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Original Research Article

Linking Degree of Deacetylation to Coagulation Efficiency of Crustacean Waste Chitosan in Water Treatment

Sinardi[1]*, Ismail Marzuki[1], Asriadi[2], Andi Sry Iryani[1], Faisal Riza Basalamah[1], Andi Irsyam Mansyur[1], Hardimas Prayudi[3]

[1] Chemical Engineering, Faculty of Engineering, Universitas Fajar, Jalan Prof. Dr. H. Abdurrahman Basalamah 101, Panakkukang, Makassar, Sulawesi Selatan, Indonesia 90231

[2] Ministry of Maritime Affairs and Fisheries, Jalan Makmur Daeng Sitakka 129, Raya, Turikale, Maros, Sulawesi Selatan, Indonesia 90512

[3] School of Metallurgy and Environment, Central South University, No. 932 South Lushan, Yuelu, Changsha, Hunan, China, 410083

* Corresponding Author, email: sinardi@unifa.ac.id

Abstract

Turbidity removal remains challenging because stable colloids resist sedimentation, motivating the use of biodegradable coagulants such as chitosan derived from crustacean waste. However, an integrated quantitative linkage between the degree of deacetylation (DD), zeta potential evolution, and dose-dependent turbidity removal across pH is still limited for shell waste-derived chitosan. This study aimed to relate DD to the zeta potential behavior and coagulation efficiency of chitosan derived from green mussel, crab, and shrimp shells at pH 5, 7, and 9. At pH 7, shrimp-shell chitosan (DD 89.05%) achieved 98.38% turbidity removal at 190 mg/L, crab-shell chitosan (DD 87.64%) achieved 96.72% at 320 mg/L, and green mussel chitosan (DD 77.80%) achieved 95.08% at 300 mg/L [mean±SD, n=3]. The zeta potential shifted from −0.81 mV (untreated synthetic water) to +1.43 to +2.80 mV after dosing, indicating dominant charge neutralization with partial overcompensation. Overall, DD provides a practical screening descriptor for selecting locally sourced chitosan and optimizing the dose for sustainable turbidity control. Valorizing shell waste into effective coagulants may reduce the reliance on inorganic coagulants and support environmentally responsible water treatment practices. This study established an integrated DD zeta potential dose relationship across pH to explain and compare the coagulation performance of locally sourced shell-waste chitosan for turbidity removal.

Keywords: Chitosan; coagulation flocculation; crustacean waste; degree of deacetylation; turbidity removal; zeta potential

1. Introduction

Turbidity remains one of the main challenges in water treatment because fine colloidal particles suspended in water are highly stable and do not easily settle under the force of gravity. The decline in water quality in several Indonesian rivers, such as the Brantas River, has been linked to rising levels of suspended solids and dissolved organic matter caused by human activities and disturbances in surrounding watersheds (Masriyanto et al., 2023; Bowo et al., 2022). Both coagulation and flocculation processes influenced water clarification under these conditions. Conventional coagulants, such as alum and ferric sulfate, are popular options; however, residual metals, sludge production, pH dependency, and potential environmental problems are some of the issues that may drive the search for more sustainable options (Coleman et al., 2024).

Among natural coagulants, chitosan is an excellent candidate because it is biodegradable, low in toxicity, and is found in the shells of shrimp, crabs, and green mussels. Several studies have suggested that its coagulation mechanism occurs simultaneously through charge neutralization, polymer bridging, and hydrogen bond interactions (Gao et al., 2024; Velasco-Aguirre and Martínez, 2021). In this respect, the large amounts of crustacean shell waste produced in Indonesia can be used to extract chitosan, which will lead to beneficial environmental and economic effects and encourage sustainable water treatment.

The functionality of chitosan is mainly ascribed to the behavior of amino groups in aqueous systems. Under acidic conditions, the amino groups are protonated and positively charged, allowing them to interact with negatively charged colloids. However, the degree of protonation mainly depends on the degree of deacetylation and solution pH (Soros and Casanova, 2025; Nouj et al., 2021). Typically, a high DD corresponds to more amino groups; however, it is not always possible to predict coagulation performance based on the DD value alone, as particle destabilization can be affected by other factors, such as polymer conformation, molecular interaction, and solubility, which vary with pH (Velasco-Aguirre and Martínez, 2021; Gao et al., 2024).

Another coagulation parameter that has been extensively studied is the zeta potential, which represents the electrostatic state of suspended particles. Typically, approaching zero from the initial zeta potential is considered the onset of destabilization, which favors particle aggregation. In this regard, Soros and Casanova (2025) suggested that changes in zeta potential can be correlated with removal efficiency. However, the correlation between DD, zeta potential, and coagulation is rather complex, since chitosan with a very high DD is not always associated with the highest surface charge because the electrokinetic behavior may be affected by molecular rearrangement, and only a limited number of amino groups are exposed to the solvent. This is because chitosan with a very high DD is not always associated with the highest surface charge, as the electrokinetic behavior may be affected by molecular rearrangement, and only a limited number of amino groups are exposed on the surface. Several studies have demonstrated various ranges of DD in chitosan from different species, such as shrimp, crab, and green mussels (Kaewprachu et al., 2023); however, the information on DD in water treatment remains insufficient, especially when considering parameters such as zeta potential, dosage, and pH in one frame of reference. Therefore, this study evaluated the relationship between the DD of chitosan derived from three crustacean waste sources (green mussels, crabs, and shrimp) and coagulation efficiency.

Therefore, this study aimed to evaluate the relationship between the DD of chitosan derived from three crustacean waste sources (green mussels, crabs, and shrimp) and coagulation efficiency. Specifically, this study analyzed how DD influences zeta potential changes under different pH conditions and identified the optimal operating conditions (pH and dose) to maximize chitosan performance as a natural coagulant. The role of molecular weight in the coagulation mechanism was also examined, and the findings are expected to strengthen the scientific basis for using local biomaterials in sustainable water treatment technologies.

2. Methods

2.1 Chitosan Raw Materials and Preparations

Waste shells of green mussels (*Perna viridis*), crabs (*Portunus pelagicus*), and shrimp (*Penaeus monodon*) were obtained from local suppliers in Makassar City and cleaned using running water to remove any remaining soft tissue. The shells were dried at 60–70 °C for 24 h and then ground to pass through a 60-mesh sieve. Chitosan production involves three stages (demineralization, deproteinization, and deacetylation) following commonly used chitin extraction routes (Gao et al., 2024; Velasco-Aguirre and Martínez, 2021).

Demineralization was carried out by soaking the shell powder in 1–2 M HCl (ratio 1:15 w/v) for 2 h to dissolve $CaCO_3$ and other minerals. Deproteinization was carried out using a 1–2 M NaOH solution at 60-80 °C for 2 h. Deacetylation is performed by immersing chitin in concentrated NaOH (40-50%) at 100–120 °C for 1–2 h to produce medium-high DD chitosan (Soros and Casanova, 2025). The sediment was washed to a neutral pH and dried. All chitosan samples were stored under airtight conditions.

2.2 DD determination

Chitosan samples were analyzed by FTIR using Prestige 21 Shimadzu to calculate the ratio of absorbance of amide group I and free amines according to standard methods (Kaewprachu et al., 2023). The DD value was calculated using the Brugner equation. FTIR was performed on chitosan powder, and the typical absorption bands of the functional groups were analyzed.

The degree of deacetylation was determined using the baseline FTIR method based on the ratio of the absorbance of the amide I and hydroxyl bands. The absorbance (A) was calculated from the transmittance values, where P_0 represents the baseline transmittance (%) and P represents the transmittance at the minimum peak (%). The DD value was calculated using Equation (1), where A_{1655} corresponds to the absorbance at 1655 cm^{-1} associated with the amine group and A_{3450} represents the absorbance at 3450 cm^{-1} corresponding to the hydroxyl group. The constant value of 1.33 represents the absorbance ratio of fully deacetylated chitin (100%).

2.3 Chitosan Solution and Test Water Preparation

A 1% chitosan stock solution (10,000 mg/L) was prepared by dissolving chitosan in a 1% acetic acid solution (v/v) by stirring for 12 h. The solution was diluted to a working concentration of 1–50 mg/L. The test water was prepared using deionized water with bentonite/kaolinite added to achieve an initial turbidity of ± 70 NTU, as used in biomaterial coagulation studies (Nouj et al., 2021). Initial quality parameters (pH, turbidity, and temperature) were measured before the coagulation process. The pH variations were organized into three categories: acidic (pH 5), neutral (pH 7), and alkaline (pH 9) uso.1 M NaOH or HCl.1 M. The selection of pH variations was intended to evaluate the differences in the amine protonation levels of chitosan and their relationship with the resulting zeta potential values.

2.4 Flocculation Coagulation Procedure (Jar Test)

The flocculation-coagulation test was performed using a six-vessel jar test according to a standard protocol (Corina et al., 2021; Soros and Casanova, 2025). The mixture was stirred rapidly at 100 rpm for 1 min and then slowly at 30 rpm for 15 min. Sedimentation for 30 minutes without stirring

Chitosan doses tested for green mussel shells were 200, 250, 300, 350, and 400 mg/L; for crab shells, 160, 200, 240, 280, 320, 360, and 400 mg/L; and for shrimp shells, 70, 90, 110, 130, 150, 170, 190, 210, and 230 mg/L, representing the low-high dose ranges commonly used in biomaterial coagulation studies (Coleman et al., 2024; De-Paz-Arroyo et al., 2025). After sedimentation, the supernatant is carefully taken and the turbidity of the residue is measured using a nephelometer.

2.5 Zeta Potential Measurement and Electrokinetic Analysis

The potential zeta was measured using a Zetasizer Nano ZS (Malvern Instruments) at each pH and dose condition. Measurements were taken to assess changes in the surface charge of the aggregate particle, determine the isoelectric point (zeta = 0), relate DD to the amine group protonation pattern, and evaluate the effects of overdose. The analysis of potential zeta changes is essential to ascertain whether the dominant mechanism is charge neutralization or bridging flocculation (Velasco-Aguirre and Martínez, 2021; Soros and Casanova, 2025).

2.6 Data Analysis

The turbidity removal efficiency was calculated from the difference between the initial and final turbidity values after treatment. This study aimed to examine the relationship between DD, zeta potential, coagulation efficiency, dosage, and pH to determine the behavior of chitosan. Each experimental condition was run independently three times, and the results were reported as the means of the measurements with standard deviations to indicate variability and consistency.

To determine whether dosage affected the efficiency of turbidity removal, one-way analysis of variance (ANOVA) was applied independently to each type of chitosan at each pH. In this case, dosage was the factor, and turbidity removal efficiency was the dependent variable. A p-value of less than 0.05 was considered to indicate a statistically significant difference in the study.

3 Result and Discussion

Chitosan performance empirically during the coagulation process is primarily influenced by the physicochemical properties of chitosan, especially the degree of deacetylation (DD). DD describes the number of free amino groups in the polymeric chain. These polymeric amino groups can be protonated in an aqueous solution and subsequently participate in charge neutralization and destabilization of the particles during the coagulation process. Therefore, rather than conducting a full characterization of the chitosans used, the present work discusses DD as the main structural parameter that links the chitosan characteristics with the zeta potential behavior and efficiency of turbidity removal. This explains how the differences in the DD of the green mussel, crab, and shrimp shell chitosans influenced the coagulation performance under different pH values and dosages.

3.1 Effect of DD on Coagulation Efficiency

DD was determined by FTIR, where the relative intensity of amide group I (approximately 1655 cm^{-1}) to that of free amines (approximately 1595 cm^{-1}) was analyzed using the Brugner equation to quantify the conversion rate of acetyl groups to amines (Kaewprachu et al., 2023; Gao et al., 2024). Using the FTIR baseline method, the DD of chitosan prepared from green mussel shells, crab shells, and shrimp shells was 77.80, 87.64, and 89.05%, respectively. The calculated DD values were 77.80% (green mussels), 87.64% (crabs), and 89.05% (shrimp). **Supplementary Figures 1-3** provide more detailed experimental data and FTIR spectra. The results of the DD calculations for the three chitosan sources are shown in table below.

Table 1. Degree of deacetylation chitosan crustaceans

Crustasea	DD (%)
Green mussel shells	77.80
Crab shells	87.64
Shrimp shells	89.05

An increase in DD increases the number of amino groups ($-NH_2$) that can be protonated to $-NH_3$, which can hypothetically contribute to the destabilization of colloidal systems through charge neutralization. An increased DD indicates an increased number of cationic active sites, which increases charge neutralization and coagulation efficiency, particularly in negatively charged colloid systems. DD can also be used to structurally describe the coagulation and electrokinetic properties.

The authors suggested that the incremental availability of amino groups ($-NH_2$) for protonation to $-NH_3^+$ results in a higher potential for charge neutralization (Gao et al., 2024; Velasco-Aguirre and Martínez, 2021). Following a study by Nouj et al. (2021), higher charge densities of chitosan samples facilitated lower doses for initial coagulation to remove turbidity. However, Soros and Casanova (2025) suggested that DD can also impact the protonation pattern and play a role in the electrostatic process during coagulation. Thus, DD can affect coagulation performance through charge neutralization. Hence, analyses of the degree of deacetylation, coupled with performance data, can provide a mechanistic rationale for selecting appropriate crustacean waste sources for the development of sustainable coagulants.

3.2 Effect of pH and Chitosan Dosage on Turbidity Removal

The coagulation performance of chitosan was significantly influenced by both pH and dosage. The experimental results are provided in **Supplementary Table 1**. Coagulation experiments were conducted under acidic (pH 5), neutral (pH 7), and alkaline (pH 9) conditions to evaluate the effects of pH and dosage on turbidity removal by the three chitosan sources. Given that the state of protonation of the amino groups changes with pH, different coagulation behaviors were anticipated for each condition. The removal efficiencies based on the varying dosage levels are displayed in **Figures 1-3**.

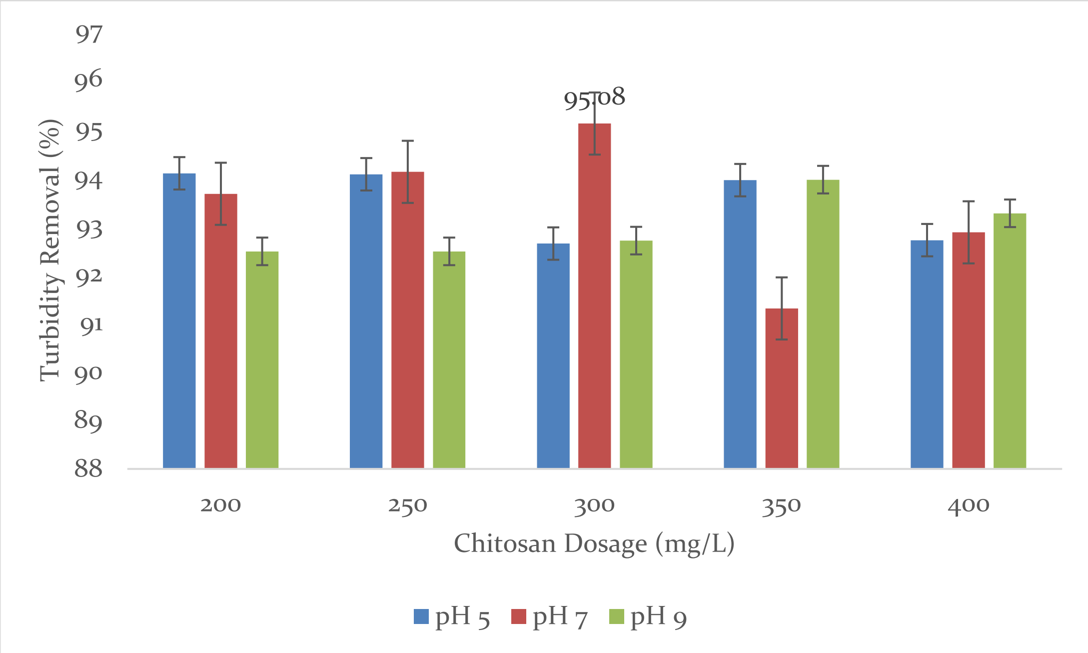

Figure 1. Turbidity removal (%) based on green mussel chitosan dose and pH

Chitosan from green mussels showed the best turbidity removal at pH 7, with an optimum dosage of 300 mg/L, showing 95.08% turbidity removal. Even with the lowest DD among the chitosan samples, particle removal was achieved at a neutral pH. This suggests that pH 7 represents an optimal compromise between the charge development and flexibility (or stiffness) of the polymer chains, thus allowing sufficient particle destabilization and floc formation. At dosages above the optimum level, no substantial improvement in the removal efficiency was observed, indicating that the addition of additional coagulants did not further enhance the treatment performance.

The performance of crab shell chitosan followed a similar trend, with the highest removal efficiency of 96.72% recorded at pH 7 and a dose of 320 mg/L. Compared with green mussel chitosan, the higher DD of crab chitosan may have contributed to stronger electrostatic interactions between the polymer and the suspended particles. Previous studies have reported that the fibrillar surface characteristics of crab-derived chitosan facilitate particle attachment and promote the formation of larger flocs (Kumar and Sharma, 2022). These factors may explain the improved removal efficiency observed under neutral pH conditions.

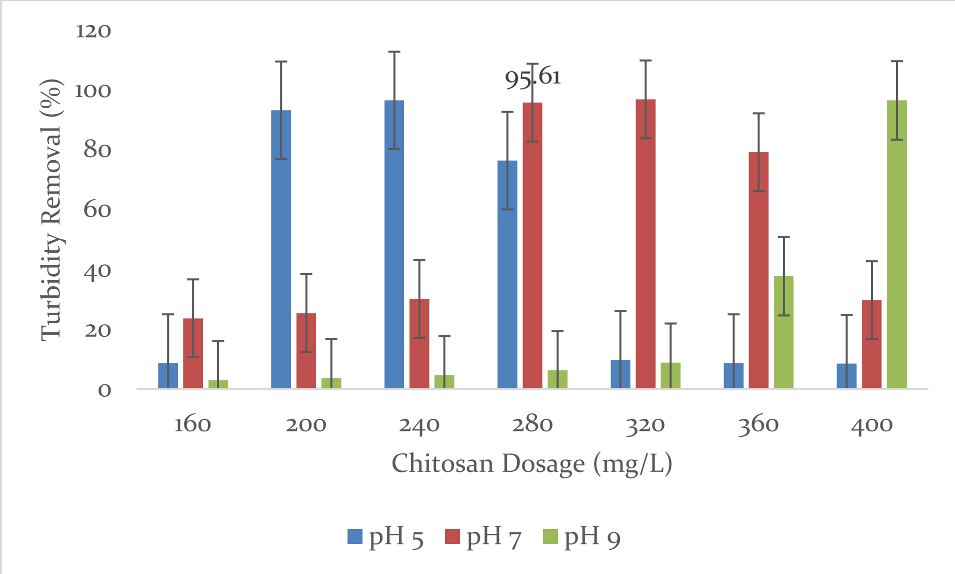

Figure 2. Turbidity removal (%) based on crab shell chitosan dose and pH

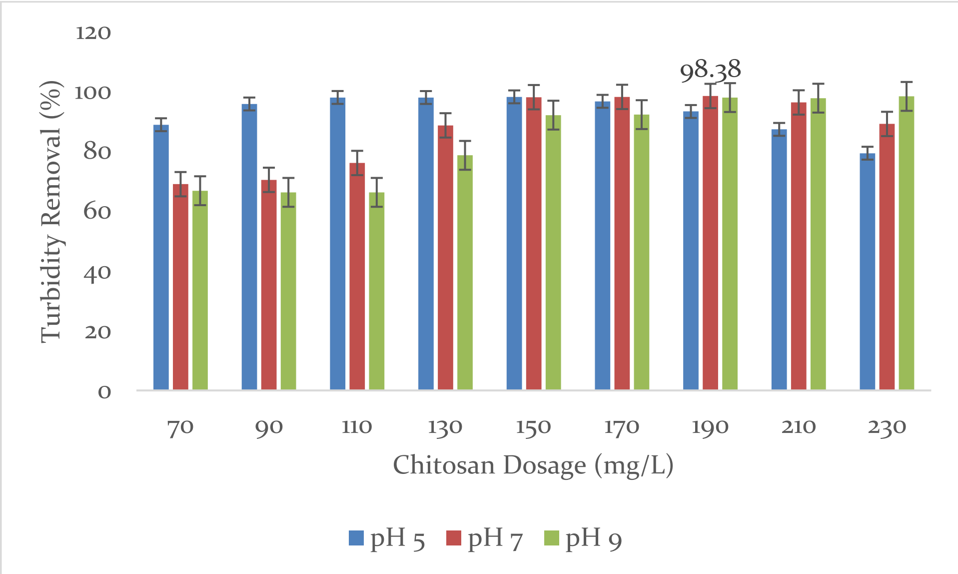

Figure 3. Turbidity removal (%) based on shrimp shell chitosan dose and pH

Among the evaluated materials, shrimp shell chitosan exhibited the best overall performance, achieving 98.38% turbidity removal at pH 7 with a dosage of 190 mg/L. Notably, this optimum efficiency was obtained at a substantially lower dosage than that required for crab and green mussel chitosan. This result is consistent with the higher DD of shrimp shell chitosan, which may increase the availability of amino groups involved in colloid destabilization. One of the benefits of using chitosan with a high degree of deacetylation (high DD) is that it can achieve a high removal efficiency at a low dosage.

The results indicate that coagulation behavior was primarily influenced by a combination of pH and dosage. Neutral pH consistently showed the highest removal efficiencies across the different chitosan sources, indicating that the optimal conditions for coagulation of suspensions were achieved at neutral pH. Similar findings have been observed in previous studies on chitosan-based coagulants (Nouj et al., 2021; Coleman et al., 2024).

The statistical analysis presented in **Supplementary Table 2** indicates that for shrimp and crab chitosan, turbidity removal was dosage-dependent, whereas for green mussel chitosan, this response was less pronounced. In acidic environments, excessive doses can negatively influence removal efficiency, indicating the possibility of destabilization of the particles due to excess positive charge. However, some removal efficiencies, even in an alkaline environment, showed that bridging and polymer entanglement can still play a role despite decreased protonation.

It should be emphasized that this study utilized synthetic turbid waters with an initial turbidity measurement of approximately 70 NTU. The absence of natural organic matter and competitive ions means that the optimal coagulation pH and dosage may differ for real-world applications. However, the trends identified may help elucidate the relationship between DD, pH, dosage, and coagulation, as well as help identify other crustacean-derived chitosan sustainability for water treatment.

3.3 Potential Zeta Relationships

The zeta potential values were measured at the respective optimal dose and neutral pH (pH 7).

Table 2. Zeta potential measurement results

Sample	Zeta Potential (mV)
Synthetic Water	-0.81
Synthetic Water + Chitosan Green Mussel Shell	+1.43
Synthetic Water + Chitosan Crab Shell	+2.65 mV
Synthetic Water + Chitosan Shrimp Shell	+2.80 mV

The untreated synthetic water had a zeta potential of -0.81 mV, indicating a slightly negative zeta potential and the availability of negatively charged particles. A distinct change in electrokinetic behavior was observed with the addition of chitosan. The zeta potential of green mussel chitosan increased to +1.43 mV, +2.65 mV for crab shell, and +2.80 mV for shrimp shell chitosan. The shift from a negative to a positive zeta potential indicates the destabilization of the particles due to the interaction of the colloidal particles with the positively charged sites of chitosan.

The shift to a more positive zeta potential can be attributed to the positively charged amino groups on the chitosan chains after protonation, which extended the electrostatic attraction to the negatively charged colloidal particles. Such behavior has also been observed in a coagulation system, where the dominant destabilization of colloidal particles is due to charge neutralization (Mroczko and Zimoch, 2019). The zeta potential shifts observed in this study provide further proof that chitosan modified the surface charge of the colloid in the suspension and induced floc formation.

The disparity in the zeta potential of the chitosan samples appeared to correlate with the varying degrees of DT. The highest DT (89.05%) was obtained with shrimp shell chitosan, with the highest positive zeta potential of +2.80 mV, indicating its greater capacity to protonate the amino groups in the polymer structure. The increased positive zeta potential most probably enhanced its interaction with negatively charged colloidal particles, thereby destabilizing them. A similar trend in coagulation behavior and zeta potential has been reported in the literature (Velasco-Aguirre and Martínez, 2021; Soros and Casanova, 2025).

The electrokinetic and coagulation test results exhibited a similar trend for all materials. Shrimp shell chitosan exhibited the highest positive zeta potential and turbidity removal efficiency. It achieved 98.38% turbidity removal at pH 7 with a 190 mg/L dosage. The results suggest that the positively charged remnant sides may have complemented the stronger interactions with the suspended matter. The relatively low dosage indicates that floc formation may have developed with a relatively greater efficiency compared to crab chitosan and green mussel chitosan.

Crab chitosan, with an intermediate DD of 87.64%, produced a potential zeta of +2.65 mV, which was slightly lower than that of shrimp chitosan but still showed a predominance of positive charges. This value was sufficient to achieve a destabilizing condition in the system and produce a large well-deposited floc. The alignment between the potential zeta values and coagulation efficiency is evident, as crab chitosan achieves an optimum turbidity removal of 96.72% at pH 7 and a dose of 320 mg/L. Higher optimum doses than shrimp chitosan reinforce the understanding that a lower DD requires a larger amount of polymers to achieve an equivalent level of protonation and neutralization of charge. This is consistent with the results of Nouj et al. (2021) and Coleman et al. (2024), who showed that chitosan with intermediate DD requires higher doses to achieve a zeta potential close to zero and a stable floc.

In contrast, green mussel chitosan had the lowest DD (77.80 %) among the three types of chitosan, indicating a much smaller number of protonated amine groups. This is reflected in the potential zeta value, which only increased to +1.43 mV, which is much lower than that of shrimp and crab chitosan. A smaller positive charge indicates that the ability of green mussel chitosan to neutralize negatively charged particles is also more limited. This condition explains why green clam chitosan requires a much higher dose (300 mg/L) to achieve an optimum clearance efficiency of 95.08% at pH 7. This efficiency remains high but is achieved with larger doses and charge changes that are not as strong as those of high DD chitosans. This conclusion is in line with the report of Kaewprachu et al. (2023), which states that chitosan from green shellfish tends to have a denser structure and lower DD, and thus, performs lower than chitosan from other crustaceans such as shrimp and crabs.

The overall data showed a direct comparison: the higher the DD, the greater the positive zeta potential value, the higher the coagulation efficiency, and the lower the optimal dose. This phenomenon is in line with the theory of electrokinetics and polymer coagulation, which states that the most efficient destabilization of colloidal systems occurs when positively charged polymers bring the system close to the isoelectric point while forming an interparticle bridge that produces large flocs (Kumar and Sharma, 2022). Thus, the relationship between DD, zeta potential, and coagulation efficiency can be used as a scientific basis for the selection of chitosan types for water treatment applications, especially when considering local biomaterial sources.

4. Conclusions

The three crustacean chitosan samples exhibited different coagulation performances. The sample with the highest DD, that is, shrimp shell chitosan, was the most efficient at removing turbidity and had the lowest optimum dosage. The other tested materials exhibited similar trends in their mechanical properties. The same tendency was observed for the zeta potential measurements, where a higher DD resulted in more positively charged surfaces and, therefore, better destabilization of the particles.

Overall, a complete analysis of several chitosan characteristics, such as DD, zeta potential, pH, and dosage, contributes to a better understanding of the mechanisms that govern the coagulation achieved with this type of polymer. These findings show that DD is a relevant factor that can be employed to predict the coagulation behavior of chitosans, thereby helping to identify the proper types of shell wastes for the production of chitosan-based coagulants.

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Ethics Statement

This study did not involve human participants or live animals. All experiments were conducted using non-living crustacean shell waste and synthetic water samples under controlled laboratory conditions. Therefore, no ethical approval was required. All procedures followed standard laboratory safety and environmental regulations.

CRediT Author Statement

Sinardi contributed to idea development, research design, formal analysis, investigation, data curation, and manuscript draft preparation. **Ismail Marzuki** contributed to research design, supervision, and manuscript review and revision. **Asriadi** contributed to resources of crustacean shell waste, data collection, and investigation. **Andi Sry Iryani** contributed to laboratory analysis, data curation, and investigation. **Faisal Riza Basalamah** contributed to data analysis, visualization, and manuscript review. **Andi Irsyam Mansyur** contributed to investigation, data validation, and technical support. **Hardimas Prayudi** contributed to formal analysis, and manuscript review and editing.

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