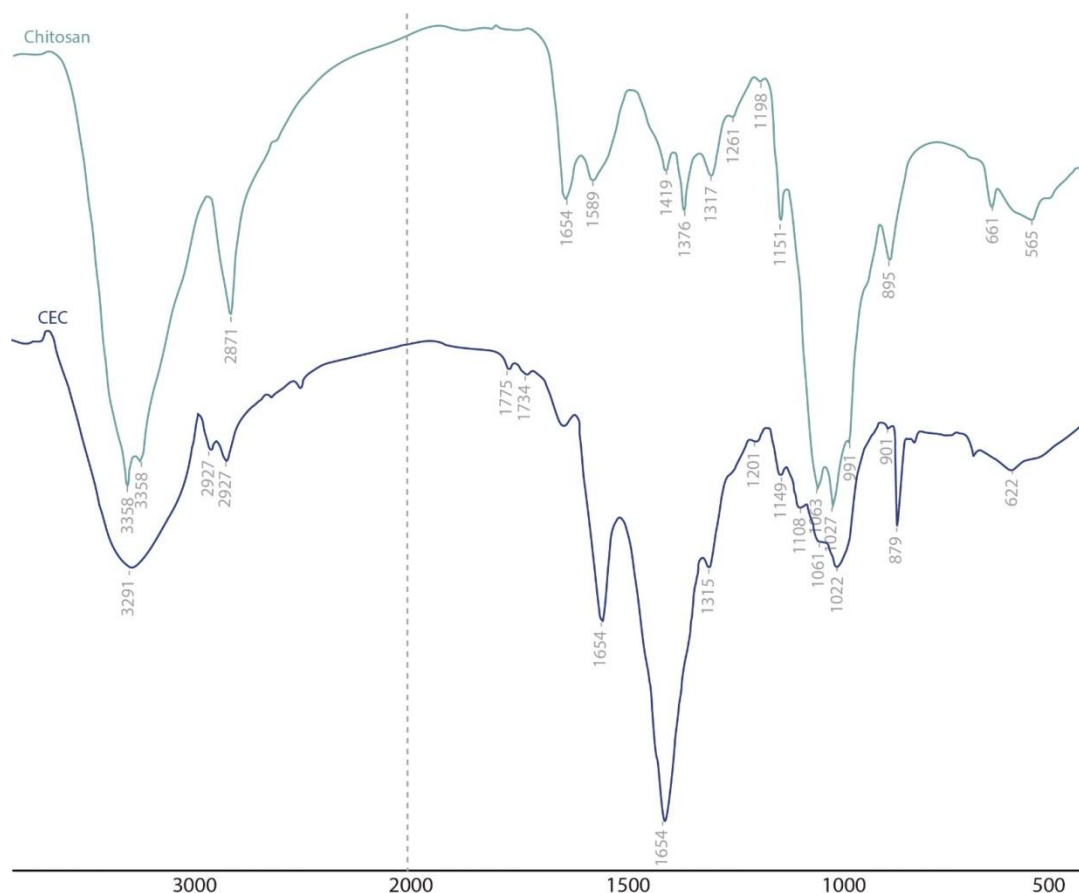


Figure 4. FT-IR spectra of Chitosan and N-CEC

3.2. Degree of substitution

Polysaccharides modification has a high impact on their physicochemical properties, improving solubility, ion-exchange capacity and adsorption [72]. In particular, the introduction of acidic groups into chitosan increases its anionic properties, enabling variable charge density along the biopolymer chain, which in turn leads to pH-dependent behavior [36]. This broadens the diversity of available raw materials and allows for the customization of biopolymer properties according to specific application requirements. Therefore, it is essential to characterize the degree of derivatization, and various analytical approaches have been reported for this purpose including nuclear magnetic resonance [60], elemental analysis [73], ultraviolet-visible and Fourier-transform infrared spectra [74]. In the present study, we investigated the degree of substitution (DS), which reflects the average number of 2-carboxyethyl groups per repeating unit (Table 2).

Table 2

Elemental analysis results

Sample	Element content, %			Degree of substitution
	C	H	N	
N-CEC 1	40.30	6.75	5.04	0.96
N-CEC 2	39.21	6.81	4.70	1.10
N-CEC 3	40.39	6.76	4.99	1.00
N-CEC 4	42.21	6.73	4.74	1.12
N-CEC 5	42.03	6.84	5.06	1.08
N-CEC 6	39.87	6.66	4.82	1.07
N-CEC 7	40.52	6.75	4.66	1.23
N-CEC 8	42.56	6.63	5.00	1.16
N-CEC 9	42.22	6.86	4.73	1.32

The data presented in Table 2 indicate a clear correlation between the DS and the reaction conditions. The DS ranges from 0.96 to 1.10 at 50 °C, from 1.07 to 1.12 at 60 °C and from 1.16 to 1.32 at 70 °C. These values are in agreement with previously reported data, with only minor deviations [56], and confirm the reproducibility of the synthesis, which is critical for future process development.

3.3. Porosity

Modifying porosity allows the improvement of mechanical stability and structure of biopolymers. The distribution of void space within the compositions can be effectively controlled by adjusting the degree of cross-linking, which in turn correlates with the degree of substitution of the starting polymer [75]. An increase in pore size enhances the pH-sensitive properties of chitosan hydrogels [64]. One of the most relevant examples is swelling, a critical factor for hydrogel performance in drug delivery applications [76]. Additionally, high porosity provides a large surface area for drug loading, while the presence of interconnected pores allows for controlled release of therapeutic agents [77]. In tissue engineering, pores and channels formed within the hydrogel matrix facilitate cell migration and proliferation into damaged tissue, ultimately supporting the regeneration or replacement of malfunctioning organs [78]. Proper porosity also contributes to optimal mechanical strength, elasticity, and structural stability of chitosan-based hydrogels [79]. The porosity characteristics of carboxyethylchitosan biopolymers synthesized in the present study are presented in Figure 5.

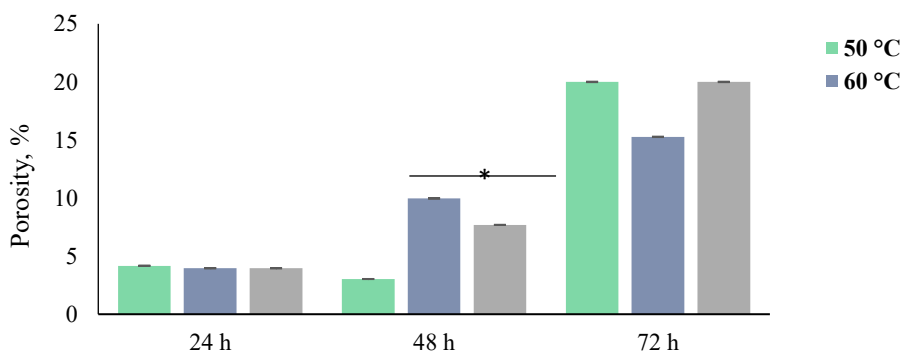


Figure 5. Porosity of N-CEC dependence on the reaction conditions. * $p < 0.05$ Kruskal-Wallis test

Based on the results presented in Figure 5, a significant effect of both temperature and reaction time on the porosity of N-CEC was observed (two-way PERMANOVA, $F(2, 26) = 2,162$, $p < 0.01$ for temperature; $F(2, 26) = 227,360$, $p < 0.01$ for reaction time). The data indicate that reaction temperature exerts a stronger influence on N-CEC porosity than reaction time. Samples synthesized at 70 °C exhibited porosity values exceeding 15 %, whereas those prepared at lower temperatures showed porosity in the range of 4–10 %, with no clear correlation to reaction time. This effect is likely attributed to a higher DS at elevated temperatures, which results in the incorporation of a greater number of $-\text{COOH}$ groups into the biopolymer structure. The increased presence of carboxylic groups enhances the potential for interchain cross-linking, thereby contributing to the formation of a more porous network (Figure 6) [80].

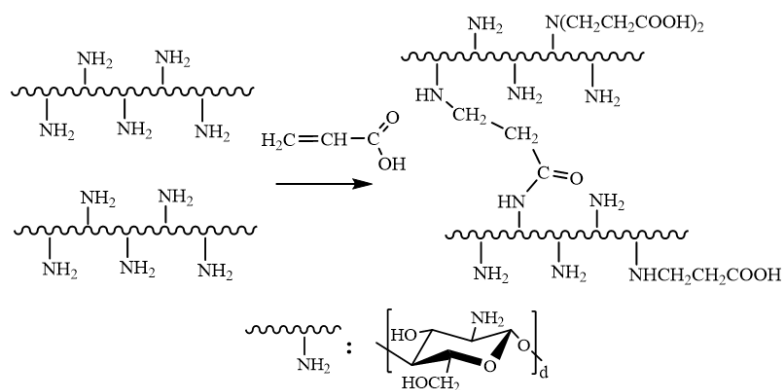


Figure 6. Cross-linking of N-CEC polymer chains occurring within the synthesis

Therefore, mild reaction conditions may lead to the formation of a slightly cross-linked polymer network; however, this assumption requires further comprehensive investigation.

Regarding pore size, N-CEC hydrogels were analyzed in both dried and swollen states. The results, presented in Figures 7a and 7b, respectively, demonstrate that the N-CEC hydrogel exhibits a layered internal structure with distinct porosity, with pore sizes ranging from 50 to 200 μm .

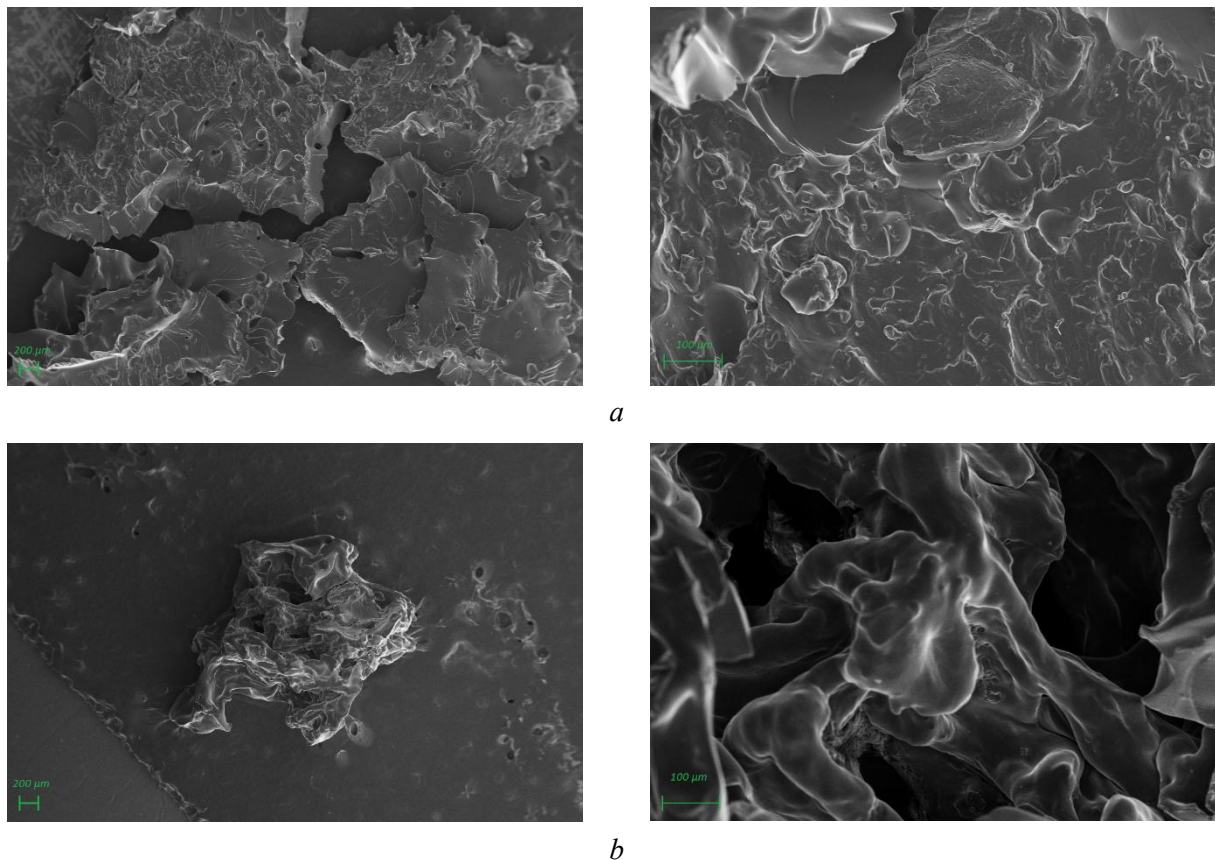


Figure 7. Representative SEM pictures of N-CEC in dried (a) and swollen (b) states

3.4. pH-Sensitive swelling

Swelling behavior is a key characteristic of biopolymers and is influenced not only by their internal structure, but also by external factors such as the pH and ionic strength of the surrounding medium. Previous studies have shown that carboxymethylchitosan (CMC) exhibits good ionic and pH sensitivity in aqueous solution, which has been attributed to the presence of amino groups in its side chains [81]. In addition, temperature-sensitive swelling of CMC-based hydrogels has been reported, with an increase in swelling observed within the temperature range of 5–55 $^{\circ}\text{C}$. This effect has been explained by the formation of additional hydrogen bonds and cross-links at lower temperatures, leading to more rigid and robust structures that are less permeable to water [82]. Overall, swelling is a highly responsive parameter, and understanding its underlying mechanisms is essential for predicting the performance of biopolymers and their compositions. The experimental data on the pH-sensitive swelling of N-CEC biopolymers synthesized under various reaction conditions are presented in Figure 8.

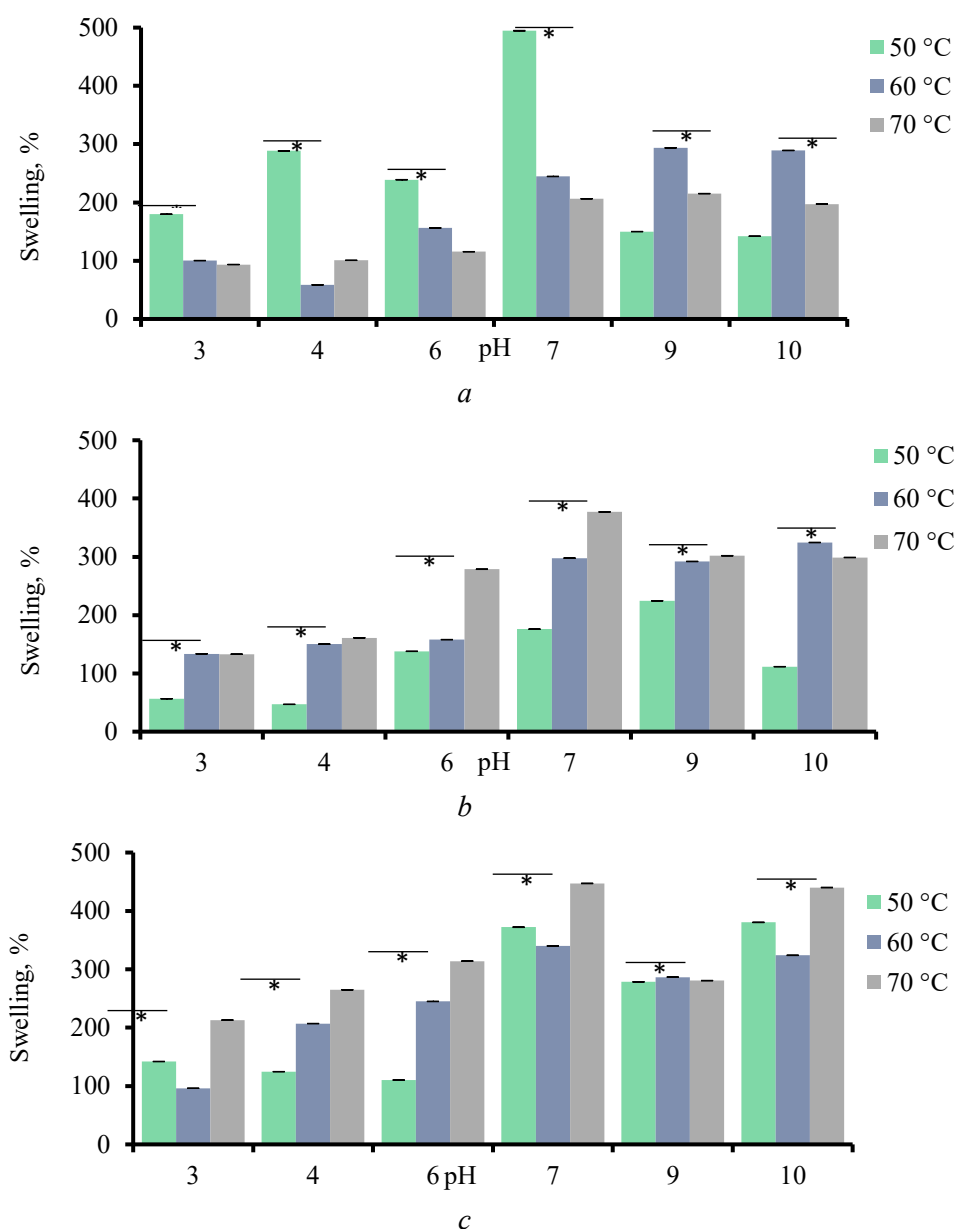


Figure 8. Swelling of N-CEC biopolymers under various pH for the samples synthesized at the temperature of 50 °C, 60 °C and 70 °C, within a). 24 h, b). 48 h, c). 72 h. * $p < 0.05$ Kruskal-Wallis test

Figure 8 demonstrates that the swelling behavior of N-CEC is strongly dependent on the pH of solution. According to two-way PERMANOVA analysis: at 24 hours, $F(2, 53) = 827,360$ $p < 0.01$ and $F(5, 53) = 835,110$ $p < 0.01$, accordingly; at 48 hours, $F(2, 53) = 171,6100$ $p < 0.01$ and $F(5, 53) = 1,049,000$ $p < 0.01$; at 72 hours, $F(2, 53) = 521,560$ $p < 0.01$ and $F(5, 53) = 1,024,200$ $p < 0.01$. All N-CEC samples exhibited limited swelling under acidic conditions, while a notable 2–3-fold increase was observed starting from pH 7, with maximum swelling reaching 450 % for biopolymers synthesized at 70 °C for 72 hours. To better interpret these results, the effects of cross-linking and pH should be considered. Cross-linking is known to significantly influence both swelling behavior and other properties of hydrogels by affecting the molecular weight [83]. In some systems, a lower degree of cross-linking provides greater network flexibility and increased hydrodynamic free volume, allowing the polymer chains to retain more solvent, which leads to increased swelling [84]. However, in our case, the high porosity of the samples synthesized at 70 °C suggests a higher degree of cross-linking, which would typically limit swelling. This apparent contradiction indicates that pH plays a more dominant role in swelling than cross-linking density under these conditions. Similar observations were reported in [85], where enhanced swelling was attributed to the deprotonation of carboxylic acid ($-\text{COOH}$) groups to carboxylate ($-\text{COO}^-$) anions, resulting in electrostatic repulsion between poly-

mer chains. This is consistent with the nature of the gel-phase synthesis, in which amino groups of chitosan are partially substituted by carboxyethyl groups. Interestingly, the unexpectedly high swelling observed for samples synthesized at 50 °C for 24 hours can be explained by a lower DS. This results in a larger number of free amino groups, which are protonated at low pH, allowing the hydrogel to swell. As pH increases, deprotonation of amino groups occurs, reducing repulsion between chains and leading to partial shrinking of the network [86, 87]. In summary, a higher degree of substitution in N-CEC synthesized at 70 °C ensures a combination of enhanced porosity and swelling, compared to mild synthetic conditions.

Conclusions

The present study demonstrates the successful synthesis of N-carboxyethylchitosan via an eco-friendly Michael addition protocol, with systematically varied reaction conditions ranging from 24 h to 72 h and from 50 °C to 70 °C. The resulting yield was strongly dependent on both time and temperature, increasing from 20 % at 50 °C (24 h) and to 98 % under optimized conditions. Structural studies of biopolymer samples were performed using FT-IR, SEM, and elemental analysis, which revealed a direct correlation between reaction temperature and the degree of substitution. Notably, higher synthesis temperatures produced samples with improved porosity (up to 15 %) and a uniform pore size distribution of 50–200 µm. The swelling behavior of N-CEC was found to be highly pH-sensitive. Under acidic conditions, swelling was limited to 100–150 %, whereas under alkaline conditions it increased 2–3-fold, reaching a maximum of 450 % for the sample synthesized at 70 °C for 72 hours. This phenomenon is likely attributable to the deprotonation of –COOH groups to –COO[–] anions, resulting in electrostatic repulsion between polymer chains. These findings highlight the potential of controllable N-CEC synthesis to achieve tunable structural and functional properties, reinforcing its applicability in the design of advanced pH-responsive biomaterials.

Funding

This research was supported by the “Priority-2030” program at Novosibirsk State Technical University (project No. SP-3 Zh-5/1).

Author Information*

**The authors' names are presented in the following order: First Name, Middle Name and Last Name*

Polina Ivanovna Dubovskaia — 3rd-year Bachelor student, Faculty of Business; Laboratory Assistant, Faculty of Physical Engineering, Novosibirsk State Technical University, 20, Prospekt K. Marksa, 630073, Novosibirsk, Russia; e-mail: dubovskaya.2022@stud.nstu.ru; <https://orcid.org/0009-0006-4769-446X>

Saeidi Arsalan — 2nd-year PhD student, Faculty of Natural Sciences, Novosibirsk State University, 1 Pirogova str., 630090, Novosibirsk, Russia; Engineer, Faculty of Physical Engineering, Novosibirsk State Technical University, 20 Prospekt K. Marksa, 630073, Novosibirsk, Russia; e-mail: saeidi@corp.nstu.ru; <https://orcid.org/0009-0006-2395-5127>

Anna Aleksandrovna Pronchenko — Junior Research Fellow, Faculty of Physical Engineering, Novosibirsk State Technical University, 20, Prospekt K. Marksa, 630073, Novosibirsk, Russia; e-mail: belousova2009@corp.nstu.ru; <https://orcid.org/0009-0006-9129-6215>

Anastasiia Ivanovna Drannikova — Engineer, Faculty of Physical Engineering, Novosibirsk State Technical University, 20 Prospekt K. Marksa, 630073, Novosibirsk, Russia; e-mail: drannikova@corp.nstu.ru

Ivan Andreevich Lukoyanov — PhD in Chemistry, Associate Professor, Faculty of Aircraft Engineering, Novosibirsk State Technical University, 20 Prospekt K. Marksa, 630073, Novosibirsk, Russia; e-mail: ivan.lukoyanov@corp.nstu.ru; <https://orcid.org/0000-0002-8706-1222>

Farida Kazbekovna Aripova — 1st-year PhD student, Institute of Fundamental and Applied Agrobiotechnologies, Novosibirsk State Agrarian University, 160 Dobrolyubova st., 630039, Novosibirsk, Russia; Junior Research Fellow, Faculty of Physical Engineering, Novosibirsk State Technical University, 20 Prospekt K. Marksa, 630073, Novosibirsk, Russia; e-mail: aripova@corp.nstu.ru

Mariia Evgenievna Savenko — 3rd-year Bachelor student, Faculty of Mechanical Engineering and Technologies; Laboratory Assistant, Faculty of Physical Engineering, Novosibirsk State Technical University, 20 Prospekt K. Marksa, 630073, Novosibirsk, Russia; e-mail: savenko.2022@stud.nstu.ru

Elizaveta Andreyevna Veretennikova — 3rd-year PhD student, I. Ya. Postovsky Institute of Organic Synthesis, Ural Branch of the Russian Academy of Sciences, 22 Sofya Kovalevskaya St., 620990, Yekaterinburg, Russia; e-mail: lizaveret@yandex.ru; <https://orcid.org/0009-0003-8588-5724>

Alexander Viktorovich Pestov — PhD in Chemistry, Research Fellow, I. Ya. Postovsky Institute of Organic Synthesis, Ural Branch of the Russian Academy of Sciences, 22 Sofya Kovalevskaya St., 620990, Yekaterinburg, Russia; e-mail: pestov@ios.uran.ru; <https://orcid.org/0000-0002-4270-3041>

Ekaterina Anatolyevna Litvinova — PhD in Biology, Research Fellow, Faculty of Physical Engineering, Novosibirsk State Technical University, 20 Prospekt K. Marksa, 630073, Novosibirsk, Russia; e-mail: e.litvinova@corp.nstu.ru; <https://orcid.org/0000-0001-6398-7154>

Aleksandr Alexeyevich Drannikov — PhD in Pharmacy, Junior Research Fellow, Faculty of Physical Engineering, Novosibirsk State Technical University, 20, Prospekt K. Marksa, 630073, Novosibirsk, Russia; e-mail: drannikov@corp.nstu.ru; <https://orcid.org/0000-0002-4839-1667>

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. **CRedit**: **Polina Ivanovna Dubovskaia** investigation, original draft preparation; **Saeidi Arsalan** visualization, original draft preparation; **Anna Aleksandrovna Pronchenko** methodology; **Anastasiia Ivanovna Drannikova** formal analysis, data curation; **Ivan Andreevich Lukoyanov** methodology, resources; **Farida Kazbekovna Aripova** methodology; **Mariia Evgenievna Savenko** methodology; **Elizaveta Andreyevna Veretennikova** methodology; **Alexander Viktorovich Pestov** investigation, methodology, review & editing; **Ekaterina Anatolyevna Litvinova** review & editing, visualization; **Aleksandr Alexeyevich Drannikov** conceptualization, methodology, project Administration, funding acquisition, supervision.

Conflicts of Interest

The authors declare no conflict of interest.

References

- 1 Hisham, F., Maziaty Akmal, M.H., Ahmad, F., Ahmad, K., & Samat, N. (2024). Biopolymer chitosan: Potential sources, extraction methods, and emerging applications. *A in Shams Engineering Journal*, 15(2), 102424. <https://doi.org/10.1016/j.asej.2023.102424>
- 2 Aranaz, I., Alcántara, A.R., Civera, M.C., Arias, C., Elorza, B., Heras Caballero, A., & Acosta, N. (2021). Chitosan: An overview of its properties and applications. *Polymers*, 13(19), 3256. <https://doi.org/10.3390/polym13193256>
- 3 Sharkawy, A., Barreiro, M.F., & Rodrigues, A.E. (2020). Chitosan-based Pickering emulsions and their applications: A review. *Carbohydrate Polymers*, 250(116885), 116885. <https://doi.org/10.1016/j.carbpol.2020.116885>
- 4 Marsili, L., Dal Bo, M., Berti, F., & Toffoli, G. (2021). Chitosan-based biocompatible copolymers for thermoresponsive drug delivery systems: On the development of a standardization system. *Pharmaceutics*, 13(11), 1876. <https://doi.org/10.3390/pharmaceutics13111876>
- 5 Khubiev, O.M., Esakova, V.E., Egorov, A.R., Bely, A.E., Golubev, R.A., Tachaev, M.V., Kirichuk, A.A., Lobanov, N.N., Tskhovrebov, A.G., & Kritchenkov, A.S. (2023). Novel non-toxic highly antibacterial chitosan/Fe(III)-based nanoparticles that contain a deferoxamine — Trojan horse ligands: Combined synthetic and biological studies. *Processes*, 11(3), 870. Basel, Switzerland. <https://doi.org/10.3390/pr11030870>
- 6 Guarnieri, A., Triunfo, M., Scieuzo, C., Ianniciello, D., Tafi, E., Hahn, T., Zibek, S., Salvia, R., De Bonis, A., & Falabella, P. (2022). Antimicrobial properties of chitosan from different developmental stages of the bioconverter insect *Hermetia illucens*. *Scientific Reports*, 12(1). <https://doi.org/10.1038/s41598-022-12150-3>
- 7 Azmana, M., Mahmood, S., Hilles, A.R., Rahman, A., Arifin, M.A.B., & Ahmed, S. (2021). A review on chitosan and chitosan-based bionanocomposites: Promising material for combatting global issues and its applications. *International Journal of Biological Macromolecules*, 185, 832–848. <https://doi.org/10.1016/j.ijbiomac.2021.07.023>

- 8 Drannikov, A.A., Blinova, E.A., Korel, A.V., Samokhin, A.G., Dubovskaya, P.I., Lazurenko, D.V., Zemlyakova, E.O., Pestov, A.V., & Litvinova, E.A. (2024). Novel biosorbents based on carboxyethyl chitosan for Allura Red dye contaminated water treatment *Chimica Techno Acta*, 11(4). <https://doi.org/10.15826/chimtech.2024.11.4.15>
- 9 Ul-Islam, M., Alabbosh, K.F., Manan, S., Khan, S., Ahmad, F., & Ullah, M.W. (2024). Chitosan-based nanostructured biomaterials: Synthesis, properties, and biomedical applications. *Advanced Industrial and Engineering Polymer Research*, 7(1), 79–99. <https://doi.org/10.1016/j.aiepr.2023.07.002>
- 10 Padhi, S., Behera, A., Hasnain, M.S., & Nayak, A.K. (2022). Uses of chitosan in drug delivery. In *Chitosan in Biomedical Applications* (pp 139–162). Elsevier.
- 11 Rajinikanth B., Rajkumar, S., Keerthika, D.S.R., & Vijayaragavan, V. (2024). Chitosan-based biomaterial in wound healing: A review. *Cureus*, 16(2), e55193. <https://doi.org/10.7759/cureus.55193>
- 12 Sultankulov, B., Berillo, D., Sultankulova, K., Tokay, T., & Saparov, A. (2019). Progress in the development of chitosan-based biomaterials for tissue engineering and regenerative medicine. *Biomolecules*, 9(9), 470. <https://doi.org/10.3390/biom9090470>
- 13 Guadarrama-Escobar, O.R., Serrano-Castañeda, P., Anguiano-Almazán, E., Vázquez-Durán, A., Peña-Juárez, M.C., Vera-Graziano, R., Morales-Florio, M.I., Rodríguez-Pérez, B., Rodríguez-Cruz, I.M., Miranda-Calderón, J.E., & Escobar-Chávez, J.J. (2023). Chitosan nanoparticles as oral drug carriers. *International Journal of Molecular Sciences*, 24(5), 4289. <https://doi.org/10.3390/ijms24054289>
- 14 Mikušová, V., & Mikuš, P. (2021). Advances in chitosan-based nanoparticles for drug delivery. *International Journal of Molecular Sciences*, 22(17), 9652. <https://doi.org/10.3390/ijms22179652>
- 15 Mura, P., Maestrelli, F., Cirri, M., & Mennini, N. (2022). Multiple roles of chitosan in mucosal drug delivery: An updated review. *Marine Drugs*, 20(5), 335. <https://doi.org/10.3390/md20050335>
- 16 Marques Gonçalves, M., Florencio Maluf, D., Pontarolo, R., Ketzner Saul, C., Almouazen, E., & Chevalier, Y. (2023). Negatively charged chitosan nanoparticles prepared by ionotropic gelation for encapsulation of positively charged proteins. *International Journal of Pharmaceutics*, 642(123164), 123164. <https://doi.org/10.1016/j.ijpharm.2023.123164>
- 17 Chuan, D., Jin, T., Fan, R., Zhou, L., & Guo, G. (2019). Chitosan for gene delivery: Methods for improvement and applications. *Advances in Colloid and Interface Science*, 268, 25–38. <https://doi.org/10.1016/j.cis.2019.03.007>
- 18 Azuma, K., Osaki, T., Minami, S., & Okamoto, Y. (2015). Anticancer and anti-inflammatory properties of chitin and chitosan oligosaccharides. *Journal of Functional Biomaterials*, 6(1), 33–49. <https://doi.org/10.3390/jfb6010033>
- 19 Zosangpuui, & Sudheer, P. (2024). Chitosan a natural anti-inflammatory and wound healing agent: A brief update. *Natural Resources for Human Health*, 4(2), 115–127. <https://doi.org/10.53365/nrfhh/177602>
- 20 Fong, D., & Hoemann, C.D. (2018). Chitosan immunomodulatory properties: Perspectives on the impact of structural properties and dosage. *Future Science OA*, 4(1). <https://doi.org/10.4155/fsoa-2017-0064>
- 21 Chang, S-H, Lin, Y-Y, Wu, G-J, Huang, C-H, & Tsai, G.J. (2019). Effect of chitosan molecular weight on anti-inflammatory activity in the RAW 2647 macrophage model. *International Journal of Biological Macromolecules*, 131, 167–175. <https://doi.org/10.1016/j.ijbiomac.2019.02.066>
- 22 Ivanova, D.G., & Yaneva, Z.L. (2020). Antioxidant properties and redox-modulating activity of chitosan and its derivatives: Biomaterials with application in cancer therapy. *BioResearch Open Access*, 9(1), 64–72. <https://doi.org/10.1089/biores.2019.0028>
- 23 Avelelas, F., Horta, A., Pinto, L.F.V., Cotrim Marques, S., Marques Nunes, P., Pedrosa, R., & Leandro, S.M. (2019). Anti-fungal and antioxidant properties of chitosan polymers obtained from nontraditional *Polybus henslowii* sources. *Marine Drugs*, 17(4), 239. <https://doi.org/10.3390/md17040239>
- 24 Ke, C-L, Deng, F-S, Chuang, C-Y, & Lin, C-H (2021). Antimicrobial actions and applications of chitosan. *Polymers*, 13(6), 904. <https://doi.org/10.3390/polym13060904>
- 25 Sarkar, S., Das, D., Dutta, P., Kalita, J., Wann, S.B., & Manna, P. (2020). Chitosan: A promising therapeutic agent and effective drug delivery system in managing diabetes mellitus. *Carbohydrate Polymers*, 247(116594), 116594. <https://doi.org/10.1016/j.carbpol.2020.116594>
- 26 Ueno, H., Mori, T., & Fujinaga, T. (2001). Topical formulations and wound healing applications of chitosan. *Advanced Drug Delivery Reviews*, 52(2), 105–115. [https://doi.org/10.1016/s0169-409x\(01\)00189-2](https://doi.org/10.1016/s0169-409x(01)00189-2)
- 27 Lavanya, K., Chandran, S.V., Balagangadharan, K., & Selvamurugan, N. (2020). Temperature- and pH-responsive chitosan-based injectable hydrogels for bone tissue engineering. *Materials Science & Engineering C, Materials for Biological Applications*, 111(110862), 110862. <https://doi.org/10.1016/j.msec.2020.110862>
- 28 Xing, L., Fan, Y-T, Shen, L-J, Yang, C-X, Liu, X-Y, Ma, Y-N, Qi, L-Y, Cho, K-H, Cho, C-S, & Jiang, H-L (2019). pH-sensitive and specific ligand-conjugated chitosan nanogels for efficient drug delivery. *International Journal of Biological Macromolecules*, 141, 85–97. <https://doi.org/10.1016/j.ijbiomac.2019.08.237>
- 29 Suryani, S., Chaerunisaa, A., Joni, I.M., Ruslin, R., Aspadiah, V., Anton, A., Sartinah, A., & Ramadhan, L.O.A. (2024). The chemical modification to improve solubility of chitosan and its derivatives application, preparation method, toxicity as a nanoparticles. *Nanotechnology, Science and Applications*, 17, 41–57. <https://doi.org/10.2147/nsa.s450026>
- 30 Desai, N., Rana, D., Salave, S., Gupta, R., Patel, P., Karunakaran, B., Sharma, A., Giri, J., Benival, D., & Kommineni, N. (2023). Chitosan: A potential biopolymer in drug delivery and biomedical applications. *Pharmaceutics*, 15(4), 1313. <https://doi.org/10.3390/pharmaceutics15041313>

- 31 Abourehab, M.A.S., Pramanik, S., Abdelgawad, M.A., Abualsoud, B.M., Kadi, A., Ansari, M.J., & Deepak, A. (2022). Recent advances of chitosan formulations in biomedical applications. *International Journal of Molecular Sciences*, 23(18), 10975. <https://doi.org/10.3390/ijms231810975>
- 32 Holme, K.R., & Perlin, A.S. (1997). Chitosan N-sulfate. A water-soluble polyelectrolyte. *Carbohydrate Research*, 302(1–2), 7–12. [https://doi.org/10.1016/s0008-6215\(97\)00117-1](https://doi.org/10.1016/s0008-6215(97)00117-1)
- 33 Dimassi, S., Tabary, N., Chai, F., Blanchemain, N., & Martel, B. (2018). Sulfonated and sulfated chitosan derivatives for biomedical applications: A review. *Carbohydrate Polymers*, 202, 382–396. <https://doi.org/10.1016/j.carbpol.2018.09.011>
- 34 Fabiano, A., Beconcini, D., Migone, C., Piras, A.M., & Zambito, Y. (2020). Quaternary ammonium chitosans: The importance of the positive fixed charge of the drug delivery systems. *International Journal of Molecular Sciences*, 21(18), 6617. <https://doi.org/10.3390/ijms21186617>
- 35 Harugade, A., Sherje, A.P., & Pethe, A. (2023). Chitosan: A review on properties, biological activities and recent progress in biomedical applications. *Reactive & Functional Polymers*, 191(105634), 105634. <https://doi.org/10.1016/j.reactfunctpolym.2023.105634>
- 36 Ardean, C., Davidescu, C.M., Nemeş, N.S., Negrea, A., Ciopec, M., Duteanu, N., Negrea, P., Duda-Seiman, D., & Musta, V. (2021). Factors influencing the antibacterial activity of chitosan and chitosan modified by functionalization International. *Journal of Molecular Sciences*, 22(14), 7449. <https://doi.org/10.3390/ijms22147449>
- 37 Li, Z., Yang, F., & Yang, R. (2015). Synthesis and characterization of chitosan derivatives with dual-antibacterial functional groups. *International Journal of Biological Macromolecules*, 75, 378–387. <https://doi.org/10.1016/j.ijbiomac.2015.01.056>
- 38 Tan, H., Ma, R., Lin, C., Liu, Z., & Tang, T. (2013). Quaternized chitosan as an antimicrobial agent: Antimicrobial activity, mechanism of action and biomedical applications in orthopedics. *International Journal of Molecular Sciences*, 14(1), 1854–1869. <https://doi.org/10.3390/ijms14011854>
- 39 Fatima, M., Mir, S., Ali, M., Hassan, S., Ul Haq Khan, Z., & Waqar, K. (2024). Synthesis and applications of chitosan derivatives in food preservation — A review. *European Polymer Journal*, 216(113242), 113242. <https://doi.org/10.1016/j.eurpolymj.2024.113242>
- 40 Yang, X., Lan, W., & Sun, X. (2023). Antibacterial and antioxidant properties of phenolic acid grafted chitosan and its application in food preservation: A review. *Food Chemistry*, 428(136788), 136788. <https://doi.org/10.1016/j.foodchem.2023.136788>
- 41 Li, Q., Li, Q., Tan, W., Zhang, J., & Guo, Z. (2020). Phenolic-containing chitosan quaternary ammonium derivatives and their significantly enhanced antioxidant and antitumor properties. *Carbohydrate Research*, 498(108169), 108169. <https://doi.org/10.1016/j.carres.2020.108169>
- 42 Aranaz, I., Acosta, N., Civera, C., Elorza, B., Mingo, J., Castro, C., Gandía, M., & Heras Caballero, A. (2018). Cosmetics and cosmeceutical applications of chitin, chitosan and their derivatives. *Polymers*, 10(2), 213. <https://doi.org/10.3390/polym10020213>
- 43 Thanou, M., Verhoef, J.C., & Junginger, H.E. (2001). Oral drug absorption enhancement by chitosan and its derivatives. *Advanced Drug Delivery Reviews*, 52(2), 117–126. [https://doi.org/10.1016/s0169-409x\(01\)00231-9](https://doi.org/10.1016/s0169-409x(01)00231-9)
- 44 Thanou, M., Verhoef, J.C., & Junginger, H.E. (2001a). Chitosan and its derivatives as intestinal absorption enhancers. *Advanced Drug Delivery Reviews*, 50, S91–S101. [https://doi.org/10.1016/s0169-409x\(01\)00180-6](https://doi.org/10.1016/s0169-409x(01)00180-6)
- 45 McCarthy, P.C., Zhang, Y., & Abebe, F. (2021). Recent applications of dual-stimuli responsive chitosan hydrogel nanocomposites as drug delivery tools. *Molecules (Basel, Switzerland)*, 26(16), 4735. <https://doi.org/10.3390/molecules26164735>
- 46 Zhou, H.Y., Chen, X.G., Kong, M., Liu, C.S., Cha, D.S., & Kennedy, J.F. (2008). Effect of molecular weight and degree of chitosan deacetylation on the preparation and characteristics of chitosan thermosensitive hydrogel as a delivery system. *Carbohydrate Polymers*, 73(2), 265–273. <https://doi.org/10.1016/j.carbpol.2007.11.026>
- 47 Xu, X., Liu, Y., Fu, W., Yao, M., Ding, Z., Xuan, J., Li, D., Wang, S., Xia, Y., & Cao, M. (2020). Poly(N-isopropylacrylamide)-based thermoresponsive composite hydrogels for biomedical applications. *Polymers*, 12(3), 580. <https://doi.org/10.3390/polym12030580>
- 48 Huang, J., Xie, H., Hu, S., Xie, T., Gong, J., Jiang, C., Ge, Q., Wu, Y., Liu, S., Cui, Y., Mao, J., & Mei, L. (2015). Preparation, characterization, and biochemical activities of N-(2-carboxyethyl)chitosan from squid pens. *Journal of Agricultural and Food Chemistry*, 63(9), 2464–2471. <https://doi.org/10.1021/jf505581n>
- 49 Chalitangkoon, J., Ronte, A., & Monvisade, P. (2023). Carboxyethylation of chitosan-based polymeric dyes for potential pH-sensing applications. *Journal of the Taiwan Institute of Chemical Engineers*, 149(105001), 105001. <https://doi.org/10.1016/j.jtice.2023.105001>
- 50 Kogan, G., Skorik, Y., Itanova, I., Krikova, L., Urakova, Z., Gomes, C., Yatluk, Y., & Krajovi, J. (2004). Antioxidant and antimutagenic activity of -(2-carboxyethyl)chitosan. *Toxicology and Applied Pharmacology*, 201(3), 303–310. <https://doi.org/10.1016/j.taap.2004.05.009>
- 51 Ibrahim, H.M., Mostafa, M., & Kandile, N.G. (2020). Potential use of N-carboxyethylchitosan in biomedical applications: Preparation, characterization, biological properties. *International Journal of Biological Macromolecules*, 149, 664–671. <https://doi.org/10.1016/j.ijbiomac.2020.01.299>
- 52 Yang, S., Dong, Q., Yang, H., Liu, X., Gu, S., Zhou, Y., & Xu, W. (2016). N-carboxyethyl chitosan fibers prepared as potential use in tissue engineering. *International Journal of Biological Macromolecules*, 82, 1018–1022. <https://doi.org/10.1016/j.ijbiomac.2015.10.078>

- 53 Huang, J., Xie, H., Ye, H., Xie, T., Lin, Y., Gong, J., Jiang, C., Wu, Y., Liu, S., Cui, Y., Mao, J., & Mei, L. (2016). Effect of carboxyethylation degree on the adsorption capacity of Cu(II) by N-(2-carboxyethyl)chitosan from squid pens. *Carbohydrate Polymers*, 138, 301–308. <https://doi.org/10.1016/j.carbpol.2015.11.037>
- 54 Jayakumar, R., Prabakaran, M., Nair, S.V., Tokura, S., Tamura, H., & Selvamurugan, N. (2010). Novel carboxymethyl derivatives of chitin and chitosan materials and their biomedical applications. *Progress in Materials Science*, 55(7), 675–709. <https://doi.org/10.1016/j.pmatsci.2010.03.001>
- 55 Koshugi, J. (1981). *Carboxyalkylated chitin and derivative* [Patent].
- 56 Pestov, A.V., Zhuravlev, N.A., & Yatluk, Y.G. (2007). Synthesis in a gel as a new procedure for preparing carboxyethyl chitosan. *Russian Journal of Applied Chemistry*, 80(7), 1154–1159. <https://doi.org/10.1134/s1070427207070282>
- 57 Adi Sulianto, A., Adiyaksa, I.P., Wibisono, Y., Khan, E., Ivanov, A., Drannikov, A., Ozaltin, K., & Di Martino, A. (2023). From fruit waste to hydrogels for agricultural applications. *Clean Technologies*, 6(1), 1–17. <https://doi.org/10.3390/cleantechnol6010001>
- 58 Hammer, Ø., Harper, D., & Ryan, P. (2001). Past: Paleontological statistical software package for education and data analysis. *Palaeontologia Electronica*, 4, 1–9.
- 59 Muzzarelli, R.A.A., Tanfani, F., Emanuelli, M., & Mariotti, S. (1982). N-(carboxymethylidene)chitosans and N-(carboxymethyl)chitosans: Novel chelating polyampholytes obtained from chitosan glyoxylate. *Carbohydrate Research*, 107(2), 199–214. [https://doi.org/10.1016/s0008-6215\(00\)80539-x](https://doi.org/10.1016/s0008-6215(00)80539-x)
- 60 Kono, H., & Kato, T. (2021). Elucidation of substituent distribution states for carboxymethyl chitosan by detailed NMR analysis. *Carbohydrate Polymer Technologies and Applications*, 2(100175), 100175. <https://doi.org/10.1016/j.carpta.2021.100175>
- 61 Jiang, H., Wang, Y., Huang, Q., Li, Y., Xu, C., Zhu, K., & Chen, W. (2005). Biodegradable hyaluronic acid/N-carboxyethyl chitosan/protein ternary complexes as implantable carriers for controlled protein release. *Macromolecular Bioscience*, 5(12), 1226–1233. <https://doi.org/10.1002/mabi.200500126>
- 62 Orienti I., & Luppi B. (1999). Chitosan and its N-carboxyethyl and N-aminoethyl derivatives as vehicles for topical formulations. *Journal of cosmetic science*, 50(5), 307–314.
- 63 do N. Ferreira, C.R., de L. Ramos, E.L., Araujo, L.F.S., da S. Sousa, L.M., Feitosa, J.P.A., Cunha, A.F., Oliveira, M.B., Mano, J.F., & da S. Maciel, J. (2021). Synthesis and characterization of scaffolds produced under mild conditions based on oxidized cashew gums and carboxyethyl chitosan. *International Journal of Biological Macromolecules*, 176, 26–36. <https://doi.org/10.1016/j.ijbiomac.2021.01.178>
- 64 Qu, J., Zhao, X., Ma, P.X., & Guo, B. (2017). pH-responsive self-healing injectable hydrogel based on N-carboxyethyl chitosan for hepatocellular carcinoma therapy. *Acta Biomaterialia*, 58, 168–180. <https://doi.org/10.1016/j.actbio.2017.06.001>
- 65 Huang, J., Xie, H., Ye, H., Xie, T., Lin, Y., Gong, J., Jiang, C., Wu, Y., Liu, S., Cui, Y., Mao, J., & Mei, L. (2016). Effect of carboxyethylation degree on the adsorption capacity of Cu(II) by N-(2-carboxyethyl)chitosan from squid pens. *Carbohydrate Polymers*, 138, 301–308. <https://doi.org/10.1016/j.carbpol.2015.11.037>
- 66 Yang, S., Dong, Q., Yang, H., Liu, X., Gu, S., Zhou, Y., & Xu, W. (2016). N-carboxyethyl chitosan fibers prepared as potential use in tissue engineering. *International Journal of Biological Macromolecules*, 82, 1018–1022. <https://doi.org/10.1016/j.ijbiomac.2015.10.078>
- 67 Ibrahim, H.M., Mostafa, M., & Kandile, N.G. (2020). Potential use of N-carboxyethylchitosan in biomedical applications: Preparation, characterization, biological properties. *International Journal of Biological Macromolecules*, 149, 664–671. <https://doi.org/10.1016/j.ijbiomac.2020.01.299>
- 68 Huang, J., Xie, H., Hu, S., Xie, T., Gong, J., Jiang, C., Ge, Q., Wu, Y., Liu, S., Cui, Y., Mao, J., & Mei, L. (2015). Preparation, characterization, and biochemical activities of N-(2-carboxyethyl)chitosan from squid pens. *Journal of Agricultural and Food Chemistry*, 63(9), 2464–2471. <https://doi.org/10.1021/jf505581n>
- 69 Shigemasa, Y., Ishida, A., Sashiwa, H., Saimoto, H., Okamoto, Y., Minami, S., & Matsushashi, A. (1995). Synthesis of a new chitin derivative, (1-carboxyethyl)chitosan. *Chemistry Letters*, 24(8), 623–624. <https://doi.org/10.1246/cl.1995.623>
- 70 Skorik, Y.A., Gomes, C.A.R., Vasconcelos, M.T.S.D., & Yatluk, Y.G. (2003). N-(2-Carboxyethyl)chitosans: regioselective synthesis, characterisation and protolytic equilibria. *Carbohydrate Research*, 338(3), 271–276. [https://doi.org/10.1016/s0008-6215\(02\)00432-9](https://doi.org/10.1016/s0008-6215(02)00432-9)
- 71 Jiang, H., Wang, Y., Huang, Q., Li, Y., Xu, C., Zhu, K., & Chen, W. (2005). Biodegradable hyaluronic acid/N-carboxyethyl chitosan/protein ternary complexes as implantable carriers for controlled protein release. *Macromolecular Bioscience*, 5(12), 1226–1233. <https://doi.org/10.1002/mabi.200500126>
- 72 Liu, X-L, Zhu, C-F, Liu, H-C, & Zhu, J-M (2022). Quantitative analysis of degree of substitution/molar substitution of etherified polysaccharide derivatives. *Designed Monomers and Polymers*, 25(1), 75–88. <https://doi.org/10.1080/15685551.2022.2054118>
- 73 Korel, A., Samokhin, A., Zemlyakova, E., Pestov, A., Blinova, E., Zelikman, M., Tkachenko, V., Bets, V., Kretien, S., Arzhanova, E., & Litvinova, E. (2023). A carboxyethylchitosan gel cross-linked with glutaraldehyde as a candidate carrier for biomedical applications. *Gels (Basel, Switzerland)*, 9(9), 756. <https://doi.org/10.3390/gels9090756>
- 74 Ogawa, K., Hirai, I., Shimasaki, C., Yoshimura, T., Ono, S., Rengakuji, S., Nakamura, Y., & Yamazaki, I. (1999). Simple determination method of degree of substitution for starch acetate. *Bulletin of the Chemical Society of Japan*, 72(12), 2785–2790. <https://doi.org/10.1246/bcsj.72.2785>
- 75 Li, Y., Qiu, Y., Hou, H., Zhang, G., Hao, H., & Bi, J. (2023). The preparation and properties of amino-carboxymethyl chitosan-based antibacterial hydrogel loaded with ϵ -polylysine. *Foods (Basel, Switzerland)*, 12(20), 3807. <https://doi.org/10.3390/foods12203807>

- 76 Craciun, A.M., Morariu, S., & Marin, L. (2022). Self-healing chitosan hydrogels: Preparation and rheological characterization. *Polymers*, 14(13), 2570. <https://doi.org/10.3390/polym14132570>
- 77 Herdiana, Y., Febrina, E., Nurhasanah, S., Gozali, D., Elamin, K.M., & Wathoni, N. (2024) Drug loading in chitosan-based nanoparticles. *Pharmaceutics*, 16(8), 1043. <https://doi.org/10.3390/pharmaceutics16081043>
- 78 Fletes-Vargas, G., Espinosa-Andrews, H., Cervantes-Uc, J.M., Limón-Rocha, I., Luna-Bárcenas, G., Vázquez-Lepe, M., Morales-Hernández, N., Jiménez-Ávalos, J.A., Mejía-Torres, D.G., Ramos-Martínez, P., & Rodríguez-Rodríguez, R. (2023) Porous chitosan hydrogels produced by physical crosslinking: Physicochemical, structural, and cytotoxic properties. *Polymers*, 15(9), 2203. <https://doi.org/10.3390/polym15092203>
- 79 Nayak, S., & Kundu, S.C. (2014). Sericin-carboxymethyl cellulose porous matrices as cellular wound dressing material. *Journal of Biomedical Materials Research Part A*, 102(6), 1928–1940. <https://doi.org/10.1002/jbma34865>
- 80 Skorik, Y.A., Pestov, A.V., Kodess, M.I., & Yatluk, Y.G. (2012). Carboxyalkylation of chitosan in the gel state. *Carbohydrate Polymers*, 90(2), 1176–1181. <https://doi.org/10.1016/j.carbpol.2012.06.072>
- 81 Khan, S., & Anwar, N. (2019). Highly porous pH-responsive Carboxymethyl chitosan-grafted-poly (acrylic acid) based smart hydrogels for 5-fluorouracil controlled delivery and colon targeting. *International Journal of Polymer Science*, 2019, 1–15. <https://doi.org/10.1155/2019/6579239>
- 82 Farasati Far, B., Omrani, M., Naimi Jamal, M.R., & Javanshir, S. (2023). Multi-responsive chitosan-based hydrogels for controlled release of vincristine. *Communications Chemistry*, 6(1), 1–14. <https://doi.org/10.1038/s42004-023-00829-1>
- 83 Elliott, J.E., Macdonald, M., Nie, J., & Bowman, C.N. (2004). Structure and swelling of poly(acrylic acid) hydrogels: effect of pH, ionic strength, and dilution on the crosslinked polymer structure. *Polymer*, 45(5), 1503–1510. <https://doi.org/10.1016/j.polymer.2003.12.040>
- 84 Kumar Singh Yadav, H., & Shivakumar, H.G. (2012). in vitro and in vivo evaluation of pH-sensitive hydrogels of carboxymethyl chitosan for intestinal delivery of theophylline. *ISRN Pharmaceutics*, 2012, 1–9. <https://doi.org/10.5402/2012/763127>
- 85 Nkana, G.R.N., Lajeunesse, A., Chabot, B., & Nguyen-Tri, P. (2024). Design of porous spherical biomaterials from carboxymethyl chitosan for removal of fluoxetine in aqueous medium. *Journal of Environmental Chemical Engineering*, 12(2), 112228. <https://doi.org/10.1016/j.jece.2024.112228>
- 86 Rohindra, D.R., Nand, A.V., & Khurma, J.R. (2004). Swelling properties of chitosan hydrogels. *The South Pacific Journal of Natural and Applied Sciences*, 22(1), 32. <https://doi.org/10.1071/sp04005>
- 87 Budianto, E., Muthoharoh, S.P., & Nizado, N.M. (2015). Effect of crosslinking agents, pH and temperature on swelling behavior of cross-linked chitosan hydrogel. *Asian Journal of Applied Sciences*, 3(5). <https://www.ajournalonline.com/index.php/AJAS/article/view/3099>