Carbohydrate Polymer Technologies and Applications 7 (2024) 100441

Contents lists available at ScienceDirect

Carbohydrate Polymer Technologies and Applications

journal homepage: www.sciencedirect.com/journal/carbohydrate-polymer-technologies-and-applications

Chitosan-based nanomedicines: A review of the main challenges for translating the science of polyelectrolyte complexation into innovative pharmaceutical products

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ARTICLE INFO

Keywords:
Chitosan nanoparticles
Nano-bio interface
Polyelectrolyte complexation
Scale-up
Quality control
Regulatory issues

ABSTRACT

Chitosan (CS) is a versatile biopolymer used in the fabrication of different types of drug delivery systems. It is recognized mainly by its pH-responsive behavior, mucoadhesive, and membrane permeation enhancer properties, which can be explored for modulated drug release and localized delivery. Other biological properties (immune adjuvant, and antitumoral) put this biopolymer at the forefront of raw materials for manufacturing new multifunctional products. Their material attributes can be improved by chemical modification, resulting in CS-based derivatives with controlled hydrophobicity, better solubility, and higher bio(mucoadhesive) capacity. The resultant CS-based materials have been used to fabricate nanoparticles (CS NPs) for the delivery of small molecules and macromolecules into body tissues. Despite many studies that have reported the efficacy and safety of CS NPs, there are still some important issues to be considered before they reach the market. Herein, we demonstrate the perceived risks of using CS NPs can be partially explained by some important gaps in basic science, technological processes, and aspects related to quality control and regulatory affairs. This review discusses the challenges and possible solutions for each of the above-mentioned points with the perspective of enabling CS NPs as technological platforms for the pharmaceutical industry.

1. Introduction

CS is a polycationic biopolymer derived from the deacetylation of chitin. It is a copolymer of glucosamine (β (1–4)-linked 2-amino-2-deoxy-D-glucose; GlcN) and N-acetylglucosamine (2-acetamido-2-deoxy-D-glucose; GlcNAc). The CS chemical structure confers better physicochemical properties than chitin, due to the possibility to dissolve the material under acidic conditions. In this regard, the presence of amino groups confers a pH-responsive behavior, a desirable property for different applications such as in food, beverages, agrochemicals, cosmetics, and pharmaceuticals (Aranaz et al., 2018; Bandara, Du, Carson, Bradford & Kommalapati, 2020; Khalaf et al., 2023; Maleki, Woltering & Mozafari, 2022; Shariatinia, 2019). Further, CS is extensively explored in the fabrication of drug delivery systems (DDS) (e.g., particulate systems, hydrogels, etc.) due to its low cost, easy processability at the bench, and controlled release capacity (Kumar, Vimal & Kumar, 2016).

The physicochemical behavior of CS is well characterized in both solid states and aqueous solutions. Particularly, the CS solution properties are highly dependent on intrinsic structural features such as the degree of polymerization (DP), degree of acetylation (DA), and extrinsic environmental factors such as pH, ionic strength, and polymer concentration (Blagodatskikh et al., 2013; Costa, Teixeira, Delpech, Souza & Costa, 2015; Sorlier, Denuzière, Viton & Domard, 2001, 2003). CS presents interesting biological activities such as immunomodulatory, antimicrobial, antitumoral, and wound healing effects (Khalaf et al., 2023; Kou, Peters & Mucalo, 2022; Shariatinia, 2019; Zhang et al., 2019). It should be noted these interesting effects are intricately linked to the chemical structure of CS as well as to their main qualitative attributes of DP and DA.

The presence of hydroxyl and amino groups in CS makes the polymer a useful backbone for chemical modification. The CS derivatives are produced to improve the physicochemical properties of CS or to add new functionalities that can be exploited during further manufacturing of dosage forms (Ahmed & Ikram, 2017; Marcondes et al., 2021). The most common derivation strategies are the quaternization, thiolation, and alkylation of CS polymer chains. The quaternized derivatives of CS (e.g.,

Abbreviations: CS, chitosan; COS, chitosan oligosaccharides; MUC, mucin; DDS, drug delivery systems.

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https://doi.org/10.1016/j.carpta.2024.100441

Available online 20 January 2024

trimethyl CS) are produced by transforming the primary amine groups of CS into quaternary salts with a permanent positive charge (Andreica, Cheng & Marin, 2020; Pathak et al., 2021). The thiolation derivatization consists of modifying the CS backbone with sulfhydryl-bearing agents like cysteine, thioglycolic acid, and 6-mercapto nicotinic. The reactions proceed via amide or amidine bonds, resulting in thiolated CS with improved adhesive properties (Federer, Kurpiers & Bernkop-schnu, 2021). The alkylated derivatives of CS are generally obtained via reductive amination of the biopolymer. The resultant amphiphilic properties of the alkylated derivatives are dependent on the alkyl chain length and impact the membrane permeation properties (Ahmed & Ikram, 2017; Pol et al., 2022).

Although CS biopolymers and their derivatives can be processed in a variety of drug delivery systems (DDS), the development of CS NPs deserves attention due to its capacity to protect drugs and macromolecules against harsh conditions. Further, CS NPs can be used to modulate drug delivery in a pH-dependent manner (Aydin & Pulat, 2012). CS NPs can be fabricated by several methods such as ionic gelation, polyelectrolyte complexation, emulsion-solvent diffusion, reverse micellization, nanoprecipitation, and spray drying (Bashir et al., 2022). The method of choice is generally dependent on the physicochemical properties of the small molecules or macromolecules to be encapsulated and other synthesis parameters such as the encapsulation and drug loading efficiency and %yield.

Ionic gelation and polyelectrolyte complexation are well-established methods for the fabrication of CS NPs based on associative interactions between CS and small ions or large polyelectrolytes of opposite charge. These techniques are frequently described as mild and cost-effective when compared to the more complex process involving emulsification and supersaturation phenomena (Ferreira et al., 2020). At present, several types of CS NPs could be prepared by combining CS with small anions (e.g., tripolyphosphate) and polyelectrolytes (dextran sulfate, hyaluronic acid, sodium alginate, etc.) (Boni et al., 2018; Carvalho et al., 2021; Moeini et al., 2018). The different combinations of polyelectrolytes offer opportunities to design new functionalities for CS NPs that go beyond those described for CS biopolymers. Indeed, it is well known that several types of polyanions have interesting biological functions such as anti-inflammatory, lubrication, and wound healing properties (Aya & Stern, 2014; Draget & Taylor, 2011).

Despite the increased interest in CS-based materials, few marketed medicines are using this biopolymer in their composition (e.g. LipoSan Ultra™, Chitocare™, Axiostat™) (Frigaard, Jensen, Galtung & Hiorth, 2022). However, the regulatory status is not clearly defined. Despite attempts by some companies to achieve GRAS status (*generally recognized as safe*) for CS, the FDA still does not recognize it as such (FDA, 2002, 2005, 2011, 2022). The International Pharmaceutical Excipients Council (IPEC) and other regulatory agencies propose some preclinical tests focusing on the short-, mid-, and longer-term clinical use of new excipients (Baldrick, 2010). This guidance could be a starting point to introduce CS-based materials in more marketed products, however, more specific considerations should be addressed for CS NPs. Currently, there are several studies demonstrating the advantages of CS NPs in both technological and biological aspects. However, it is important to address some unresolved questions.

This review focuses on the current scenario of CS NPs fabricated by ionic gelation or polyelectrolyte complexation as possible technological platforms for the encapsulation of different types of drugs. The fundamental understanding of CS NPs mechanisms of formation and biological interactions as well as technological barriers and regulatory issues are discussed as the main obstacles hindering the development of marketed products.

2. The global market of CS: is there an opportunity for nanoparticulate systems?

The global CS market is projected to reach USD 47.06 billion by 2030, registering a compound annual growth rate (CAGR) of 20.1 % from 2023 to 2030 (GVR, 2022). This expected increase in CS values is somewhat explained by the demands for bio-based polymers in different industrial sectors. Recently, an in-depth patentology analysis of the chitinous biomaterials was reported, aiming to provide an outlook for the scientific background on the trends of intellectual property protection on chitinous materials (Kertmen, Dziedzic & Ehrlich, 2023a, 2023b). The patent analysis was carried out in the 2nd quarter of 2022 and identified 3650 patent families related to CS-based products. Particularly, the cosmetic, pharmaceutical, and biotechnological sectors can be the main drivers for the increased CS value.

A total of 58,329 publications were found under the term "chitosan nanoparticles" in the Science Direct platform for the last decade (2013–2023). This indicates that the science and technology behind CS NPs evolved greatly at least quantitatively. However, most studies are exploratory by nature, with a major focus on evaluating possible application possibilities without careful consideration of the mechanistic understanding of how the ideas should work. Examples of distinct applications are described in Table 1. Despite this high number of research studies found in periodic journals only 11 initiatives were found in clinical trials (ClinicalTrials.Gov, n.d.) Such a low number of clinical trials reflects the uncertainties around CS NPs, which could be attributed not only to the CS properties but also to the uncertainty of nanotechnology methods and claims.

The application achievements of CS-based materials put this biopolymer at the forefront of the development of new functional products. Many applications in the pharmaceutical-related areas of drugs, vaccine adjuvants, and biopharmaceuticals are now well-established on the lab scale (Dmour & Islam, 2022; Khalaf et al., 2023). The influence of NP size and surface charge on the biological responses is frequently reported in those studies, but little is known about how the specific ranges of values of the CS NPs physicochemical attributes interfere with the desired response. In addition, considerations about stability under biorelevant conditions are not very well documented, which creates uncertainty from a regulatory point of view. Therefore, it is expected the future development of CS NPs should focus on conducting more systematic studies aiming to better describe these aspects to reach market opportunities. Further, initiatives on the mass production of CS NPs will demand new process technologies, especially those that can be easily implemented by the industry.

3. Major challenges to establishing CS NPs as a platform technology

Despite Chitin and CS-based materials being extensively studied in both basic science and technological aspects, there are some focal points to be better understood to establish the CS as a functional excipient and CS NPs as a versatile technology platform for the pharmaceutical and biotechnological industry. We present here the three main points that should be addressed in the next years to provide suitable scenarios for launching CS NP-based products: i. the fundamental questions related to mechanisms of CS NPs formation and interaction with biological interfaces, ii. technological advances regarding scaling up processes and, iii. development of quality control methods and clarification of regulatory issues.

3.1. Fundamental science gaps of CS NPs

Most basic science questions for CS NPs revolve around developing a greater understanding of the molecular details of the ionotropic gelation or polyelectrolyte complexation process (Ferreira et al., 2020). The elucidation of the predominant supramolecular forces in nanoparticle formation and stabilization is important to achieve nanoparticles with adjustable properties. Furthermore, the understanding of how CS NPs interact with biological surfaces can help in the development of bioresponsive systems. Regardless of whether such fundamental questions

Table 1
Description of main technological attributes and functional claims of CS NPs produced with CS or CS derivatives.

CS-based raw material	Other components	Technological attributes	Performance and functional claims	Reference
Low Molecular Weight CS	Poly-glutamic acid, oligodeoxynucleotides (ODN)	DA and mannosylated moieties of CS	The decreased charge density on the CS backbone resulted in enhanced intracellular ODN release, which promoted in vitro cytokine secretion. The mannose grafted on the CS- backbone promoted the uptake of CS NPs through the mannose receptor-mediated recognition.	(Babii et al., 2020)
COS	Polyelethyleglycol, Cyclosporin (CsA)	Zeta potential and triggered release	The charge conversion of COS NPs enabled the efficient delivery of CsA and AZD9291 in vivo, in response to the weakly acidic tumor environment. The presence of COS had positive charge attributes, which enhanced the affinity between tumor cells and NPs, enhancing the cell uptake.	(Chen et al., 2022)
TMC	Tripolyphosphate, protective antigen, Poly I:C	*In vitro* release profile, particle size distribution, and zeta potential	The protective antigen-loaded TMC nanoparticles as well as CpG and Poly I:C adjuvanted TMC-PA formulations promoted strong IgG antibody response via subcutaneous, intramuscular and subcutaneous routes in mice.	(Malik, Gupta, Mani, Gogoi & Bhatnagar, 2018)
Oleoyl-carboxymethyl chitosan	Hyaluronic acid	Particle size distribution, zeta potential, DNA loading and release.	The transfection efficiency of hyaluronic acid modified-NPs was 5-fold higher than that of non-modified NPs under the same conditions.	(Liu et al., 2013)

have more technological or biological implications, the scientific answers are crucial for the correct production of innovative products and the implementation of personalized therapies in a lot of complex diseases.

The understanding of the role of supramolecular interactions on the colloidal properties of CS NPs (e.g., hydrodynamic and charge properties) is a central axis of research (Aibani, Rai, Patel, Cuddihy & Wasan, 2021). Most of this physicochemical knowledge basis was consolidated in the last decades due to extensive research in the material properties of CS in solid state and aqueous solution, which enabled a more rational production of CS NPs (Schatz, Viton, Delair, Pichot & Domard, 2003; Sorlier, Rochas, Morfin, Viton & Domard, 2003).

The relationship between CS DP and DA and polymer chain conformation and ionization in solution are now well characterized. It was concluded these structural factors dictate much of CS solution behavior during CS NP synthesis. The DP is formally defined as the number of monomer units in the CS polymer chain. It is calculated as the ratio of molecular weight (Mw) of a CS and Mw of the GlcN and GlcNAc repeat units. The degree of acetylation (DA, %) is defined as the molar fraction of GlcNAc in the copolymers of CS composed of GlcNAc and GlcN (Jiang et al., 2017). This factor determines de overall ionization state of CS and together with Mw can influence the CS solution conformation and supramolecular interactions with other biomolecules. In this respect, it is known that while low Mw CS polymer chains usually extend as stiffer linear chains, medium and high Mw CS polymer chains entangle due to the increase in the length of the molecular chain. The higher the Mw, the higher the entanglement, and the polymer chains usually adopt coil conformation (Cho, Heuzey, Bégin & Carreau, 2006). The overall charge of CS dependent on DA modulates this intramolecular entanglement due to charge repulsion. It is known that CS monomers have distinct physical-chemical properties, while GlcN provides ionic characteristics, and the GlcNAc is hydrophobic. The distribution and size of the sequence of comonomers affect the charge distribution, but little is known about how this interferes with the self-assembly of CS NPs (Cord-Landwehr et al., 2020; Wattjes et al., 2020).

The other category of synthesis factors that influence the mechanisms of CS NPs formation is the extrinsic or environmental variables such as pH, ionic strength, and polymer concentration. In this respect, it is reported that pH and ionic strength modulate the charge of CS molecules by interfering with the ionization equilibria and ion-based charge screening, respectively (Gucht, Spruijt, Lemmers & Cohen Stuart, 2011). It has been demonstrated that pH can modulate the strength of polyelectrolyte complexation and the ionic strength of the solution modulates the kinetics of CS NPs assembly (Ferreira et al., 2023; Lalevée et al., 2016). However, most studies were performed with monovalent charged ions and the influence of ion specificity and higher valency ions has yet to be determined. The concentration regimes affect mainly the intermolecular chain entanglement. The semidilute regime of CS chains is characterized by unentangled polymeric chains and exists above the chain's overlap concentration c^* within the polymer concentration range $c^* < c <$ ce, where ce is the polymer concentration corresponding to the onset of entanglements (Cho et al., 2006). For example, the dependency relationship between CS entanglement and the total polymeric concentration of the starting solution has been shown to affect the hydrodynamic size of the nanoparticle (Sreekumar, Goycoolea, Moerschbacher & Rivera-Rodriguez, 2018).

CS NPs are generally administered via mucosal routes due to their well-recognized mucoadhesive and permeation-enhancing properties. However, the interactions of NPs with mucus barriers have proved to be much more complex than the considerations made by mucoadhesion theories (Mansuri, Kesharwani, Jain, Tekade & Jain, 2016). Indeed, an opposing line of research has presented the concept of mucopenetration, which has been studied in some formulations of CS NPs (Cheng et al., 2021; Lai, Wang & Hanes, 2010). Both mucoadhesion and mucopenetration concepts are limited drug delivery strategies as they hinder some important details of the phenomenology of mucus interactions that are dependent on the action of combined supramolecular forces between the CS NP and the biological system. Therefore, research studies searching to understand and modulate the interactions at the so-called nano-bio interface is a mandatory topic to enable more effective and safer therapies with CS NPs (Nel et al., 2009). Herein, the emerging mucus-modulating concept shares some fundamentals of colloidal and supramolecular chemistry responsible for CS NPs formation and stabilization (Fig. 1). They are dynamic and are dependent on the biophysicochemical characteristics of the CS NPs, the biological surface, and the interaction environment (Ferreira et al., 2023; Nordgård & Draget, 2018).

Nano-bio interfacial interactions that occur between the synthetic and biological worlds require a new type of approach on the part of scientists. The concept of a static DDS whose function is to direct the drug to the desired location for release has been confronted with the most recent evidence in pharmaceutical nanotechnology. Several works report that the dynamics and structure of the system in the environment in which the biological response occurs are essential components that must be considered (Nel et al., 2009). Herein, the biomolecular corona formation around NPs is a key hallmark of events occurring at the nano-bio interface (Biology et al., 2016; Docter et al., 2015; Maskos & Stauber, 2017). Particularly, it has been demonstrated the serum source affects the reliability of in vitro experiments of CS NP−breast cancer cell interactions, which can partially explain the limited clinical success of drug targeting by CS NPs in some types of cancer (Ezzat et al., 2022). Other studies have pointed protein corona can significantly affect the in

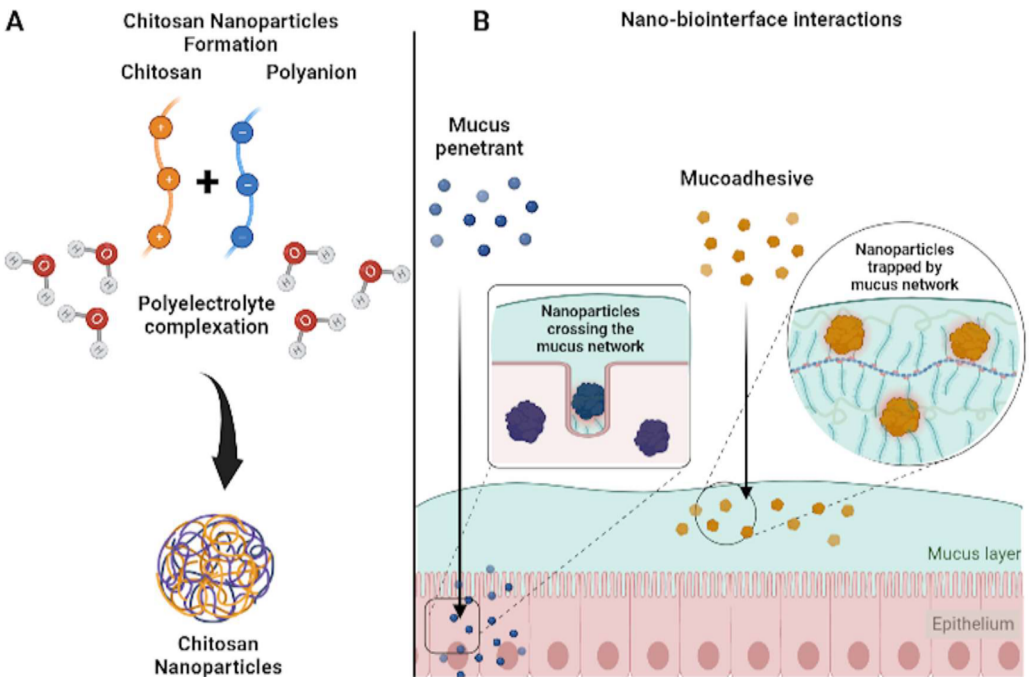

Fig. 1. Main fundamental sciences gaps of CS NPs applied to mucosal delivery. (A) The CS NPs formed by polyelectrolyte complexation are explained by associative forces between CS and polyanions. The role of other elements during CS NPs formation (e.g. ions and water) is not well understood. (B) Nano-bio interface interactions is responsible for the mucoadhesive or mucopenetrant behavior of CS NPs. The knowledge of how to rationally manipulate these interactions is now an advanced concept of mucus modulation.

vivo fate of CS NPs including biodistribution and half-life (Tekie et al., 2020). This complexity of nano-bio interfacial interactions brings DDS remarkably close to biological systems, in which the dynamics of supramolecular interactions form the basis of the biochemical and biophysical processes involved in physiological or pathological events. Therefore, studies focused on understanding the main factors affecting protein corona formation on CS NPs and strategies to overcome some deleterious effects must be a field of intense future research (Caprifico, Foot, Polycarpou & Calabrese, 2021; Moraru, Mincea, Menghiu & Ostafe, 2020).

Much of the knowledge of CS interactions with biological interfaces was generated by biophysical studies with mucin (MUC) and biomembrane models (Ferreira et al., 2023; Haugstad et al., 2015; Pavinatto et al., 2007; Silva, Nobre, Pavinatto & Oliveira, 2012; Thongborisute & Takeuchi, 2008). Although conventional scientific explanations fall on the role of electrostatic interactions, recent research indicates that other interactions must be considered. Particularly, it is becoming more and more evident that the hydrophobic effect and H-bonds can play significant roles in mediating CS-MUC and CS-biomembrane interactions (Pedro, Pereira, Oliveira & Miranda, 2020; Pereira et al., 2020). In this respect, some important gaps should be addressed in future research such as the role of water and environmental factors of pH and ionic strength in modulating those interactions (Ferreira et al., 2023).

3.2. Main technological demands for producing CN NPs on a large scale

The scalability of CS NPs is not straightforward, and it is highly dependent on the properties of the raw material and on the fabrication method. The scale-up of nanomaterials is intricately linked to some fundamental science topics of molecular/nanoscale phenomena. Despite a lot of basic research has elucidated the influence of the molecular features (DA and DP) of CS on their charge and hydrodynamic properties, and we have gained a lot of insights on how these factors affect CS NPs formation in the bench, little is known about how to consider this knowledge at large scale of CS NPs production. Particularly, as discussed before, nanostructures are formed and stabilized by multiple supramolecular forces (e.g., electrostatics, H-bonds, hydrophobic effect, etc.) in which the combined effects are scale-dependent (Savyasachi et al., 2017). More specifically, it is important to consider the spatiotemporal evolution of those supramolecular interactions together with mass transport phenomena during scale-up (Herdiana, Wathoni, Shamsuddin & Muchtaridi, 2022; Panariello, Mazzei & Gavriilidis, 2018).

The use of scalable methods is another crucial factor in establishing CS NPs as a technological platform. Both ionic gelation and polyelectrolyte complexation occur in an aqueous solution upon mixing CS with a charged (poly)anion. There are dozens of studies demonstrating the feasibility of these processes at the bench, but the transposition of scale requires considerations about the mixing operations in the length and time scales of polyelectrolyte complexation (Gucht et al., 2011). Currently, this knowledge is somewhat limited, and it is important to investigate how the hydrodynamics, charge, heat, and mass transfer can affect the thermodynamic and kinetic parameters of CS NPs formation during scale-up.

The understanding of mixing mechanisms as a function of length and timescales can lead to the optimization of industrial batches. These mechanisms are usually employed to control crystallization processes, but they can be applied in other types of reactions since the mixing step is a critical operation (Abiev, Kudryashova, Zdravkov & Fedorenko, 2023). The mixing mechanism is usually divided into macro, meso, and micromixing and, in each scale, the set of interfering factors can be different. The macromixing occurs on the vessel scale, which represents the uniformity of the local concentrations of CS and other polyelectrolytes within the entire vessel. The mesomixing refers to the dispersion of the feed stream shortly after it enters the intravortex. It is characterized by a turbulent exchange of fresh feed with the surrounding fluid. Finally, micromixing comprises the molecular diffusion and engulfment of different fluid elements at the scale of the smallest turbulent eddies, which represents molecular scale mixing.

A key issue is to understand how the main supramolecular interactions responsible for CS NPs formation behave in these different mixing scales. From this knowledge, it is important to design operations

able to control these interactions through scalable techniques. Currently, three possible scaling methodologies are used to obtain CS NPs (Fig. 2). The bulk mixing operates at the macromixing scale and frequently produces unstable particles and batches with great variability. On the other hand, methodologies focused on phenomena occurring at the meso-micro mixing scales are more suitable for obtaining CS NPs with reproducible properties. In this respect, the flash nano complexation (FNC) and microfluidic technologies enabled more precise control of the molecular diffusion processes and, consequently slowed down the kinetics of CS NPs formation (Ahmed et al., 2021; Hu et al., 2021; Zhang, Chen, Ma & Sun, 2020; Zoratto et al., 2021). Recently it was demonstrated the mixing efficiency provided by FNC promotes higher compactness and increases the aggregation number of CS inside each NP formed by ionic gelation with tripolyphosphate anions (Yuan & Huang, 2019; Yuan et al., 2022).

Despite the outstanding results of CS NPs produced by FNC, the transposition of scales must be accompanied by a risk assessment. This could be done by focusing on a systematic process that involves identifying, analyzing, and controlling hazards and risks. Currently, there are no wide risk assessment tools defined for CS NPs production, but the concepts of Safe by design (SbD) and Quality by Design (QbD) can bring an important framework for this need (Marques, Som, Schmutz, Borges & Borchard, 2020). Both concepts share some similarities and focus on the systematic development of formulation attributes by studying the potential influence of the material's properties and process factors. Usually, the development is made by the application of statistical tools and the design of experimental methods.

3.3. Regulatory and quality control issues

The regulation of CS-based raw materials is a pressure matter to enable its applications. Despite CS and its derivatives can be found in dietary supplements, the application to medicines and vaccines still demands more research. More specifically, the application of CS NPs is subject to the same regulatory agenda as other types of nanomaterials. Hence, the particle size, charge, and surface properties should be extensively characterized not only with a focus on the desired response but also on their potential adverse effects (Foulkes et al., 2020; Mühlebach, 2018). This type of study should be a collaborative effort between regulatory agencies, academia, and industry and, it will probably demand articulated action on the product and analytical development (Fig. 3).

The development of quality control (QC) tests is a critical topic that must run in parallel with the establishment of the regulatory agenda of CS-based materials and CS NPs. The main sources of CS come from the conventional means of crustacean shells, or alternative fungal and insect biomass. The extraction process occurs through chemical or biotechnological methods. The last one can be categorized as fermentation-based or enzyme-based. The chemical methods have a lot of disadvantages (e.g., use of harsh chemicals and environmental pollution), but they demonstrate more commercial viability, as the process and the product are more controllable. On the other hand, biotechnological methods are considered more environmentally friendly. The different extraction routes demand different process control strategies as the risk of microbiological contamination cannot be assumed to be the same (Islam, Hoque & Taharat, 2023; Joseph, Krishnamoorthy, Paranthaman, Moses & Anandharamakrishnan, 2021). Further, it is important to establish critical microbiological parameters to be monitored, especially of CS destined for the production of sterile formulations. The purity of the raw material is an important quality control attribute. Some studies have demonstrated that protein contaminants can be the main causes of allergic reactions of CS-based products than the CS polymer per se (Amaral et al., 2016; Baldrick, 2010).

There are many methods for characterization of the main structural or colloidal parameters of CS (e.g., DA and MW) and CS NP formulations (concentration of NPs, hydrodynamic diameter, zeta potential, polydispersity index), however, there is a lack of standardization in the protocols. One critical issue that must be evaluated is the suitability of

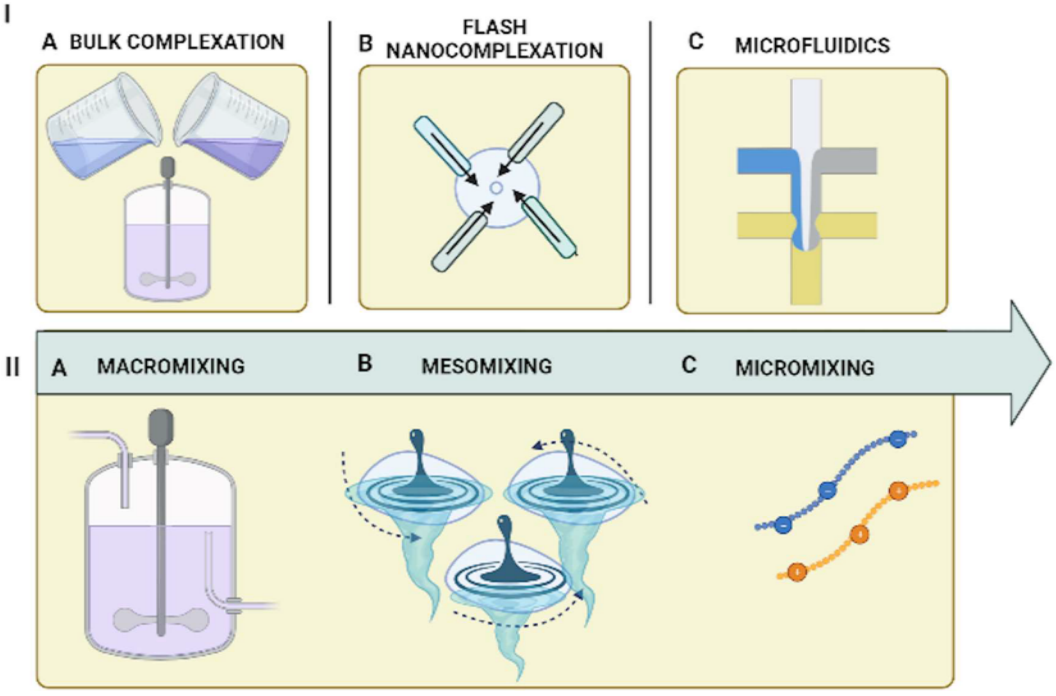

Fig. 2. Main technological gaps of CS NPs are concentrated in understanding the scaling processes. I – Techniques for obtaining NPs differ in their capacity to control the properties of the particles and in the level of reproducibility of the batches: (A) Bulk complexation is usually characterized by low reproducibility and unstable particle formation; (B) Flash nanocomplexation and (C) Microfluidics enables better control of reproducibility and properties of NPs in distinct batches. II – Mixing mechanisms for polyelectrolyte complexation of CS as a function of scale: (A) macromixing occurs at the vessel scale, (B) meso-mixing occurs at vortex scale and (C) micromixing occurs at the molecular scale.

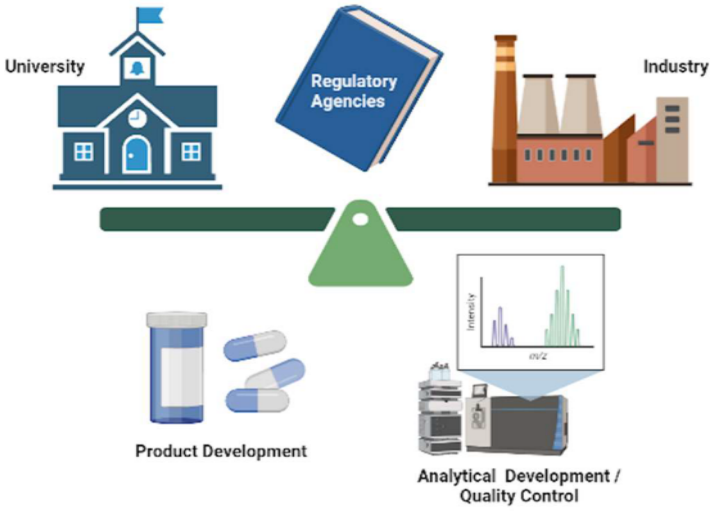

Fig. 3. The mitigation of risks of CS NPs-based products must start by integrating different expertise in the same framework: a collaborative effort among university-regulatory agencies and industry. Articulated actions between product development and analytical/quality control can facilitate research and registration of innovative new products.

the technique to measure properties of the nanometric scale as it usually differs considerably from macroscopic properties. Many properties of chitin and CS-based materials are scale-dependent (Lee, Hao, Park, Oh & Hwang, 2022). In this aspect, it should be discussed among specialists whether method validation is an important issue to be considered and what validation parameters should be evaluated in each case. Methods for simple characterization of physicochemical attributes are different from performance and stability indicating methods and, therefore, different rationales must be adopted (Bellich, D'Agostino, Semeraro, Gamini & Cesàro, 2016).

Although extensive monitoring of biological interactions is not mandatory for the quality control of conventional products, nanotechnology-based products must follow a different path. One of the main functionalities of nanotechnology-based products is in the nano-bio interface and, therefore, the reproducibility of CS NP dynamics in these environments should be considered a potentially critical attribute (Aibani et al., 2021). This will require a great deal of effort on the part of the researchers, as the control methods must have a great discriminative capacity to the point of detecting alterations in the interaction signatures that could impact the performance of the product. Here, it is also worth mentioning that these interactions will also depend on the validity of the chosen model and the main biological features that it can recapitulate.

4. Conclusions and perspectives

CS NPs are excellent DDS platforms. This review pointed to the research efforts that have been carried out to generate an overall cumulative knowledge that could culminate in innovative CS-based pharmaceutical products soon. The methods of ionotropic gelation and polyelectrolyte complexation allow the encapsulation of biomolecules with different physicochemical characteristics. There are several successful examples of obtaining CS NPs by combining this polycation with polyanions such as hyaluronic acid, alginates, and dextran sulfate, among others. This diversity of combinations translates into a therapeutic arsenal for cancer treatment, wound healing, immunomodulation, etc. However, the translation of the scientific knowledge of CS NP's material and biological properties into useful pharmaceutical products is not straightforward. It demands more in-depth evaluations of risk-benefit assessments as well as advances in different lines of research.

The perceived risk of using CS NPs in the development of pharmaceutical products must be mitigated by closing some important basic and applied gaps before consolidating CS NPs as reliable technological platforms. Here we raised the importance of constructing robust scientific knowledge combined with technological, regulatory, and CQ frameworks. Despite decades of active research on CS-based materials and CS NPs, a clear relationship unambiguously explaining the molecular mechanisms that control the particle properties and how the nano-bio interfacial interactions influence the biological performance of formulations has been still elusive. Further, new outstanding modern technologies (e.g., microfluidics and flash nano complexation) can be used for mass production of CS NPs, but it will require significant work to understand the scale-up rules. Advances in CQ nanoscale-specific methods and a descriptive regulatory model should be accomplished. This great demand for action must be articulated among the different stakeholders represented mainly by the regulatory agencies, academia, and industry.

Funding sources

This research was funded by the São Paulo Research Foundation (FAPESP) (Grant number 20/14062-1).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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