BIOLOGY, MEDICINE, & NATURAL PRODUCT CHEMISTRY ISSN 2089-6514 (paper)
Volume 14, Number 2, October 2025 | Pages: 905-909 | DOI: 10.14421/biomedich.2025.142.905-909 ISSN 2540-9328 (online)

Synthesis, Characterization, and Micellar Behavior of Amphiphilic Chitosan Bearing Sulfate and Anisaldehyde Imine Groups

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Manuscript received: 05 August, 2025. Revision accepted: 14 October, 2025. Published: 24 October, 2025.

Abstract

Amphiphilic chitosan derivatives bearing sulfate and anisaldehyde imine (anisimine) groups were successfully synthesized and characterized for potential applications as functional biomaterials. The synthesis involved two key steps: (1) sulfation of chitosan using chlorosulfonic acid to introduce O-sulfate groups, and (2) Schiff base formation via reaction with p-anisaldehyde to generate N-anisimine functionalities. Structural modifications were confirmed by Fourier-transform infrared (FTIR) spectroscopy, which showed characteristic absorption bands at ~1250 cm^{-1} and ~820 cm^{-1} corresponding to O=S=O stretching of sulfate, and a C=N stretch at ~1640 cm^{-1} indicating imine formation. Proton nuclear magnetic resonance (1H-NMR) spectra further verified the successful attachment of aromatic protons from p-anisaldehyde and the disappearance of primary amine peaks, confirming imination. The amphiphilic behavior and micelle-forming ability of the N-anisimine-O-sulfated (NAOS) chitosan were evaluated using pyrene as a hydrophobic fluorescent probe for determining the critical micelle concentration (CMC), which was found to be 0.012 mg/mL. The observed low CMC value indicates strong self-assembly capability in aqueous media. These results suggest that NAOS chitosan possesses promising structural and surface-active properties suitable for advanced applications in drug delivery and cosmetic formulations.

Keywords: amphiphilic polymers; chitosan derivatives; delivery systems; Schiff base.

INTRODUCTION

Chitosan, a natural polysaccharide derived from chitin, has garnered significant attention due to its biocompatibility, biodegradability, and versatile chemical functionality. Its primary amino and hydroxyl groups offer extensive opportunities for chemical modifications, enabling the development of tailored materials for applications in drug delivery, cosmetics, and tissue engineering. However, the inherent hydrophilicity of chitosan often limits its ability to interact effectively with hydrophobic compounds, thereby restricting its use in systems that require amphiphilic properties.

The introduction of amphiphilic functionalities to chitosan has emerged as a promising strategy to overcome these limitations. Amphiphilic chitosan derivatives, capable of forming self-assembled structures such as micelles or nanoparticles, have been extensively investigated in recent years. For instance, Sharma et al. (2017) synthesized amphiphilic chitosan derivatives by grafting stearic acid onto the chitosan backbone, demonstrating their ability to form nanomicelles to deliver nonviral gene (Sharma & Singh, 2017). Similarly, Pol et al. (2022) reported the preparation of amphiphilic chitosan modified with Palmitoyl and quarternary ammonium moities, demonstrating its ability to encapsulate plasmid DNA (Pol et al., 2022).

In addition to hydrophobic modifications, the introduction of hydrophilic groups such as sulfate or carboxymethyl functionalities further enhances the versatility of chitosan. Dimassi et al. (2018) explored sulfated chitosan derivatives and highlighted their improved biocompatibility and ionic characteristics, making them suitable for biomedical applications (Dimassi et al., 2018). Moreover, amphiphilic chitosan bearing both hydrophobic and hydrophilic modifications has shown exceptional potential in forming stable emulsions and hydrogels for cosmetic and pharmaceutical formulations (Lazaridou et al., 2025).

Among hydrophobic modifications, aromatic groups such as anisimine offer unique advantages due to their ability to enhance the interaction of material with hydrophobic drugs and aromatic compounds through π-π interactions which is an essential driving force for self-assembly (Gao et al., 2023). Sulfation, on the other hand, introduces negatively charged sulfate groups that

improve water solubility and provide ionic functionality. Combining these modifications creates a synergistic effect, enhancing the amphiphilic nature of chitosan and broadening its application potential.

In this study, novel amphiphilic chitosan derivatives functionalized with anisimine and sulfate groups were synthesized and characterized. Their structural features and amphiphilic properties were thoroughly evaluated. This work aims to elucidate the potential of such dual-functionalized chitosan derivatives for advanced applications in drug or cosmetic delivery systems.

MATERIALS AND METHODS

Materials

Commercially available chemicals and solvents from Merck were utilized in this research for their specific roles and high purity. Chitosan was derived from shrimp shells (Litopenaeus vannamei). Chlorosulfonic acid ($HClSO_3$, Merck, Germany) used as the sulfonation agent. Anisaldehyde (Merck, Germany) was utilized for synthesizing amphiphilic chitosan derivatives. Additional materials included ethanol (Merck, Germany), glacial acetic acid (Merck, Germany), deuterated oxide (Merck, Germany), trifluoroacetic acid (Acros Organics, USA), pyrene, dialysis membranes with molecular weight cut-off (MWCO) 12,000–14,000 (Sigma-Aldrich, USA), and cellulose-acetate membrane filters (0.45 µm, Merck Millipore, Ireland). These were used for critical micelle concentration determination.

Synthesis of Chitosan

Chitosan was prepared from shrimp shell waste through a multistep chemical process involving demineralization, deproteinization, and deacetylation, following a modified procedure based on Zvezdova (2010) and Suendo et al. (2010). Initially, shrimp shells were thoroughly cleaned to remove residual organic matter, then washed, dried, and milled into a fine powder (particle size ~0.3–0.5 mm) (Zvezdova, 2010) (Suendo et al., 2010). The demineralization process was conducted by soaking the powdered shells in 7% hydrochloric acid (HCl) at room temperature using a solid-to-liquid ratio of 1:10 (w/v) for 24 hours to dissolve calcium carbonate and other mineral components. The sample was then filtered, washed repeatedly with distilled water until neutral pH was achieved, and subsequently dried. The deproteinization step involved treating the demineralized residue with 10% sodium hydroxide (NaOH) at 60 °C for 24 hours to remove proteins. After thorough washing and drying, the resulting material (chitin) was subjected to deacetylation using 50% NaOH at 60 °C for 8 hours to convert it into chitosan.

Synthesis of Chitosan

Preparation of O-Sulfate Chitosan

Chitosan (1 g, degree of deacetylation (DD) 88.6%, molecular weight (MW) 3300 kDa was reacted with chlorosulfonic acid (4 mL) in distilled water (30 mL) at room temperature overnight. The reaction mixture was neutralized using a 20% (w/v) NaOH solution to achieve a pH of 7. The neutralized solution was dialyzed against deionized water using a dialysis membrane with a molecular weight cutoff (MWCO 12,000–14,000) for two days, followed by lyophilization an additional two days to obtain a yellow powder.

Preparation of N-anisimine -O-sulfate Chitosan

O-sulfate chitosan (0.5 g) was reacted with p-anisaldehyde (30 µL) in dimethylformamide (DMF, 1 mL) at a molar ratio of 1:1 and 1:3 (O-sulfate chitosan: p-anisaldehyde). The reaction was carried out at 78°C for 4 hours. The resulting heterogeneous mixture was filtered, and the precipitate was washed with methanol. The product was dried under vacuum at room temperature overnight to yield a fine powder.

Structural Characterization

The chemical structures of chitosan and its derivatives were confirmed using the following methods. The chemical structure of chitosan and chitosan derivatives was confirmed with ATR-FTIR, 1H-NMR and 13C-NMR. ATR-FTIR analysis was performed on a Nicolet 6700 FTIR spectrometer. 1H-NMR and 13C-NMR were performed on a Bruker 400 MHz spectrometer. Chitosan, O-sulfate-chitosan, N-anisimine-O-sulfate-chitosan were dissolved in the mixed solvent of D2O and CF3COOH. The degree of substitution (DS) for the derivatives was calculated using the integration of NMR peaks (Jaidee et al., 2012):

$$\%\text{DS of anisaldehyde} = \frac{(\int H_{7.0} + H_{7.78} + H_{9.63})}{5 \int H_{3,05}}$$

Determination of Critical Micelle Concentration

The CMC of chitosan derivative was determined using pyrene as a hydrophobic probe (Aguiar et al., 2003). A solution of pyrene in acetone (6×10^{-5} M) was prepared, and 10 µL was added to eppendorf tubes. The acetone was evaporated before adding 1 mL of chitosan derivative solutions (concentrations ranging from 0.01 to 0.6 mg/mL). The solutions were sonicated at room temperature for 30 minutes and equilibrated at 65°C for 3 hours. Fluorescence emission spectra (350–450 nm) were recorded using an excitation wavelength of 336 nm on a fluorescence spectrophotometer. Both excitation and emission bandwidths were set at 5 nm. The CMC values were calculated based on the pyrene emission spectra.

RESULTS AND DISCUSSION

The result of IR and ^{1}H-NMR analysis

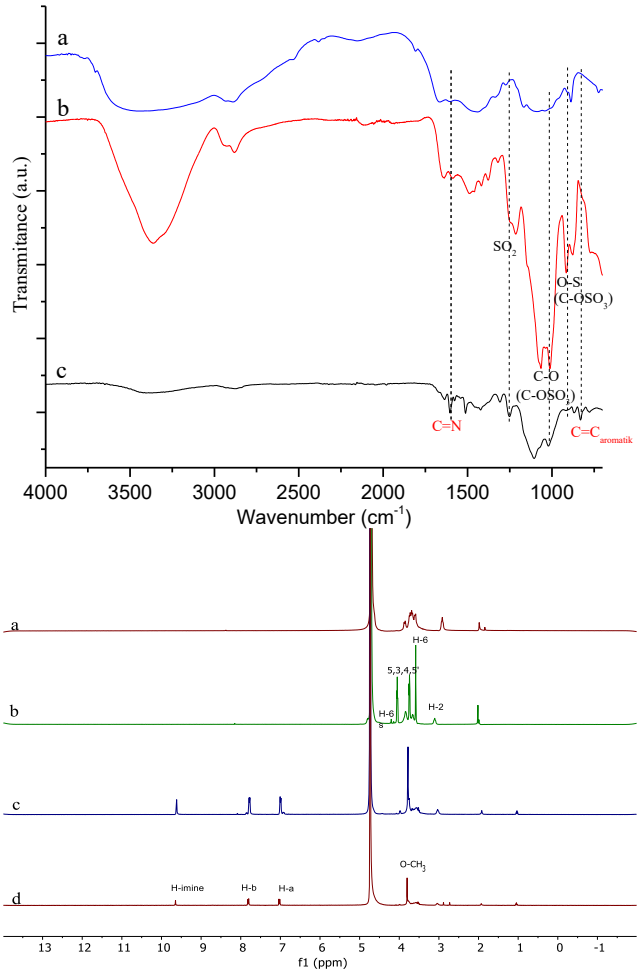

Figure 1. FTIR and 1H-NMR spectra of chitosan (a), O-sulfate-chitosan (b) and NAOS-chitosan (DS 96% anisimine (c) and DS 49% anisimine (d))

Table 1. Degree of substitution of amphiphilic chitosan derivatives.

Mol ratio of CHO : NH_2	Integration value				DS(%)
	$H_{3.05}$	$H_{7.08}$	$H_{7.81}$	$H_{9.65}$	
3 : 1	2.49	4.84	4.95	2.16	96
1 : 1	2.22	2.05	2.28	1.11	49

The result of micelar behaviour

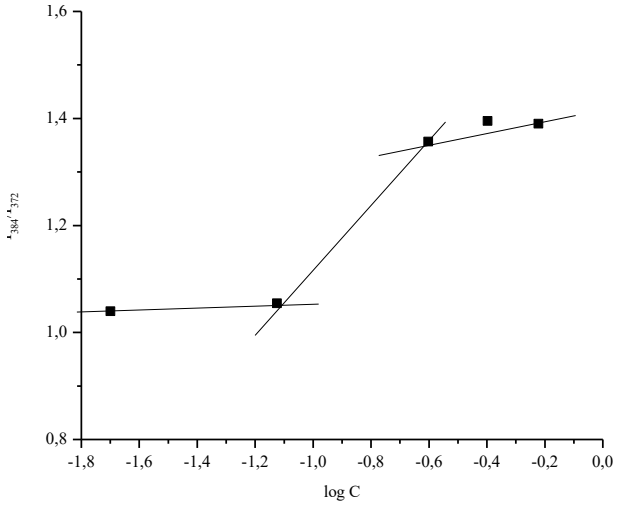

Figure 2. Intensity ratio plots of I384/I372 versus log C for NAOS-Chitosan (96% Degree of Substitution) in water

Discussion

The synthesis of the novel amphiphilic chitosan derivatives bearing sulfate and anisimine functional groups were achieved through a stepwise reaction pathway, as depicted in the reaction scheme (Figure 3). The first step involved the sulfation of chitosan using chlorosulfonic acid, which selectively reacted with the hydroxyl groups of the glucosamine units in the chitosan backbone. The introduction of sulfate groups ($–OSO_3^-$) into the polymer structure significantly enhanced its hydrophilic properties, making the modified chitosan more soluble and reactive in aqueous environments.

The presence of 20% sodium hydroxide (NaOH) played a crucial role in this step, acting to neutralize the reaction mixture and terminate the sulfation process. By reacting with excess chlorosulfonic acid, NaOH prevented over-sulfation and degradation of the chitosan backbone (Diab et al., 2012), ensuring the preservation of the polymer's structural integrity. This controlled reaction allowed the incorporation of sulfate groups while maintaining the desirable physical and chemical characteristics of chitosan. This sulfated intermediate served as a critical precursor for the subsequent functionalization step, providing the necessary hydrophilic balance to support amphiphilic behavior after the introduction of hydrophobic groups.

In the subsequent step, the sulfated chitosan was subjected to Schiff base formation by reacting with p-anisaldehyde in a dimethylformamide (DMF). This reaction facilitated the covalent attachment of the aromatic imine group via condensation between the primary amine groups of chitosan and the aldehyde functionality of p-anisaldehyde, yielding the desired imine bond (C=N). The aromatic group from p-anisaldehyde introduced hydrophobicity, resulting in an amphiphilic structure.

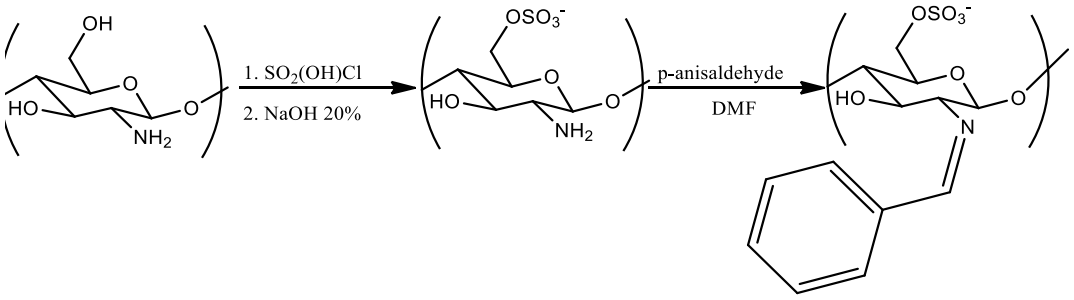

Figure 3. Reaction scheme of synthesis of NAOS-Chitosan

The Fourier-transform infrared (FTIR) spectra (Figure 1) provide critical evidence supporting the structural transformations at each step of the reaction. The characteristic absorption bands of chitosan were observed at 3400–3200 cm^{-1}, representing O–H and N–H stretching vibrations, and at ~1600 cm^{-1}, corresponding to N–H bending vibrations. The polysaccharide backbone was further identified through the C–O stretching peaks at ~1070 and ~1020 cm^{-1}. The introduction of sulfate groups was confirmed by the appearance of new absorption bands at ~1250 cm^{-1} and ~1025 cm^{-1}, assigned to S=O and C–O–S stretching vibrations, respectively. The reduced intensity of the O–H stretching band supports the substitution of hydroxyl groups by sulfate groups. The imination step was evidenced by the emergence of a new absorption band at ~1650 cm^{-1}, attributed to the C=N stretching vibration of the imine bond. Additionally, the presence of an aromatic C=C stretching band at ~1500 cm^{-1} confirms the incorporation of the aromatic p-anisaldehyde group. Importantly, the sulfate-specific bands at ~1250 cm^{-1} and ~1025 cm^{-1} remain visible, indicating the preservation of sulfation after Schiff base formation.

The structural changes were further validated by ^1H NMR spectroscopy, which provided insight into the chemical environment of the protons in the chitosan derivative. Signals corresponding to the glucosamine ring protons were identified, with H-1 appearing at ~5.0 ppm and the other protons (H-2 to H-6) resonating between 3.0–4.0 ppm. The sulfation step induced deshielding effects, resulting in the appearance of new signals at ~3.5 ppm, corresponding to protons adjacent to the introduced sulfate groups. The imine proton (C=N–H) was detected as a distinct signal at ~8.0–9.0 ppm, confirming the formation of the imine bond. The aromatic protons of the p-anisaldehyde group were observed as multiplets in the region of ~6.5–7.5 ppm, consistent with the structure of the attached aromatic group.

The NMR spectrum of the final product showed overlapping signals from both the sulfation and imination steps. The retention of sulfate-specific peaks and the presence of the aromatic imine signals confirm the successful synthesis of the amphiphilic chitosan derivative. The degree of substitution (DS) of chitosan derivatives was calculated using the integration of NMR peaks as shown in Equation (1). The equation quantifies the degree to which anisaldehyde groups have been grafted or chemically attached to the chitosan backbone. The numerator sums up the integral values of anisaldehyde-specific protons, indicating the amount of anisaldehyde bound to the chitosan. The denominator normalizes this value based on the reference signal of the chitosan backbone, ensuring that the calculation accounts for the number of available reactive sites (proton equivalent). The table shows the degree of substitution (DS) results for a chitosan derivative substituted with anisaldehyde. The results are presented for two different molar ratios of aldehyde to amino groups in chitosan. The data clearly show that increasing the molar ratio of anisaldehyde to amino groups enhances the degree of substitution, as evidenced by the higher DS value for the 3:1 ratio compared to the 1:1 ratio. This suggests that the availability of anisaldehyde molecules relative to reactive sites on Chitosan is a critical factor in determining the extent of substitution. The FTIR and NMR spectra provide conclusive evidence of the successful sequential modification of chitosan. The structural features introduced during sulfation and Schiff base formation are clearly reflected in the spectral data, supporting the chemical integrity of the final product. The presence of both hydrophilic sulfate and hydrophobic aromatic groups substantiates the amphiphilic nature of the synthesized derivative.

The amphiphilic behavior of NAOS-chitosan was evaluated by determining its critical micelle concentration (CMC), as shown in Figure 2. The CMC is the concentration at which amphiphilic molecules begin to self-assemble into micelles, a key property for applications in encapsulation and delivery systems. The graph for NAOS-chitosan shows a gradual increase in the intensity ratio (I_{338}/I_{372}) as the concentration increases. The intensity ratio remains constant at approximately -1.0. This suggests that NAOS-Chitosan molecules do not aggregate significantly in this region. The ability of NAOS-Chitosan to exhibit a sharp transition in intensity ratio confirms its amphiphilic nature. As concentration increases, hydrophobic interactions drive the formation of self-assembled structures. This trend indicates the transition from individual amphiphilic molecules to micelle formation. The distinct plateau in intensity ratio

at higher concentrations suggests the stability of these self-assembled structures, which is a hallmark of amphiphilic compounds.

The Critical Micelle Concentration (CMC) of approximately 0.1 mg/mL indicates promising potential for NAOS-Chitosan as a material in cosmetic or drug delivery applications. A low CMC indicates that micelle formation occurs at a relatively low concentration. The amphiphilic behaviour of NAOS-Chitosan (hydrophilic chitosan backbone and hydrophobic anisaldehyde modification) makes it ideal for encapsulating hydrophobic active ingredients (e.g., drugs, vitamins, essential oils) within micelles.

CONCLUSIONS

In this study, amphiphilic chitosan derivatives bearing sulfate and aromatic imine groups were successfully synthesized through a two-step functionalization process. The sulfation of chitosan and subsequent Schiff base formation with p-anisaldehyde were confirmed by FTIR and 1H NMR spectroscopy, which provided clear evidence of the incorporation of sulfate and aromatic imine (C=N) groups. These structural modifications enhanced the amphiphilic nature of the chitosan derivatives. The critical micelle concentration (CMC) study demonstrated that NAOS-chitosan forms micelles at low concentrations. This micellar behavior highlights the potential of the synthesized derivatives for encapsulating hydrophobic molecules, making them suitable for applications in drug or cosmetic delivery.

Acknowledgements: This research is supported by Direktorat Jendral Pendidikan Tinggi, Riset, dan Teknologi under Research Grant: Luaran Wajib Hibah Kemendiktisaintek Skema Penelitian Dosen Pemula (PDP) Tahun 2025 No. Kontrak 1483bc/IT9.2.1/PT.01.03/2025.

Authors' Contributions: Herayati & Deana Wahyuningrum designed the study. Herayati carried out the laboratory work. Damar & Indah analyzed the data. Herayati wrote the manuscript. All authors read and approved the final version of the manuscript

Competing Interests: The authors declare that there are no competing interests.

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