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REVIEW

Chitosan/Alginate-Based Nanoparticles for Antibacterial Agents Delivery

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Abstract: Nanoparticle systems integrating alginate and chitosan emerge as a promising avenue to tackle challenges in leveraging the potency of pharmacological active agents. Owing to their intrinsic properties as polysaccharides, alginate and chitosan, exhibit remarkable biocompatibility, rendering them conducive to bodily integration. By downsizing drug particles to the nano-scale, the system enhances drug solubility in aqueous environments by augmenting surface area. Additionally, the system orchestrates extended drug release kinetics, aligning well with the exigencies of chronic drug release requisite for antibacterial therapeutics. A thorough scrutiny of existing literature underscores a wealth of evidence supporting the utilization of the alginate-chitosan nanoparticle system for antibacterial agent delivery. Literature reviews present abundant evidence of the utilization of nanoparticle systems based on a combination of alginate and chitosan for antibacterial agent delivery. Various experiments demonstrate enhanced antibacterial efficacy, including an increase in the inhibitory zone diameter, improvement in the minimum inhibitory concentration, and an enhancement in the bacterial reduction rate. This enhancement in efficacy occurs due to mechanisms involving increased solubility resulting from particle size reduction, prolonged release effects, and enhanced selectivity towards bacterial cell walls, stemming from ionic interactions between positively charged particles and teichoic acid on bacterial cell walls. However, clinical studies remain limited, and there are currently no marketed antibacterial drugs utilizing this system. Hence, expediting clinical efficacy validation is crucial to maximize its benefits promptly.

Keywords: alginate, chitosan, polymeric nanoparticle, antibacterial agents, drug delivery, controlled release, nano-delivery systems

Introduction

One of the primary challenges encountered by drug molecules, particularly newly developed ones, is their limited solubility. Studies indicate that nearly 40% of newly discovered chemical entities exhibit low solubility in water.[1,2] This low solubility adversely affects their bioavailability, resulting in restricted absorption by the body.[3] Consequently, drugs often need to be administered in high doses to achieve the desired therapeutic effect.[4] A concrete example of this problem can be found in drugs classified as Biopharmaceutics Classification System (BCS) class II and IV.[5] Class II drugs exhibit low solubility but high permeability, whereas class IV drugs display low solubility and permeability.[6] Such circumstances present a significant challenge in the development of effective and efficient drug formulations.

Among the many drug candidates with solubility issues in water, antibacterial agents are the most affected. For instance, the use of marketed antibiotics, such as amoxicillin, faces challenges due to its low solubility in water. This limits the oral use of amoxicillin, as the reconstitution process with water is restricted to 150 mg/mL. With the need for higher doses, clinicians generally prefer intravenous injection, which poses issues regarding patient comfort and increases healthcare facility costs.[7] In addition, curcumin, a prominent example characterized by low water solubility,

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measuring a mere 11 ng/mL.[8] This inherent limitation translates to poor bioavailability of curcumin, whether administered orally or topically.[9] Furthermore, curcumin is prone to rapid degradation during distribution processes.[10,11] Consequently, despite the minimal amount of the drug that reaches the bloodstream, its swift elimination significantly limits its effectiveness in treating diseases.

The insufficient effectiveness of antibacterial drugs often serves as a major trigger for bacterial resistance.[12] Resistant bacteria have the ability to adapt and evade the effects of antibacterial drugs.[13] Compounding this issue, approximately 70% of the existing antibacterial agents have already encountered resistance.[14] While the main cause of this resistance is generally attributed to patient non-compliance with medication instructions,[15] the root of the problem may also lie in the complexity of dosing and frequency of drug intake due to the low bioavailability of the drug.[16,17] These findings underscore the need for innovative breakthroughs to address the multifaceted challenges surrounding antibacterial agents, whether they are still in the developmental stage or already available on the market. To address these critical solubility and resistance challenges, nanotechnology offers targeted solutions through enhanced drug delivery mechanisms.

Nanotechnology emerges as a leading solution to address the challenge of low drug solubility in water.[18–21] Among the array nano-delivery systems available, polymeric nanoparticles stand out with additional advantages. Beyond enhancing drug solubility, polymeric nanoparticles provide excellent stability during storage and distribution through the bloodstream.[22] The optimal drug absorption capacity within the polymer matrix enables sustained release of the drug.[23] Notable examples of drugs already leveraging polymeric nanoparticles in the market include Abraxane (paclitaxel encapsulated in nano-albumin), Zilretta (triamcinolone acetonide dispersed in PLGA hydrogel), and Taxotere (docetaxel encapsulated in PLGA micelles).[24–26] However, it's crucial to acknowledge that the widespread application of polymeric nanoparticles in antibacterial agents remains limited, leading to a gap in the development of antibacterial drugs using this technology until they attain marketable status.

Natural polymers are generally preferred in their application to form polymeric nanoparticle systems.[22] Their main advantage lies in their biodegradable nature, minimizing the risk of toxicity within the body.[27] Among biodegradable polymers, chitosan and alginate emerge as preferred choices due to their facile solubility in water, simplifying the manufacturing process.[28] Moreover, they sourced from abundant and unused materials, with chitosan derived from crustacean shells like shrimp and crab, and alginate extracted from brown algae.[29,30] The use of alginate for drug encapsulation in nanoparticle systems began in the 1980s, while chitosan-based nanoparticles were first developed for delivering the antitumor agent 5-fluorouracil in 1994.[31,32] Progress continued, leading to the development of nanoparticles combining both materials in 2004 by Douglas et al for gene delivery as non-viral transfection agents.[33]

In terms of their chemical properties, alginate and chitosan possess pH-sensitive release properties. Alginate, with its carboxyl groups (COO^-), undergoes protonation under acidic conditions, causing it to contract, and swells under basic conditions due to the exchange of Ca^{2+} ions with Na^+.[34] Conversely, chitosan exhibits opposite behavior due to its amine groups along its polymer chain. The protonation of amine groups in chitosan under acidic conditions causes it to swell, enabling drug release at that pH.[35] The combination of both materials can be advantageous for controlling drug release at specific pH levels by adjusting the ratio of alginate to chitosan used. The promising potential of alginate and chitosan-based polymeric nanoparticles in addressing problems without creating new issues is evident. This review endeavors to provide a comprehensive overview of the evidence-based development of alginate and chitosan-based polymeric nanoparticles for delivering antibacterial agents. It is hoped that this review will catalyze continued development until these formulations evolve into marketable products.

Method

This present study conducted a literature review, by utilizing Google Scholar, PubMed, and Scopus databases. Keywords such as "alginate", "chitosan", "nano", "nanoparticle", "nanoformulation", "nanodrug", "nano-delivery systems", "controlled release", "antibacterial", "antimicrobial", "antimicrobe", and "antibacterial" were employed to ensure comprehensive search. The search was performed without any time restrictions to maximize the inclusivity of the review. Selection criteria were established, delineating specific inclusion and exclusion criteria. Inclusion criteria comprised literature focusing on polymeric nanoparticle development utilizing a combination of chitosan and alginate, employing

antibacterial agents as loaded drugs, and conducting antibacterial activity evaluations. Exclusion criteria encompassed review papers, case reports, case series, editorial letters, and publications lacking full-text availability.

Chitosan and Alginate as Basis for Nanoparticle Delivery System

Polymeric Nanoparticle

Polymeric nanoparticles, an innovation in drug delivery, offer a revolutionary solution with extremely small particle sizes ranging from 1 to 1000 nm.[36] Divided into two main categories, nanospheres and nanocapsules, these types of nanoparticles combine the advantages of polymers in drug delivery with the sophistication of nano-technology.[37,38] Nanocapsules, as one type, involve the formation of a polymeric membrane encapsulating drugs on a nano scale.[39] Meanwhile, nanospheres involve the formation of a polymeric matrix in which drugs are dispersed both molecularly and on a nano scale within the matrix.[40] The process of forming polymeric nanoparticle systems occurs through a self-assembly mechanism, where polymer chains cross-link independently to form the desired nanoparticle structure (Figure 1).[38] This enables the nanoparticles to exhibit controlled release kinetics and targeted delivery, addressing limitations of conventional drug delivery systems.[41] The versatility and tunability of polymeric nanoparticles make them promising candidates for various biomedical applications, including drug delivery, imaging, and diagnostics.[23] Through continuous advancements in polymer science and nanotechnology, polymeric nanoparticles hold significant potential to revolutionize modern medicine by enhancing therapeutic efficacy, reducing side effects, and improving patient outcomes.[42]

Polymeric nanoparticles emerged historically as a solution to the challenge of drug bioavailability.[23] Drugs, typically organic compounds, often struggle with solubility in water, complicating their effective delivery.[1] Polymers offer a solution due to their high molecular weight, enabling the creation of hollow structures capable of encapsulating drugs.[43] Engineered to improve drug solubility, polymeric nanoparticles are crafted to transport drugs more efficiently to target sites in higher concentrations.[44] This improved solubility stems from the increased surface area resulting from the reduction in particle size.[45] By leveraging polymer properties and principles of nanotechnology, polymeric nanoparticles represent a promising approach to overcoming the limitations of traditional drug delivery systems, eventually enhancing therapeutic outcomes. Their ability to increase drug solubility and target specific areas holds significant potential for advancing medical treatments, particularly in areas where conventional methods fall short. As research in this field progresses, polymeric nanoparticles are poised to revolutionize drug delivery, offering new avenues for improving patient care and treatment efficacy.

Polymeric nanoparticles stand out among various nano-delivery systems due to their numerous advantages. These nanoparticles leverage intermolecular interactions between drugs and polymers for absorption, enabling sustained release

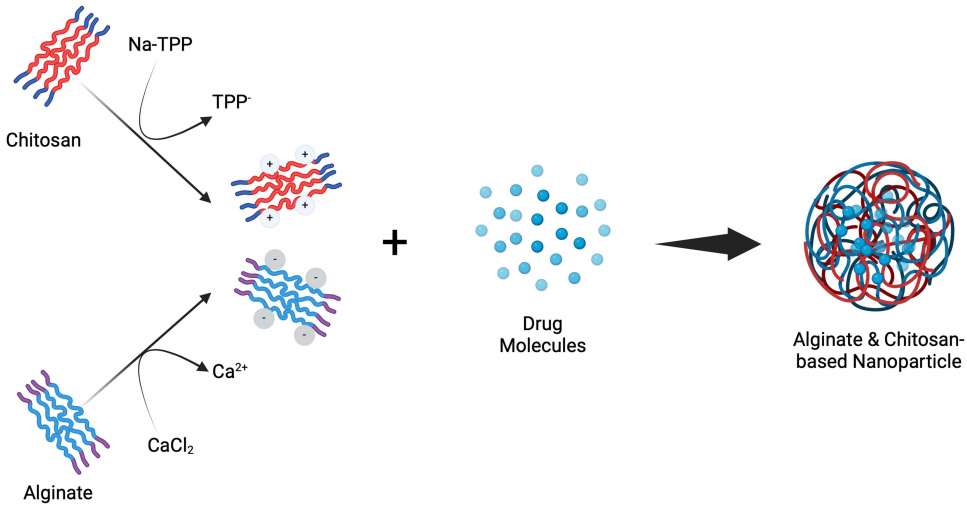

Figure 1 The mechanism of forming alginate and chitosan-based nanoparticles through chemical cross-linking involves several steps. Created with BioRender.com.

capabilities.[46] Within polymeric nanoparticle systems, drug release can be finely tuned for specific durations, enhancing therapeutic outcomes. Moreover, various polymers exhibit unique physical properties.[47] For instance, polymethacrylic acid, chitosan, dextran, and alginic acid are known for their high pH sensitivity, allowing for controlled drug release at specific pH levels.[48] Additionally, the production process for polymeric nanoparticles is relatively straightforward; stabilizers are not required as the polymer itself serves this function.[49] This inherent simplicity streamlines manufacturing processes and significantly reduces production costs, rendering polymeric nanoparticles an appealing option for drug delivery applications. With their unparalleled versatility, precise control over drug release kinetics, and ease of production, polymeric nanoparticles offer a promising avenue for advancing targeted drug delivery systems. By harnessing the capabilities of polymeric nanoparticles, researchers can explore innovative approaches to drug delivery, ultimately improving patient outcomes and expanding the possibilities of medical treatment.

Chitosan and Alginate in Developing Nanoparticle

The use of natural polymers presents significant appeal due to their ease of acquisition and relative avoidance of toxicity issues.[50] Among these, chitosan stands out as a linear polysaccharide family composed of varying amounts of (β1→4)-linked residues of N-acetyl-2-amino-2-deoxy-D-glucose (glucosamine, GlcN), and 2-amino-2-deoxy-D-glucose (N-acetyl-glucosamine, GlcNAc) residues.[51] Chitosan exhibits solubility in aqueous acidic media through primary amine protonation and is the sole natural polycation, with its charge density, governed by the degree of acetylation and pH of the medium.[52] Furthermore, alginate, another natural polymer sourced from algae, emerges as a promising material due to its abundant presence in nature. Comprising (1,4)-linked β-d-mannuronic and α-l-guluronic acids, alginates forms diverse structures, molecular weights, and physicochemical properties, driven by homogeneous (MM or GG) and heterogeneous (MG or GM) blocks.[53] As an anionic polymer, alginate also demonstrates adequate capability in forming polymeric matrices sensitive to pH, similar to chitosan.[54] These natural polymers offer versatile platforms for various applications, including drug delivery, owing to their inherent properties and environmental sustainability.

The synthesis of nanoparticle systems from chitosan and alginate involves cross-linking processes (as depicted in Figure 1).[55,56] Chitosan, with its polycationic nature, features NH_3^+ groups along its polymer chain, enabling them to bind to cross-linking agents, typically polyanions such as tripolyphosphate ions.[57] In contrast, alginate, cross-links through interaction with bivalent cations like calcium, presenting a contrasting mechanism to chitosan.[54] This cross-linking event results in the formation of interconnected structures from polymers, giving rise to nano-sized cavities capable of entrapping drugs.[58] This robust cross-linking process leads to the development of stable nanoparticle structures with enhanced drug encapsulation and controlled release properties, making chitosan and alginate promising candidates for drug delivery applications.[59]

In addition to their inherent biocompatibility advantages, both chitosan and alginate stand out for their distinctive benefits compared to other polymer types. Chitosan, with its polycationic chains, excels in enhancing specificity in drug delivery to negatively charged surfaces, such as bacterial cell walls.[57] While inherently insoluble in water, chitosan can dissolve under acidic conditions, making it invaluable for drug release in acidic environments.[60] However, the utilization of chitosan's release profile varies significantly depending on polymer chain modifications, which can be easily performed.[61] On the other hand, alginate not only exhibits pH-dependent release capabilities but also boasts high mucoadhesive properties.[62] This additional advantage enables efficient drug adhesion in topical applications on mucosal surfaces, further enhancing drug delivery efficacy. Consequently, both chitosan and alginate offer versatile options for drug delivery systems, each with unique properties that can be tailored to specific therapeutic needs, rendering them invaluable assets in pharmaceutical research and development.

However, despite their numerous advantages, the utilization of natural polymers often comes with inherent limitations, with stability emerging as a primary concern in polysaccharide applications.[63] Specifically, Chitosan tends to exhibit aggregation due to its inherent ionic instability.[64] This challenge can be mitigated by combining chitosan with other polysaccharides. In this context, alginate emerges as a compatible partner, given the contrasting nature of chitosan's polycationic properties and alginates anionic characteristics.[65] Leveraging the difference in their pH dissolution tendencies becomes advantageous for forming a stable composite system with a tunable release profile based on their composition ratio.[66] The complementary nature of their utilization ultimately yields a synergistic effect in nano-scale

drug delivery, where the drawbacks of one polymer are compensated by the strengths of the other, resulting in enhanced overall performance and efficacy.

Preparation Methods in Developing Chitosan/Alginate-Based Nanoparticles

Ionic Gelation Method

The ionic gelation method is widely employed in producing nanoparticles using alginate or chitosan as the polymer matrix. Nanoparticle formation arises from the interaction between positively and negatively charged entities. Alginates, as negatively charged polymers, engage with positively charged polymer cations, typically calcium (Ca^{2+}), to establish cross-links.[67] Conversely, chitosan, a cationic polymer, interacts with polyanions such as sodium tripolyphosphate (Na-TPP) to form cross-links.[68] The presence of cross-linking agents induces a sol-gel transition within polymer, leading to nanoparticle formation. Through this method, nanoparticles with precise control over size and properties can be crafted, enabling tailored applications in various fields such as drug delivery, tissue engineering, and food technology.[69] This approach offers distinct advantages in scalability, reproducibility, and versatility, positioning it as a preferred choice for nanoparticle synthesis. Additionally, the ability to manipulate parameters like polymer concentration, cross-linker type, and reaction conditions allows fine-tuning of nanoparticle characteristics, facilitating the design of materials with specific functionalities and performance attributes.[67]

The ionic gelation method offers distinct advantages over other chitosan and alginate-based nanoparticle fabrication techniques. Its relative simplicity, devoid of sophisticated equipment and organic solvents, coupled with shorter processing times, renders it exceptionally efficient. Moreover, optimizing the polymer-to-drug ratio can enhance drug encapsulation efficiency within the formed nanoparticles.[70] Additionally, the reversible nature of ionic interactions reduces the potential for toxicity compared to covalent interactions.[69] However, this reversibility also poses challenges, as it compromises the mechanical stability of the nanoparticles. To address this drawback, increasing the degree of ionic interaction through the modification of polymer functional groups to introduce more ionic charges emerges as a viable strategy.[68] By enhancing the extent of ionic interactions, the stability and performance of nanoparticles can be improved, thereby overcoming this limitation and further advancing the applicability of the ionic gelation method in drug delivery and related fields.

Polyelectrolyte Complexation Technique

Nanoparticle fabrication based on chitosan and alginate is facilitated through the polyelectrolyte complexation technique, offering a straightforward approach to synthesis. This method capitalizes on electrostatic interactions to induce cross-linking within the polymer matrix, akin to the principles underlying ionic gelation.[71,72] Through this technique, cross-linking occurs via ionic interactions, leading to the formation of stable nanoparticles. Notably, these ionic interactions can occur between polymers themselves, between polymers and drugs, or within the polymer-drug-polymer complex.[71] In the context of alginate and chitosan utilization, ionic interactions occur between the two polymers due to their opposite charges—alginate being negatively charged and chitosan positively charged.[73] This electrostatic attraction fosters the formation of stable nanoparticles.

The polyelectrolyte complexation technique offers a simpler alternative to the ionic gelation method, notably eliminating the need for organic solvents. This simplicity, coupled with the ability to fine-tune nanoparticle properties by adjusting parameters such as polymer composition and drug loading, enhances its appeal for drug delivery applications. Its streamlined nature is evident as it eliminates the requirement for cross-linking agents, thereby optimizing material usage.[71] However, the absence of cross-linking agents does entails certain drawbacks. One significant consequence is the compromised mechanical stability of the resulting nanoparticles compared to those produced by ionic gelation. This instability arises from the relatively immobile polymer-polymer interactions, often hindered by steric effects.[74] To address this limitation, the use of polymers with shorter monomer chains emerges as a viable option for nanoparticle manufacturing using this technique. By employing shorter monomer chains, the mobility of the polymers is enhanced, leading to improved mechanical stability of the nanoparticles.[74,75] Despite these drawbacks, the

polyelectrolyte complexation technique remains a viable option for nanoparticle synthesis, offering simplicity and versatility in material selection and fabrication processes.

Ionic Gelation Method Combined with Polyelectrolyte Complexation Technique

The combination of both methods has been developed to address their respective limitations effectively. The process commences with the formation of alginate nanoparticles via the ionic gelation method. Alginate undergoes cross-linking through calcium ions (Ca^{2+}), inducing a sol-gel transition.[76] Subsequently, chitosan is introduced using the polyelectrolyte complexation technique. The presence of chitosan complements the unbound anionic functional groups in alginate, enhancing nanoparticle stability. By integrating these techniques, the resulting nanoparticles benefit from the mechanical stability conferred by ionic gelation and the simplicity of material usage and fabrication offered by polyelectrolyte complexation.[77] This synergistic approach enables the production of nanoparticles with improved properties suitable for various applications, including drug delivery and tissue engineering. Moreover, the versatility of this combined method allows for fine-tuning of nanoparticle characteristics, ensuring compatibility with specific biomedical requirements.[78] In summary, the integration of ionic gelation and polyelectrolyte complexation stands as a promising strategy for overcoming the inherent limitations of each technique, advancing nanoparticle synthesis towards enhanced efficacy and versatility.

Ionic Gelation Method Combined with Emulsification Technique

The combined use of ionic gelation and emulsification techniques is often employed for the encapsulation of oil-based active pharmaceutical ingredients, such as essential oils.[79] Alginates undergo sol-gel transition facilitated by calcium ion cross-linkers, complemented by emulsification with stabilizers like Tween 80 to form micelles.[80] The addition of chitosan in the final stage aims to enhance the stability of the formed micelles. This approach offers an effective strategy for encapsulating oil-based drugs, ensuring improved stability and controlled release properties for pharmaceutical applications.

However, this method tends to be relatively costly and time-consuming due to the need for multiple additives and sequential processing steps. Moreover, the emulsification process often requires the use of organic solvents like dichloromethane to disperse the oil into the stabilizer, potentially leading to residual organic solvent in the formed particles.[69] Nevertheless, nanoparticles produced through this combined approach exhibit excellent physical stability, owing to the role of the added stabilizers during the emulsification process.[80,81] Despite its drawbacks, the utilization of both techniques offers a promising strategy for achieving enhanced stability and controlled release properties in drug-loaded nanoparticles.

Layer-by-Layer Self-Assembly Technique

The Layer-by-Layer (LbL) self-assembly technique is employed to coat nanoparticles using polyelectrolyte polymers.[82] Typically utilized in drug delivery systems, it accommodates a wide array of active pharmaceutical ingredients, including inorganic substances and coarse nanosuspensions.[83] Encapsulated nanoparticles are obtained by gradually adding polyelectrolyte polymers such as chitosan and alginate alternately drop by drop through titration.[84] The layering process can be repeated multiple times, depending on the desired nanoparticle size. This method offers precise control over nanoparticle properties, making it suitable for tailored drug delivery applications with enhanced efficacy and stability.

In addition to providing advantages in physical stability, the Layer-by-Layer (LbL) self-assembly technique offers relative safety from potential toxicity by circumventing the need of organic solvents.[85] Through the layering process, drug release kinetics can be precisely tailored by adjusting the thickness and number of layers as needed to meet specific requirements. Despite its benefits, the method is inherently time-consuming due to the meticulous drop-by-drop layering process.[86] Furthermore, while the physical stability increases with each added layer, careful consideration is required regarding the impact on particle size increment, ensuring it aligns with the desired application requirements for effective drug delivery and biomedical applications.

Factors Affecting the Stability of Chitosan/Alginate-Based Nanoparticles

There are several factors that can influence the stability and effectiveness of a nanoparticle system based on a combination of alginate and chitosan. As depicted in Figure 2, there are six main factors that significantly affect the stability of the nanoparticle system produced. Below is a detailed explanation of their respective influences and strategies that can be used to address them.

pH

Alginates and chitosan require ionization processes to form polyelectrolyte complexation, where the strength interaction is significantly influenced by the environmental pH.[87] This presents a significant challenge, given that alginate ionizes in basic environments, while chitosan ionizes in acidic conditions. Thus, achieving optimal animalization competitions is imperative to ensure effective nanoparticle formation. Ionization occurs when the pH exceeds the pKa for acids and falls below the pKa for bases.[88] The pKa of alginate typically ranges between 3.4 to 4.4, while that of chitosan is approximately 6.5.[58,89] Consequently, the optimal pH conditions for forming alginate/chitosan-based nanoparticles lie between the pKa values of alginate and chitosan, facilitating effective polyelectrolyte interaction and stable nanoparticle formation.

Organic Solvents

The utilization of organic solvents is commonly employed in processes requiring co-solvency. Organic solvents play a crucial role in aiding the dispersion of drugs with extremely low solubility in water during the bottom-up nanoparticle fabrication process.[90] While these solvents typically evaporate readily, even at ambient temperatures, the potential for leaving behind residues cannot be overlooked. Such residues may pose negative implications for the toxicity profile of the resultant formulations. To address this concern, before opting for methods necessitating the use of organic solvents,

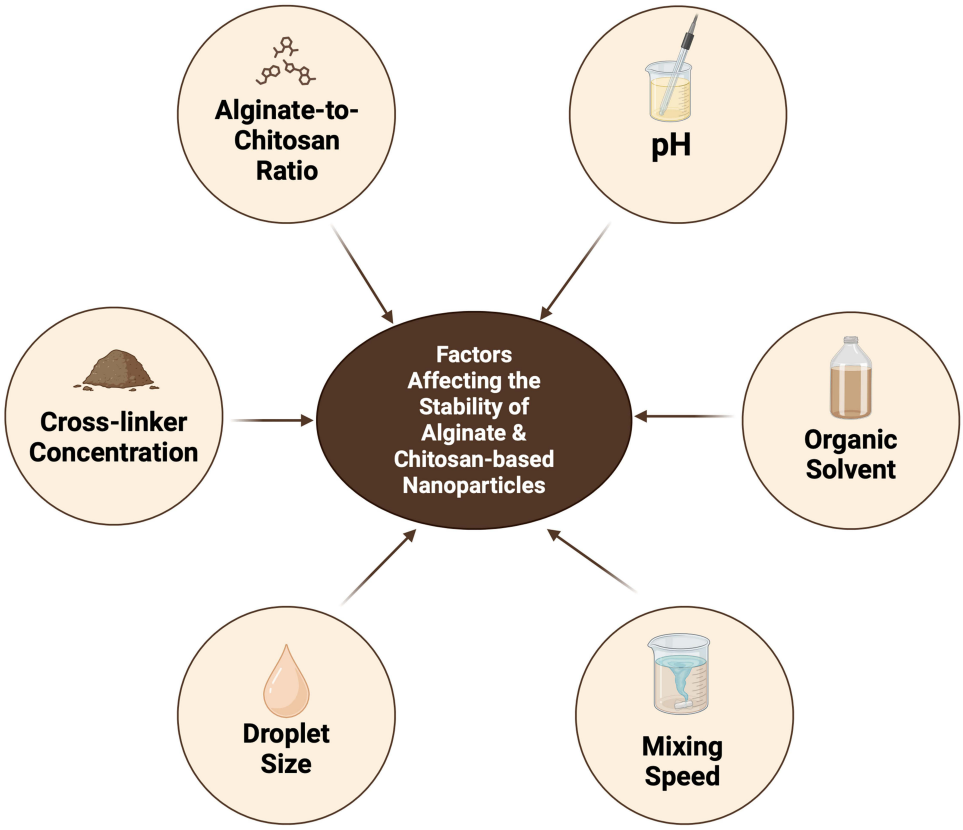

Figure 2 Factors influencing the stability of alginate and chitosan-based nanoparticle systems. Created with BioRender.com.

careful consideration of their risk-to-benefit ratio is paramount. Additionally, the selection of organic solvents should prioritize those not only effective for application but also safe. Among the safe choices of organic solvents are triacetin, ethyl formate, butyl lactate, benzyl alcohol, and ethyl acetate.[91,92]

Mixing Speed

In the manufacturing of nanoparticles, the collision frequency between particles increases proportionally with the stirring speed, thereby augmenting the likelihood of particle breakup and size reduction.[93] This phenomenon has been extensively investigated in studies focused on alginate/chitosan-based nanoparticle synthesis. Notably, Emami et al and Samprasit et al conducted experiments where stirring speeds ranging from 500 to 1500 rpm were explored, unveiling that the highest stirring speeds yielded the smallest particle sizes.[94,95] However, it is imperative to carefully consider the optimal stirring speed, particularly when transitioning to larger production scales, to ensure reproducibility and scalability while maintaining the desired particle characteristics. Striking this delicate balance between stirring speed and nanoparticle properties is paramount for the successful implementation of nanoparticle technology across various industrial applications.

Droplet Size

In the layer-by-layer self-assembly method, the droplet size plays a pivotal role, determining the thickness of the nanoparticle coating process. As the droplet size of the polymer solution decreases, thinner polymer layers are formed.[96] While a monolayer is generally desired, in certain situations, such as enhancing stability, multilayer conditions may be necessary. Small droplet sizes can be achieved through the use of nozzles with small diameters. However, when selecting the nozzle size, it is imperative to consider not only the desired droplet size but also the viscosity characteristics of the polymer solution.[97] Viscous fluids typically face challenges in flowing through small nozzles.[98] Achieving optimal conditions for layer-by-layer self-assembly requires careful consideration of these factors to ensure precise control over the coating thickness and the desired properties of the resulting nanoparticles.

Crosslinker Concentration

The key to fabricating nanoparticles using alginate and chitosan polymers lies in the cross-linking process. Therefore, the quantity or concentration of cross-linking agents becomes exceptionally crucial as a determining factor for successful nanoparticle formation. Previous studies, such as those conducted by Mokhtari et al, have indicated that the use of high concentrations of $CaCl_2$ can yield small-sized nanoparticles with high encapsulation efficiency.[99] This phenomenon arises from the fact that elevated concentrations of cross-linking agents enhance polymer chain folding, leading shorter chains.[100] However, it's important to note that an excessive concentration of cross-linker can also yield negative consequences. Such a scenario may result in a reduction in crystallinity, rendering the particles brittle and impacting the mechanical stability of the nanoparticles.[101] Thus, achieving an optimal balance in cross-linker concentration is essential for the successful synthesis of alginate and chitosan-based nanoparticles.

Alginate to Chitosan Concentration Ratio

Considerations regarding the ratio of alginate to chitosan usage are essential to achieve optimal conditions for nanoparticle formation, tailored to specific objectives. A higher proportion of chitosan relative to alginate may inadvertently diminish encapsulation efficiency.[102] This phenomenon arises due to an excess of amino groups in chitosan, which competes for interaction with M residues in alginate with the crosslinker (calcium ions).[103] Furthermore, this imbalance fosters an increased propensity for electrostatic interactions among particles, leading to aggregation and a subsequent decrease in the physical stability of the formed nanoparticles.[104]

While mitigating such occurrences remains imperative, it is crucial to also weigh the impact of the alginate-chitosan ratio on release profile dynamics. Utilizing a higher quantity of chitosan can yield sustained release profiles for drugs under basic pH conditions. This attribute holds significant promise for the treatment of infected wounds, given that wound pH typically resides in basic conditions ($\geq$7.3).[105]

Evidence of Antibacterial Agents' Delivery Using Chitosan/Alginate-Based Nanoparticles

In its function as a drug delivery system, alginate and chitosan-based nanoparticles generally can enhance and improve antibacterial efficacy through four main mechanisms (Figure 3). Firstly, reducing the size of drug particles increases drug solubility, resulting in improved drug absorptivity.[106] Secondly, drugs are protected during delivery to the target site of action, in this case, bacterial cells, ensuring high bioavailability.[107] Thirdly, the ionic interaction between the positive charge on chitosan and the negative charge on the surface of bacterial cells enhances drug selectivity.[108] Lastly, drugs are sustainably released, providing prolonged bacterial-killing effects.[109] These benefits can be applied to various types of drugs, ranging from pure chemical drugs (marketed drugs), natural sourced drugs, biologic agents, to inorganic drugs. Evidence-based findings regarding the benefits of using alginate and chitosan-based nanoparticles for each type of active substance are detailed as follows.

Application in Delivering Marketed Antibacterial Drugs

Nanoparticles based on alginate and chitosan have been extensively explored to enhance the efficacy and overcome limitations of existing drugs. The evidence of their utilization is summarized in Table 1, reflecting their broad applicability, notably in augmenting the effectiveness of antibiotics across various classes. From penicillins, aminoglycosides, fluoroquinolones, rifampicin, to polypeptides such as daptomycin, and glycopeptides like vancomycin, all can be harnessed through this approach.[108–117] These nanoparticles adeptly tackle various physicochemical challenges associated with antibiotics, notably by ameliorating solubility issues. For instance, the solubility of amoxicillin, typically sparingly soluble in water, can be significantly enhanced through this strategy.[111] Moreover, the protective effects conferred by alginate and chitosan-based nanoparticles play a pivotal role in safeguarding water-sensitive drugs such as amoxicillin and peptide antibiotics, ensuring stability throughout storage and transportation processes.[118]

The selection of fabrication methods for alginate and chitosan-based nanoparticles is pivotal, especially considering the physical and chemical characteristics of the intended drug. Modern antibiotics often exhibit relative instability in aqueous environments; for instance, amoxicillin, with its active beta-lactam moiety, is prone to beta-lactam ring hydrolysis.[118] In such cases, the oil-in-water emulsification method proves more suitable for manufacturing such medications.[111] Moreover, evidence underscores the prevalence of the ionic gelation method due to its

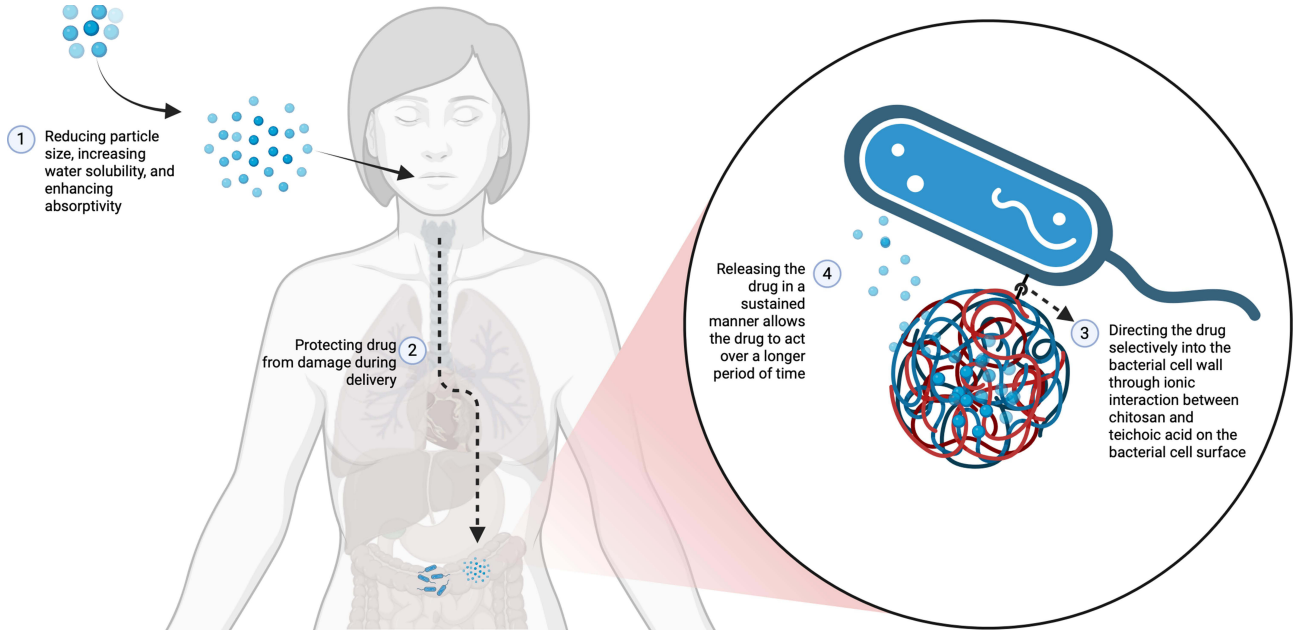

Figure 3 The mechanism underlying the enhancement of antibacterial efficacy through the delivery of antibacterial drugs into the alginate and chitosan-based nanoparticle system. Created with BioRender.com.

Table 1 Evidence of Alginate/Chitosan Nanoparticles Utilization for Delivering Marketed Antibacterial Drugs

Antibacterial Agent	Preparation Method	Study Design	Bacteria	Results	Mechanism of Action	Ref
Doxycycline	Ionotropic Gelation Method	In vitro	*Salmonella typhi, Pseudomonas aeruginosa, Enterobacter cloacae*, and *Staphylococcus aureus*	Bacterial cell count (against *S. typhi* and *P. aeruginosa*): Negative control = 10^8 Free doxycycline = more than 3×10^3 Doxycycline-loaded NPs = zero	Synergistic effects occur between doxycycline and the nanoparticle system, resulting in the improvement of the drug's physicochemical properties, thereby enhancing doxycycline's antibacterial effects	[108]
Vancomycin	Ionic gelation method	In vitro	*Staphylococcus aureus*	Vancomycin-loaded NPs exhibit sustained action until 24 h with the inhibitory zone of 18 mm	The sustained release of vancomycin within the nanoparticle system provides long-term inhibition of bacteria.	[109]
Rifaximin	Ionic gelation method	In vitro	*Escherichia coli, Pseudomonas aeruginosa*, and *Bacillus haynesii*	Inhibitory zone (against B. haynesii): Free rifaximin = 28 mm Rifaximin-loaded NPs = 34 mm	The surface ionic interaction mechanism between nanoparticles and bacterial cell membranes enhances the bacterial disruption process by rifaximin.	[110]
Amoxicillin	Oil-in-water emulsification combined with ionic gelation method	In vivo	*Helicobacter pylori*	Growth inhibition rate level: AMX-loaded NPs > Blank NPs	The incorporation of amoxicillin into nanoparticles enhances its antibacterial effects compared to conventional amoxicillin due to increased drug bioavailability.	[111]
Amoxicillin	Ionotropic pre-gelation combined with polyelectrolyte complexation method		*Helicobacter pylori*	In vivo mucopenetration studies revealed that amoxicillin-loaded NPs exhibit higher percentage drugs accumulated than free amoxicillin	The nanoparticle formulation helps amoxicillin in maintaining its stability during adhesion process in gastric juice environment. It also increase uptake of amoxicillin into gastric mucosa due to reduced particle size.	[119]
Amoxicillin (AMX) combined with docosahexaenoic acid (DHA)	Oil-in-water emulsification combined with ionic gelation method	In vivo	*Helicobacter pylori*	Growth inhibition rate level: AMX-DHA-loaded NPs > AMX-loaded NPs > DHA-loaded NPs > Blank NPs	The system is capable of loading multiple components, providing additive properties from the addition of DHA	[111]
Enrofloxacin	Ionic gelation method	In vitro and in vivo	*Escherichia coli*	MIC: Free Enrofloxacin = 1 μg/mL Enrofloxacin-loaded NPs = 0.125 μg/mL	Drug delivery in the nano-system protects the drug from negative exposure, thereby indirectly increasing efficacy.	[112]

Doxycycline	Ionotropic Gelation Method	In vitro	*Proteus mirabilis, Escherichia coli*, and *Enterococcus faecalis*	Inhibition rate: Doxycycline-loaded NPs > Blank NPs > Free Doxycycline > Negative Control	Synergistic effects arise from the nano-charge of alginate guided by non-ionic interactions between chitosan and bacterial peptidoglycan teichoic acid.	[113]
Rifampicin (RIF) and ascorbic acid (ASC)	Ionic gelation method	In vitro	*Staphylococcus aureus*	MIC: Free Rifampicin = 0.2 μg/mL RIF-ASC mixture = 0.2 μg/mL Blank NPs = no inhibition RIF-ASC-loaded NPs = <0.025 μg/mL	The primary mechanism of nanoparticle delivery is through the destruction of bacterial cell walls, allowing the encapsulated active substances to efficiently reach their targets within bacterial cells.	[114]
Daptomycin	Ionotropic pre-gelation combined with polyelectrolyte complexation method	In vitro	*Methicillin-susceptible Staphylococcus aureus* (MSSA), MRSA, *S. epidermidis, Staphylococcus capitis, Staphylococcus hominis, Staphylococcus lugdunensis, Staphylococcus haemolyticus*, and *Staphylococcus warneri*	MIC (against *S. capitis*): Free daptomycin = 1 μg/mL Daptomycin-loaded NPs = 0.5 μg/mL	In enhancing antibacterial activity, the concentration of chitosan is crucial for obtaining optimal nanoparticle parameters for the delivery process.	[115]
Tobramycin	Ionotropic pre-gelation combined with polyelectrolyte complexation method	In vitro and in vivo	*Pseudomonas aeruginosa*	Similar MIC value (0.625 mg/mL) for both unencapsulated tobramycin and tobramycin-loaded NPs indicate that there is no negative effect due to nano formulation.	The controlled release effect of the nanoparticle system extends the drug's therapeutic window.	[116]
Benzoyl peroxide	Ionic gelation method	In vitro	*Propionibacterium acnes*	Bacterial reduction level (log reduction): NPs > Chitosan > Control	The primary mechanism of this nano-system is the disruption of bacterial cell walls initiated by the surface ionic interaction of chitosan with bacterial cell walls.	[117]

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straightforwardness. Selecting the drug's base or salt form is also critical to ensure optimal compatibility with alginate and chitosan. The variance in ionization properties significantly impacts the molecular interactions within the resultant nanoparticle system.[108–110,113] Therefore, a thorough consideration of these factors is imperative to develop effective drug delivery systems that enhance stability and efficacy while mitigating potential degradation issues in aqueous environments.

Enhancing the efficacy of marketed drugs can be achieved through mechanisms aimed at improving drug bioavailability. One such approach involves loading drugs into nano-sized particles based on alginate and chitosan. Through this method, drug release can occur in a sustained manner.[109] For instance, in the treatment of ocular infections, daptomycin has been encapsulated within chitosan and alginate-based polymeric nanoparticles. Consequently, drug release proceeds steadily, with only 9.37% of the drug released at the 240th minute. This stands in contrast to the free delivery of daptomycin, which exhibits a permeability profile of up to 21.21% at the 240th minute.[115] The rapid release kinetics associated with the free drug delivery system may result in transient drug presence at the target site, necessitating more frequent dosing. Thus, sustaining drug presence on the ocular surface over an extended period through nanoparticle utilization can significantly enhance overall drug efficacy.

Application in Delivering Antibacterial Agents from Natural Sources

The challenge in utilizing natural-source drugs lies in their complex composition, where the efficacy is often intertwined with the synergy among their constituents. This complexity arises as the pharmacological activity of natural-source drugs typically depends on the interplay between various constituents, each complementing and augmenting the pharmacological activity of the others.[120] Moreover, the relatively poor solubility of natural-source constituents in water further compounds these challenges.[121] However, by employing alginate and chitosan-based nanoparticle systems, these issues can be addressed effectively. These systems excel in encapsulating the chemical constituents of natural-source drugs, ensuring there protection and sustained release Additionally, the reduction in particle size to the nano-scale indirectly enhances the solubility of active substituents in water by increasing the surface area available for interaction with the solvent.[122] As a result, the use of nanoparticle systems offers a promising avenue for overcoming the challenges associated with natural-source drugs, thereby potentially unlocking their therapeutic potential.

In formulating natural-source drugs into nanoparticle systems based on alginate and chitosan, careful consideration of the physicochemical properties of the natural materials to be encapsulated is crucial. Table 2 presents various instances of natural-source materials successfully loaded into these systems. These range from single chemical constituents such as quercetin and curcumin to oils like oregano oil and various essential oils, as well as crude extracts.[106,123–134] Ionic gelation, either alone or in combination with polyelectrolyte complexation techniques, is predominantly employed across the encapsulation of various types of natural-source materials. Particularly for organic substituents like oils, a more suitable approach involves formulating them using the oil-in-water emulsification method, followed by combining them with ionic gelation.[106,124,127,130,133] This method not only enhances the encapsulation of oils but also improves the stability of the active ingredients, preventing their premature release during storage.

Emerging evidence suggests that the application of alginate and chitosan-based nanoparticle systems significantly enhances the potency and efficacy of natural-source drugs. These nanoparticle systems offer superior targeting capabilities, particularly against bacteria, leveraging the positively charged chitosan's remarkable affinity to interact with teichoic acid on bacterial cell walls.[123,128,132] Furthermore, the increased solubility of substances such as oregano oil reduces the required dosage for effective treatment.[106] The Minimum Inhibitory Concentration (MIC) of oregano oil experiences a significant reduction upon loading into alginate and chitosan-based nanoparticle systems. Across various bacteria strains, including Streptococcus aureus, methicillin-resistant Staphylococcus aureus (MRSA), Enterococcus faecalis, Streptococcus pyogenes, and Escherichia coli, the MIC of oregano oil decreases from 0.0625% to 0.0078%.[106,124] Similar to marketed drugs, active constituents are encapsulated efficiently and can be released in a sustained manner. For instance, curcumin achieves an impressive loading efficiency of 93% with sustained release extending up to 58%.[125,128] Such compelling findings shows the immense potential of nanoparticle system in optimizing the therapeutic effects of natural-source drugs.

Table 2 Evidence of Alginate/Chitosan Nanoparticles Utilization for Delivering Antibacterial Agents from Natural Sources

Antibacterial Agent	Preparation Method	Study Design	Bacteria	Results	Mechanism of Action	Ref
Quercetin	Ionic gelation method	In vitro	*Staphylococcus aureus* and *Escherichia coli*	Inhibitory zone against *S. aureus*: Free quercetin = 14.1 ± 0.9 mm Blank Nanoparticles (NPs) = 9.8 ± 0.17 mm Quercetin-loaded NPs = 17.3 ± 0.30 mm	The positive charge on the surface of nanoparticles interacts with the negatively charged bacterial wall, enhancing drug adsorption into bacterial cells.	[123]
Oregano oil (OrO)	Emulsification and electrostatic gelation method	In vitro	*Streptococcus aureus, methicillin resistant streptococcus aureus* (MRSA), *Enterococcus faecalis, Streptococcus pyogenes, Escherichia coli, Pseudomonas aeruginosa*, and *Yersinia enterocolitica*	Minimum inhibitory concentration (MIC) against MRSA: Gentamicin-loaded NPs = 0.25 mg/L Ciprofloxacin-loaded NPs = 0.25 mg/L OrO-loaded NPs = 0.0625 mg/L	The improved solubility profile of oregano oil encapsulated in nanoparticles provides higher bacterial inhibition efficacy at low oregano oil concentrations.	[106,124]
Curcumin (Cur)	Ionic gelation method	In vitro	*Staphylococcus aureus* and *Escherichia coli*	Inhibitory zone (against *E. coli*): Free curcumin = 10 mm Cur-loaded NPs = 17.5 mm	Curcumin encapsulated in nanoparticles exhibits better bioavailability and permeability, resulting in higher antibacterial efficacy.	[125]
Syzygium aromaticum L. essential oil (EO)	Ionic gelation method	In vitro	*Escherichia coli, Klebsiella pneumonia, Salmonella enteritidis, Staphylococcus aureus, methicillin-resistant Staphylococcus aureus* (MRSA), and *Listeria monocytogenes*	MIC (in *S. aureus*): Free EO = 14/250 µg/mL Alginate NPs = >2000 µg/mL Chitosan NPs = 8/2000 µg/mL EO-loaded NPs = 9/1000 µg/mL	Besides alginate's role in enhancing the physicochemical properties of essential oil, chitosan preserves the availability of eugenol as the main constituent of the essential oil, capable of breaking down bacterial cell walls.	[126]
Melaleuca alternifolia (tea tree) Oil (TTO)	Oil-in-water emulsification combined with thin film dispersion method	In vitro	*Bacillus cereus, Staphylococcus aureus, Escherichia coli*, and *Salmonella enterica*	MIC (in *B. cereus*): Alginate NPs = 250 µg/mL Chitosan NPs = 250 µg/mL Free TTO = 62.5 µg/mL TTO-loaded NPs = 62.5 µg/mL	Nanoparticle systems do not enhance the antibacterial effect of tea tree oil but maintain antibacterial efficacy during delivery through extended-release mechanisms.	[127]
Curcumin	Polyelectrolyte complexation technique	In vitro	*Streptococcus mutans*	Curcumin-loaded NPs exhibit higher bacterial reduction than free curcumin	Apart from the gradual release mechanism of curcumin, strong ionic interactions between nanoparticles and bacterial cell walls help increase drug contact time with bacteria.	[128]

(*Continued*)

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Table 2 (Continued).

Antibacterial Agent	Preparation Method	Study Design	Bacteria	Results	Mechanism of Action	Ref
Solanum nigrum L. leaf extract	Ionic gelation technique	In vitro	*Streptococcus aureus, Pseudomonas aeruginosa, Escherichia coli*, and *Bacillus subtilis*	MIC (in *P. aeruginosa*): NPs = <230 μg/mL Raw extract = 1560 μg/mL Extract-loaded NPs = <230/195 μg/mL	Nanoparticle systems enhance antibacterial effects due to the synergistic effect between chitosan and the extract.	[129]
Thyme essential oil (TEO) and garlic essential oil (GEO)	Oil-in-water emulsification combined with ionic gelation method	In vitro	*Staphylococcus aureus, Escherichia coli*, and *Aeromonas hydrophila*	MIC (in *S. aureus*): TEO-loaded NPs = 10 μg/mL GEO-loaded NPs = 12.5 μg/mL TEO-GEO-loaded NPs = 7.5 μg/mL	Chitosan components, besides serving as nanostructure formers, also provide a synergistic effect through interactions with essential minerals on bacteria and forming a film on the bacterial surface, preventing nutrient entry into bacterial cells.	[130]
Grape pomace extract	Ionic gelation method	In vitro	*Methicillin-susceptible Staphylococcus aureus* (MSSA), *Escherichia coli, Pseudomonas aeruginosa, Salmonella enteritidis*, and *Listeria monocytogenes*	Encapsulation of grape pomace extract into NPs exhibit highest bacterial reduction against MMSA: 5-log reduction (higher than raw extract, 3-log reduction)	The use of encapsulation systems into chitosan and alginate provides protection effects for antibiotic components in the extract (anthocyanins and neochlorogenic acid), thus enabling antibacterial effects even upon delivery to the intestines.	[131]
Artocarpus lacucha Roxb. extract	Ionic gelation method	In vitro	*Corynebacterium sp., Staphylococcus aureus, Staphylococcus epidermidis, Bacillus sp., Micrococcus luteus, Pseudomonas aeruginosa, methicillin resistant Staphylococcus aureus* (MRSA), and *Propionibacterium acnes*	Highest efficacy reflected by inhibitory zone toward *Micrococcus luteus*: Raw extract = 17.0 ± 0.0 mm Extract-loaded NPs = 17.7 ± 0.6 mm	The primary mechanism of nanoparticles in enhancing the antibacterial potency of the extract occurs due to increased surface area, enhancing surface interactions with bacterial walls.	[132]
Turmeric oil and Glycyrrhiza glabra L. extract	Oil-in-water emulsification combined with ionic gelation method	In vitro	*Staphylococcus aureus, Enterococcus faecalis, Acinetobacter baumannii, Bacillus cereus, Pseudomonas aeruginosa*, and *Klebsiella pneumonia*	Highest efficacy reflected by inhibitory zone and MIC toward *Klebsiella pneumonia*: Raw extract = 1.5 cm and 140 μg Extract-loaded NPs = 2 cm and 150 μg	High loading capacity enables the incorporation of organic contents into the nano-system, indirectly enhancing physicochemical properties and antibacterial efficacy.	[133]
Ocimum sanctum (OS) leaf extract	Ionotropic pre-gelation combined with polyelectrolyte complexation method	In vitro	*Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus*	Bacterial reduction towards *Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus*, respectively: OS leaf extract only = 69, 75, 82, and 84% OS leaf extract-loaded NPs = 100, 97, 100, and 100%	Small particle size provides a large active surface area accompanied by extended-release properties.	[134]

Application in Delivering Inorganic Antibacterial Agents

The utilization of alginate and chitosan-based nanoparticles confers benefits similar to those observed with natural compound-derived counterparts, even extending to pharmacologically active inorganic substances. Notably, inorganic substances such as metals generally exhibit low solubility in water, presenting a notable challenge.[135] For instance, silver, renowned for its practical antibacterial efficacy, which exhibits poor solubility in aqueous environments. To address this challenge, silver nanoparticles are often engineered at the nanoscale for medical applications.[136] However, mere size reduction does not consistently enhance silver's antibacterial efficacy, owing to the nanoparticles' low selectivity in reaching their intended targets within the body. Hence, in numerous applications, inorganic nanoparticles like silver undergo modifications to enable targeted action.[137] Evidence indicates that loading silver nanoparticles can enhance their effectiveness as antibiotics by precisely targeting silver ions towards bacterial cell walls, ensuring a potent action against microbial pathogens. Similarly, other inorganic substances, such as titanium dioxide (TiO_2), copper oxide (CuO), and zinc oxide (ZnO), exhibit comparable phenomena.[138–142]

In Table 3, several pieces of evidence underscore the utilization of alginate- and chitosan-based nanoparticles for the delivery of inorganic metals. Despite the general suitability of the layer-by-layer self-assembly method for inorganic substances, the ionic gelation method is more commonly employed due to its procedural simplicity. However, it's noteworthy that formulations involving copper oxide (CuO) combined with zinc oxide (ZnO) utilize the layer-by-layer self-assembly technique, particularly advantageous for loading mixed active ingredients.[142] The modification of inorganic materials into alginate- and chitosan-based nanoparticles yields promising outcomes. Notably, processes aimed at reducing particle size significantly enhance the permeability of inorganic pharmaceutical ingredients into bacterial cells, as observed in the delivery of zinc oxide (ZnO).[141] Release reaches maximum values when ZnO concentration is at its peak; nevertheless, release remains sustained regardless of concentration, rendering it suitable for chronic antibacterial therapy.

Application in Delivering Biologic Antibacterial Agents

The challenge in delivering biologic drugs lies in their susceptibility to degradation due to environmental factors.[143] Enzymes and proteins composed of polypeptide chains of amino acid monomers, are particularly vulnerable to denaturation triggered by extreme temperature and pH conditions within the body.[144] This susceptibility extends not only to enteral delivery, but also in parenteral delivery, necessitating modifications to shield biologic substances from direct environmental effects.[107] In this context, alginate- and chitosan-based nanoparticles play a crucial role in preserving the stability of drug substances throughout storage and delivery to the target site.

Various biologic materials have been effectively incorporated into alginate- and chitosan-based nanoparticles, as outlined in Table 4. Despite the often-complex molecular structures of biologic substances, straightforward techniques such as ionic gelation and polyelectrolyte complexation technique are sufficient to form optimal nanoparticle systems. Although biologic substances typically have large molecular sizes, their loading into alginate- and chitosan-based nanoparticle systems is not restricted. This versatility stems from the Zwitterionic properties of biologic materials, where both ion charges can strongly interact ionically with NH_4^+ from chitosan and COO^- from alginate.[145] In contrast to loading probiotic materials, the layer-by-layer self-assembly method is preferable as it provides multilayered vesicular systems capable of maintaining bacteria viability until reaching their intended delivery target.[146]

As previously explained, the primary function of using alginate- and chitosan-based nanoparticle systems for biologic delivery is to enhance the physical and chemical stability of the substances.[146–151,153] Although nanoparticle size is advantageous, it is not the primary benefit obtained, owing to the constraints imposed by the relatively large molecular size of biologic agents. Consequently, the critical point of this modification for biologic agents lies in their ability to achieve high entrapment efficiency and proven stability against temperature and pH fluctuations. Experimental evidence concerning antibacterial peptides (Ib−M1) demonstrates that the system exhibits good stability against pH changes (pH 2 and 11), temperature variations (4 and 100°C), and proteinases (trypsin and pepsin), thus ensuring no reduction in antibacterial efficacy of the active substance.[153] This is a promising indication that antibacterial peptides can also be delivered orally.

Table 3 Evidence of Alginate/Chitosan Nanoparticles Utilization for Delivering Inorganic Antibacterial Agents

Antibacterial Agent	Preparation Method	Study Design	Bacteria	Results	Mechanism of Action	Ref
Titanium oxide (TiO_2) combined with geraniol (GRL)	Ionic gelation method	In vitro and in vivo	*Streptococcus pyogenes*	Minimum inhibitory concentration (MIC): Alginate-GRL = 0.312 μg/mL Alginate-TiO_2-GRL = 0.156 μg/mL Alginate-Chitosan-TiO_2-GRL = 0.156 μg/mL	The effectiveness of bacterial killing is enhanced through the synergy between the mechanisms of bacterial photodynamic inactivation by TiO_2 and cell wall destruction by geraniol.	[138]
Silver (AgBr) nanoparticle	Ionic gelation method	In vitro	*Staphylococcus aureus* and *Escherichia coli*	MIC: Blank nanoparticles (NPs) = 128 μg/mL AgBr-loaded NPs = 32 μg/mL	The effectiveness of bacterial killing is increased.	[139,140]
Zinc Oxide (ZnO)	Ionic gelation method	In vitro	*Staphylococcus aureus* and *Escherichia coli*	Inhibitory zone based on concentration of ZnO-loaded NPs: 1% < 0.1% < 0.01% < 0.001% < 0%	Reduction in the size of ZnO particles results in easier penetration of ZnO into the core of bacteria through the pores of the bacterial cell wall.	[141]
Copper oxide (CuO) combined with ZnO	Layer-by-layer self-assembly	In vitro	*Staphylococcus aureus* and *Escherichia coli*	Bacterial growth inhibition in *Staphylococcus aureus* and *Escherichia coli respectively*: Light condition = 95.03% and 92.71% Dark condition = 64.20% and 64.03%	The effectiveness of bacterial killing with the loading of a combination of these two types of metal provides an increase in antibacterial efficacy due to the localization of the metals towards the bacterial cell wall.	[142]

Table 4 Evidence of Alginate/Chitosan Nanoparticles Utilization for Delivering Biologic Antibacterial Agents

Antibacterial Agent	Preparation Method	Study Design	Bacteria	Results	Mechanism of Action	Ref
ε-Polylysine (ε-PL)	Ionic gelation method	In vitro	*Escherichia coli, Bacillus subtilis, Staphylococcus aureus*, and *Micrococcus luteus*	Minimum inhibitory concentration (MIC): Free ε-PL = 31.25–125 μg/mL ε-PL -loaded nanoparticles (NPs) = 7.81–38.67 μg/mL	Encapsulation of ε-PL into nanoparticle systems provides protection of the active substance from degradation and offers sustained-release profiles.	[147]
Nisin	Ionic gelation combined with complexation method	In vitro	*Listeria monocytogenes, Staphylococcus aureus, Micrococcus luteus, Pseudomonas aeruginosa, Salmonella enterica*, and *Enterobacter aerogenes*	MIC: Free nisin = 2 mg/mL Nisin-loaded NPs = 0.5 mg/mL	Stability improvement of the nisin peptide by nanoparticles offers protection of the peptide during storage, thereby maintaining antimicrobial effectiveness over an extended period.	[148–151]
Combination of ε-Poly -Lysine and epigallocatechin gallate (ε-PL-EGCG)	Alginate ionotropic pre-gelation followed by chitosan polyelectrolyte complexation	In vitro	*Escherichia coli* and *Staphylococcus aureus*	Bacterial concentration: Blank = 7.3log CFU/g Non-NPs = 4.1log CFU/g NPs = 3log CFU/g	Continuous release of ε-PL-EGCG provides prolonged bacterial inhibition effects as the encapsulated drug is released from the nanoparticles.	[152]
Antibacterial Peptide (Ib-M1)	Ionic gelation method	In vitro	*Escherichia coli*	MIC: Free Ib-M1 = 12.5 μM Ib-M1-loaded NPs = 0.5 μM	The enhancement of effectiveness occurs not through mobilization but rather through the stabilization of peptides contained within the nanoparticles, thereby providing a prolonged effect.	[153]
Endolysin (LysMR-5)	Ionotropic gelation combined with polyelectrolyte complexation technique	In vitro	*Staphylococcus aureus*	Inhibitory zone: Alg-Chi NPs = 14 ± 1.5 mm Free LysMR-5 = 18 ± 2 mm LysMR-5-loaded Alg-Chi NPs = 22.5 ± 3.1 mm	Nanoparticle encapsulation opens the gateway into bacteria through peptidoglycan lysis and provides sustained-release effects of the encapsulated drug.	[154]
Probiotic *Pediococcus acidilactici* (PA) combined with phthalyl inulin (PI)	Layer-by-layer self-assembly technique	In vitro	*Salmonella gallinarum*	Pediocin concentration: PA = 7.44 μg/mL PA-PI = 5.56 μg/mL PA-PI-loaded NPs = 10.98 μg/mL	Nanospheres offer protection against microbiota agents during the delivery process and provide extended-release effects.	[146]

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Challenges and Future Perspective

The use of natural polymers presents significant advantages compared to synthetic and semi-synthetic polymers due to their ease of acquisition and relative avoidance of toxicity issues. In contrast, the use of synthetic polymers such as Tween 80 and PLGA (polylactic-co-glycolic acid) has been associated with hypersensitivity problems, particularly in pediatric consumers.[155,156] Moreover, the use of semi-synthetic polymers like poloxamer carries the risk of producing toxic residues following the ultrasonication process.[157] While the utilization of alginate- and chitosan-based nanoparticle systems presents a promising solution, it encounters limitation and hurdles that may hinder its translation into practical solution for patient use. The availability of antibacterial drug formulations loaded into nanoparticle systems remains scarce in the market, with VivaGel® being a notable example of a dendrimer nanoparticle system for bacterial vaginosis treatment.[158] This scarcity is attributed to the difficulty in technology transfer from lab-scale to production scale settings. Although these systems may exhibit efficacy at the lab scale, the same assurance may not be obtained at the production scale due to factors such as equipment quality and capacity. For instance, the use of high-speed mixers necessitates sophisticated technology equipment on a large scale. In contrast to the use of technology in the development of drugs at the micro scale, such as microemulsions, the technology required for microemulsion development tends to be simpler and does not necessitate equipment with high-energy capacity for the mixing process.[159] Consequently, these limitations ultimately contribute to high production costs.[160] Therefore, the development of production systems with the simplest possible approach may be the best effort to facilitate smooth technology transfer and indirectly reduce formulation production costs, thus fostering smoother pathways for the integration of nanoparticle based antibacterial drug formulations into clinical practice. Additionally, the development of drugs in the form of nanoparticles frequently encounters regulatory challenges. These issues include the absence of standardized testing and characterization protocols for nanoparticle drugs, as well as the lack of an established nomenclature for naming drugs that have been modified into nanoparticles.[161]

Despite its drawbacks, alginate- and chitosan-based nanoparticle systems hold considerable promise for the future of antibacterial drug delivery. Based on existing evidence, not all antibacterial agents have been explored for integration into this advanced delivery system. For example, in tuberculosis treatment, where resistance poses a significant challenge, none of the anti-tuberculosis drug regimens have been modified into this system. Such a system would not only aid in combating resistance but also potentially reduce the dosage requirement, thereby enhancing patient compliance and treatment outcomes.[162] The use of alginate and chitosan in nanoparticle development is also considered to support green nanotechnology. This is not only due to the simplicity of the development process but also because alginate and chitosan are readily sourced from various unused marine natural materials.[163] Utilizing these materials before they become harmful waste has a beneficial impact on the environment. Furthermore, it's the development of alginate- and chitosan-based nanoparticle systems remain highly tunable. Researchers have the flexibility to modify the side chains of chitosan and the salt form of alginate to fine-tune the physicochemical properties, to address the specific challenges of each antibacterial drug.

Conclusion

The medical applications of alginate and chitosan in drug delivery offer an effective solution to maximize the efficacy of various antibacterial agents. Alginate and chitosan have been extensively utilized as the basis for forming polymeric nanoparticle systems for antibacterial drug delivery purposes. With a variety of suitable methods, these systems have proven to be applicable not only to well-established drugs that have broad approval for public use but also to with various materials presenting challenges due to their unique physical and chemical properties. This review findings indicate that alginate and chitosan-based nanoparticles exhibit promising capabilities in enhancing the efficacy of various types of antibacterial agents, ranging from marketed antibiotics to natural-source, inorganic, and biologic antibacterial agents. Understanding the success achieved with this system holds promise for providing solutions to various challenges encountered in antibacterial treatment in the future, thereby opening opportunities for researchers to conduct further

clinical trials until licensure is obtained for alginate and chitosan-based nanoparticle-based products that can be marketed.

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