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# Food biopolymers-derived nanogels for encapsulation and delivery of biologically active compounds: A perspective review

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ABSTRACT

Nanotechnology is an emerging field in the food and pharmaceutical industries since it can fabricate various delivery systems for the encapsulation, protection, and controlled release of many different drugs, nutraceuticals, and bioactive compounds. The use of materials in the nanoscale ($10^{-9}$ m) may display distinct physicochemical and biological properties to overcome problems associated with poor water-solubility, stability, bioaccessibility, and bioavailability of these active agents. Food-derived proteins and polysaccharides or their combination are attractive materials to form complex encapsulation and delivery systems (e.g., emulsions, capsules, and micelles). Among these carriers, nanogels have emerged as a versatile hydrophilic delivery system to encapsulate hydrophilic and hydrophobic compounds with high load capacity and a capability to respond to environmental stimuli. Biopolymers-based nanogels can be used for various manipulations in food applications (functional and fortified foods) or biomedical applications (drug delivery) based on the nanotechnology concept. This review presents specific food proteins and polysaccharides used to fabricate protein-polysaccharide nanogels as the carrier of biologically active compounds and the corresponding characterization methods. The encapsulation, protection, and release of these components and the possible factors influencing the formation of biopolymers-based nanogels are also discussed.

## 1. Introduction

Bioactive compounds are components found in small amounts in fruits, nuts, cereals, and other plant sources (e.g., essential oils, vitamins, minerals, polyphenols, carotenoids, etc.). Certain bioactive compounds are natural antioxidants, which have numerous health benefits and anti-disease biological activities in humans. They appear to have beneficial health effects in the body by modulating physiological or cellular activities and have been particularly explored in the pharmaceutical and, more recently, in the food industry (de Souza Simões et al., 2017; Shishir, Xie, Sun, Zheng & Chen, 2018; Wei & Huang, 2019). Some of these bioactive compounds are natural antioxidants and anti-inflammatory. They also present other beneficial effects such as the decreased incidence of some diseases, such as cardiovascular, metabolic, and degenerative diseases, also acting in various forms of cancers (Shishir et al., 2018). Such effects may be positive or negative and it depends on the nature of the substance, the dose, and the bioavailability (Walia, Gupta & Sharma, 2019).

Developing functional food products enriched with bioactive compounds (e.g., phenolics) has been an emerging focus in the food industry. However, most bioactive compounds' functionality may be lost during food processing and storage (Peng et al., 2016). The oxidation of phenolic compounds by the action of oxygen (oxidative degradation) may lead to the formation of free radicals altering some sensory attributes (e.g., aroma, flavor) in functional foods, which may negatively impact shelf stability, sensorial features, and consumer acceptability (Gómez-Mascaraque et al., 2017). Furthermore, there are numerous challenges associated with the development of novel and more efficient functional food products enriched with pure bioactive compounds in biological formulations because of their particular characteristics, i.e., poor bioaccessibility and bioavailability, off-flavor, poor solubility, weakened physical stability, and inferior chemical stability during food processing and storage (Wei & Huang, 2019). To overcome these problems, novel encapsulation and delivery systems such as lipid-based techniques (e.g., nanoemulsions, liposomes), biopolymer-based techniques (e.g., single biopolymer nanocarriers, complex nanocarriers), nature-inspired techniques (e.g., cyclodextrins, caseins), and specialized equipment-based techniques (e.g., nanospray dryer, electrospinning) have recently been used for micro/nanoencapsulation of bioactive compounds in the food industry (Abaee, Mohammadian & Mahdi, 2017).

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Protein-polysaccharide nanostructures are generally developed at the nanoscale (<1000 nm) and may have different morphologies and properties depending on the material used in their construction. The small size of these nanostructures offers various advantages, in comparison to conventional delivery systems, due to the higher surface area-volume ratio obtained at this scale, and precisely targeted and controlled release of the loaded bioactive compounds and other active agents (Fasolin et al., 2019; Joye, Davidov-Pardo & McClements, 2014). This category of the delivery system includes emulsion-based and capsule-based delivery systems, molecular complexes, core-shell particles, composite nanoparticles, and micelles, including other systems. Among these carriers, biopolymer-based nanogels are of particular interest due to their high water stability, biocompatibility, biodegradability, well-defined structure, and a multifunctional possibility to be used in the fields of food, pharmaceuticals, and biomedicine, with a possible gradual and target release of the encapsulated compound (Lin et al., 2015; Wei & Huang, 2019; Zhang, Zhai, Wang & Zhai, 2016). Food biopolymers such as proteins and polysaccharides have gained attention as promising biomaterials for the fabrication of various encapsulation and delivery systems for bioactive molecules and nutraceuticals due to their abundance in nature, water-solubility, non-toxicity, low-cost processing, biodegradability, biocompatibility, and bioactivity. Furthermore, proteins and polysaccharides have several reactive functional groups on their backbone (e.g., carboxylic acid, hydroxyl, and amino groups), which contributes to their structural and functional diversity. They also show the capacity of assembling protein−polysaccharide complexes through hydrogen bonding and hydrophobic interactions, being suitable for specific applications in the delivery of bioactive compounds and other substances (Debele, Mekuria & Tsai, 2016; Lin et al., 2015; Peng et al., 2016; Zhou, Hu, Wang, Xue & Luo, 2016).

Nanoencapsulation of bioactive compounds produced by self-assembly of food-derived proteins and polysaccharides provides the possibility to create nanostructures that have various characteristics, such as biodegradability, biocompatibility, effectively controlled release, and site-specific delivery, combing the advantages of both biopolymers (Q. Zhang et al., 2021). Nanostructures can enhance some sensory features (e.g., masking undesirable flavors), improve bioaccessibility and bioavailability, prevent oxidative reactions preserving the bioactivity and control the release of bioactive agents, or even increase their quality and stability as well as enhance their bioactivity due to the reduced size of materials at the nanoscale (Fasolin et al., 2019; Rezaei, Fathi & Jafari, 2019).

Compared to other nano delivery systems, nanogels present some unique advantages, such as the higher loading capacity of bioactive compounds due to their swelling properties. For food industry applications, they can be produced from GRAS materials and, therefore, they have the potential to be secure, non-immunogenic, and fully/semi biodegradable. Nanogels allow the grouping of several bioactive ingredients in the same formula. They are capable of encapsulating both hydrophobic and hydrophilic compounds. For pharmaceutical industry applications, nanogels have the potential for passive or active targeting depending on specific disease phenotype, with decreased side effects. They are accessible to scale-up and biofriendly formulation routes, appropriate to various bioactive substances (medicines, proteins, antibodies, peptides, and vaccines). In addition, nanogels can be tailor-made to sense and respond to environmental changes to ensure the encapsulation stability and engineering sustained release of bioactive substances (Fasolin et al., 2019; Neamtu, Rusu, Diaconu, Nita & Chiriac, 2017; Wei & Huang, 2019). This review emphasizes the research progress in the formulation of novel food-derived biopolymer-based nanogels through a green self-assembly method by using the two major food macromolecules, proteins and polysaccharides, and their application in the encapsulation of bioactive compounds and active agents. In addition, different characterization techniques, challenges, and safety issues about the assembly of protein-polysaccharide complexes in the processing of functional food and pharmaceuticals are also discussed.

## 2. Food-grade materials used for nanogels fabrication

### 2.1. Food proteins

Proteins are biopolymers built with different amino acids linked by peptide bonds. They can be found in various food sources such as animals, plants, algae, or fungi. Despite the biological function of proteins in living organisms, proteins can improve the sensory perception of food products such as taste, flavor, and texture. Protein molecular structure and functionality and their roles in determining the functionality and chemosensory properties of food depend on their four levels of structure (primary, secondary, tertiary, and quaternary), mainly influenced by amino acids composition and the processing condition (Q. Zhang et al., 2021). Chemical properties also influence protein association and folding, such as amino acid groups, density or distribution of charges, electrostatic interactions, hydrogen bonding, hydrophilicity, and hydrophobicity. Environmental parameters (e.g., pH, ionic strength, solvent type, and temperature) influence the functional properties of proteins (Munialo, Euston & de Jongh, 2018). Consequently, food proteins have different active functional properties such as water-holding, fat holding, gel, foam, and emulsion formation capacities. Therefore, the protein functionalities can be differentially applied in food processing. For instance, gel-forming is one protein functionality of great importance to possibly produce nanostructures to be used as bioactive compounds encapsulant and delivery systems, using different sources of food-derived proteins (Aryee, Agyei & Udenigwe, 2018; Munialo et al., 2018; Q. Zhang et al., 2021).

#### 2.1.1. Soy protein

Soy protein is a natural polymer mainly used in the food industry due to its high nutritional content and ability to improve the texture of foods. Soy protein isolate (SPI) consists of two major globulin components, $\beta$-conglycinin (7S) and glycinin (11S) (Abaee et al., 2017). Consequently, because of their abundance, they are the main factors responsible for determining the functional properties of soy-based protein formulations (Munialo et al., 2018). Due to its natural abundance, good biocompatibility, and biodegradability, soy protein has been extensively tested as a starting material for the preparation of polymeric-based systems (e.g., nanogels) to be used as a carrier to encapsulate and deliver bioactive compounds (Abaee et al., 2017; Jin et al., 2016; Munialo et al., 2018). Stable and pH-sensitive soy protein-based nanogels were developed via self-assembly through heat treatment (95 $^{o}$C) protein dispersion at a pH value of 5.9. The authors concluded that disulfide bonds and hydrophobic interactions were the main forces involved in forming these nanogels (Chen, Lin, Sun & Zhao, 2014). In another study, soy $\beta$-conglycinin−dextran nanogels, prepared by the self-assembly of $\beta$-conglycinin-dextran Maillard conjugates followed by heat-gelation at the isoelectric point of the protein, were used to fabricate stable core−shell nanogels with a hydrodynamic diameter of about 90 nm (Feng et al., 2015). Modified soy protein and dextran were used to produce nanogels via self-assembly, which were used as a delivery vehicle and enabled the controlled release of riboflavin (Jin et al., 2016). The developed nanogels exhibited particle size in the range of 32–40 nm and encapsulation efficiency up to 65.9%. The resultant electrostatic interactions and hydrogen bonding between modified soy protein-dextran and riboflavin were stable against various pH. In a recent study, zein-soybean nanoparticles with 200 nm were fabricated to encapsulate the quercetin, which presented 82.5% of encapsulation efficiency (Li et al., 2019). The electrostatic, hydrogen bonding, and hydrophobic interactions were proposed as the main driving force for the entrapment of quercetin within the nanoparticles. These nanoparticles were relatively stable at high ionic strength and temperature.

#### 2.1.2. Whey proteins

Whey proteins are a mixture of highly nutritional proteins isolated from the cheese industry, such as $\beta$-lactoglobulin, $\alpha$-lactoalbumin,

bovine serum albumin, and immunoglobulins with molecular weights between 14 and 1000 kDa. It has been widely used in various foods due to its nutritional and functional characteristics, such as gelling and emulsification properties (Z. Zhang, Zhang, Chen, Tong & Mcclements, 2015). The physicochemical properties of the whey proteins and specifically $\beta$-lactoglobulin, the major whey protein, make them suitable for novel food and non-food applications. For instance, whey proteins are mainly used as hydrogels in nanoparticles systems for encapsulation, protection, and controlled release of different bioactive compounds. Depending on the technique, whey proteins can form hydrogels via heat-set and cold-set conditions (Gunasekaran, Ko & Xiao, 2007). Whey proteins have a negative charge above their isoelectric point (pH about 5) and a positive charge below it. Therefore, they can be used to assemble structures based on electrostatic interactions in the same way as the other biopolymers. Additionally, whey proteins show properties of emulsifiers and, thus, they can be used to form oil-in-water nanoemulsions in different encapsulation methods (El-salam & El-shibiny, 2012; Gunasekaran et al., 2007; Z. Zhang et al., 2015).

#### 2.1.3. Caseins

Caseins are a group of reasonably flexible proteins derived from milk, and in bovine milk, approximately 95% of the caseins exist as casein micelles. There are four main types of caseins ($\alpha$s1, $\alpha$s2, $\beta$, and $\kappa$), and each has unique structural and functional properties. These properties include binding with molecules and ions (e.g., ion binding, binding with hydrophobic molecules), surface activity, self-assembly, and co-assembly properties, gelation properties, pH-responsive gel swelling and contraction behavior, interactions with other proteins/polymers (e.g., covalent conjugations and non-covalent interactions), shielding capabilities, and biocompatibility and biodegradability (Gunasekaran et al., 2007; Z. Zhang et al., 2015). Casein micelles appear as roughly spherical shape aggregates with diameters ranging from 50 to 500 nm (average 150 nm) and a molecular weight between $10^6$ and $3 \times 10^9$ Da. The amphiphilic structure of caseins confers excellent surface properties and may be used to stabilize lipid droplets. Casein molecules have an isoelectric point around pH 4.6 and are positively charged at lower pH and negatively charged at higher pH. Due to the substantial reduction in electrostatic repulsion at the isoelectric point of casein molecules, casein-coated oil droplets, or casein molecules, the structure and the physicochemical properties of the caseins may be changed, which contribute to obtaining the precipitate fraction (casein-rich) at pH close to pI (El-salam & El-shibiny, 2012). Additionally, casein micelles are more resistant to thermal treatments than globular proteins due to their structure and flexibility. All these properties make caseins micelles useful for bioactive compounds and nutraceuticals due to their ability to host water-insoluble ingredients in their hydrophobic interior (e.g., carotenoids, vitamins, polyphenols, omega 3 oils, and other fatty acids, and minerals), which improves the water stability and bioavailability (Livney, 2010; Z. Zhang et al., 2015).

#### 2.1.4. Gelatin

Gelatin is a hydrocolloid polymer composed of a mixture of protein fractions. It is typically obtained either by partial thermal denaturation or by enzymatic degradation of animal collagen derived from skin, bones, and cartilages tissues. The primary sources of industrial gelatin are porcine skin or bovine hide and bone. Still, alternative sources such as poultry and fish byproducts can also be employed to manufacture gelatin (Devi, Sarmah, Khatun & Maji, 2017). Even though the molecular weight of gelatin is significantly lower than that of collagen, it may exceed 30 kDa, which is required for its gelation properties. Structurally, gelatin comprises different amino acids, and glycine, proline, and hydroxyproline are the predominant amino acid residues in collagen. The characteristics and functionalities of gelatin depend on its molecular structure and may have a better effect on the quality and its potential applications (Kang et al., 2019). Due to its specific features like amphoteric nature, good biocompatibility, biodegradability, low antigenicity, and multi-functionality, gelatin is extensively used in the food, nutraceutical, and pharmaceutical industries to manufacture capsules, coating of tablets, stabilization of emulsions, and drug delivery (Ranasinghe et al., 2020). The isoelectric point of gelatin occurs at pH 4.7, and below this value, the net charge on protein is negative. It has many applications, likewise as a form of complex coacervates, polyelectrolyte complex, and more recently, nanogel particles for the encapsulation and delivery of different bioactive compounds and small-molecules (Devi et al., 2017; Hamman, 2010; Kang et al., 2019; Ranasinghe et al., 2020).

#### 2.1.5. Hen egg proteins

Hen egg is considered a polyfunctional ingredient as it contains many functionally important proteins (egg white and yolk proteins). Due to its unique functional properties, including emulsifying, foaming, gelling, thickening, coloring, and aromatic characteristics, it can be used in various food products and non-food products. Ovalbumin and lysozyme are two main egg white proteins commonly used as delivery systems for bioactive compounds or pharmaceutical agents. Ovalbumin, a globular protein, is the major egg white protein ($^{>}$50%) and has an isoelectric point around 5. Its structure and properties are well-known, with a molecular weight of 45 kDa and 386 residues of amino acids. Each molecule has four free sulfhydryl groups (-SH) and one internal disulfide bond (S-S). Lysozyme is another highly functional globular food protein found in egg white (3.5%) and has an isoelectric point of 10.7. It is a small protein with a molecular weight of 14.3 kDa, having 129 amino acid residues, and is very rigid, stabilized by four disulfide bridges that lead to high thermal stability. Its many functional properties as a natural antibacterial and antiviral agent have led to several applications in different areas. In the food industry, lysozyme from egg white has been applied as a food preservative to promote shelf life and enhance the safety of products (Abeyrathne, Lee & Ahn, 2013; Anton, Nau & Lechevalier, 2009; Silvetti, Morandi, Hintersteiner & Brasca, 2017). Ovalbumin and lysozyme can be utilized to fabricate nanogels. Once these proteins are heated above their thermal denaturation temperature, the exposed functional groups can be linked to other biopolymers by intermolecular hydrophobic interactions, hydrogen bonds, and even electrostatic interactions. These protein interactions can be effectively used to stabilize the surface structure of nanogels in an aqueous solution, and they have been used to fabricate nanogels for controlled release of bioactive compounds (Chen et al., 2014; Yu, Yao, Jiang & Zhang, 2006; Zhou, Wang, Hu & Luo, 2016).

### 2.2. Food polysaccharides

Polysaccharides are the other commonly used food biopolymer for the fabrication of nanostructures to encapsulate and deliver bioactive compounds. Polysaccharides are polymeric carbohydrate molecules composed of at least ten monosaccharide units linked by glycosidic bonds. Depending on the composition of monosaccharide units, polysaccharides can be classified as homopolysaccharides, containing only a single type of monomer, or heteropolysaccharides, having two or more different kinds. Among several polysaccharides, the homopolysaccharides starch, cellulose, dextran, and the heteropolysaccharides chitosan, xanthan gum, pectin, and Arabic gum, are widely used in food matrixes. Many reactive functional groups, variable chemical composition, and different molecular weight ranges determine the polysaccharides' structure and available properties. For instance, various hydrophilic groups (e.g., aldehyde, carboxyl, hydroxyl, and amino groups) present on their molecular backbone can interact with other polymers to create a bioadhesive state, allowing appropriate tailoring of polysaccharide-based delivery systems for encapsulation of bioactive compounds (Barclay, Day, Petrovsky & Garg, 2019; Noreen, Nazli, Akram, Rasul & Mansha, 2017; Q. Zhang et al., 2021).

#### 2.2.1. Starch

Starch is the major digestible storage polysaccharides in nature, predominantly found in staple crops, cereals, and plant roots, including rice, wheat, maize, corn, barley, potato, cassava, etc. It is mainly composed of two types of biopolymers, amylose, and amylopectin, which are different in the chain structure. Amylose is a linear chain biopolymer composed of single monosaccharide glucose linked by $\alpha$-(1–4)-D-glycoside bonds. Conversely, amylopectin is a branched-chain biopolymer composed of $\alpha$-(1–4)-D-glycoside backbone with branches of $\alpha$-(1–6)-D-glycoside bonds. These two biopolymers are assembled in granules with dimensions ranging from 1 to 100 μm. Starch has been widely used in various foods and non-food products due to its thickness, gelling, and stabilizing properties. However, there are many limitations associated with native starches such as low solubility and poor functional properties, retrogradation, and limited enzymatic digestion. Currently, many chemical, enzymatic, and physical treatments have been employed to improve their available properties. Native and modified starch products have been extensively studied as promising biomaterials for applications in the food and pharma industries. Starch is a flexible polysaccharide to be used for drug and bioactive delivery systems due to its biodegradability and biocompatibility. Native starch hydrogel particles with a size range between 10 and 1000 nm have been widely tested for encapsulation and delivery of bioactive compounds (Ahmad, Gani, Hassan, Huang & Shabbir, 2020; Chin, Yazid & Pang, 2014; Kim, Park & Lim, 2015).

#### 2.2.2. Cellulose

Cellulose is considered the most abundant polysaccharide derived from renewable resources in nature, found in plants, natural fibers, and biologically synthesized by many living organisms such as sea animals, bacteria, fungi, and different plants. Cellulose comprises linear chains of glucose monomers linked by $\beta$−1–4 glycosidic bonds. Cellulose and its derivates are attractive components in nanotechnology for encapsulation and delivery of several compounds in many fields because of their suitable mechanical properties, biocompatibility, and biodegradability. In addition, cellulose has various reactive functional groups, (hydroxyl groups) on its backbone, which could be biochemically or chemically altered (esterification or etherification) to form different types of cellulosic compounds such as methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropyl methylcellulose, carboxymethylcellulose, and sodium carboxymethylcellulose. These cellulose derivatives usually have new functional diversity, such as better hydrophilicity, and facilitate the synthesis of nanogels with complex structures, which have been widely used as vehicles for bioactive compounds (Pan, Liu, Yang, Cai & Xiao, 2021; Siqueira, Bras & Dufresne, 2010; Syed, Garg & Sarkar, 2018).

#### 2.2.3. Pectin

Pectin is a natural heteropolysaccharide contained in plant cell wall components, basically composed of poly $\alpha$−1–4 galacturonic acid residues with varying degrees of methylation of carboxylic acid residues and a variety of neutral sugars such as rhamnose, galactose, and arabinose. The degree of esterification (DE) is an important parameter that influences the gelation mechanism, processing conditions, and properties of the pectin and may be defined as the percentage of the total number of carboxyl groups esterified. Regarding the DE classification, pectin can be separated as: (i) high-methoxyl pectin (HMP: DE>50%), and (ii) low-methoxyl pectin (LMP: DE<50%). Pectin is considered a food safety ingredient and has various other benefits such as emulsion stabilizer, gelling properties, and bonding abilities. Furthermore, pectin is biocompatible and mucoadhesive, and it is not digested by human gastric or intestinal enzymes, being almost totally degraded by microbial enzymes in the colon (intestinal microbiota). The pKa value of LMP is about 2.9–3.0. Above pH 4, LMP is highly negatively charged due to deprotonation of the carboxylic groups on the molecule segments, which can interact with positively charged segments of other biomolecules, such as proteins, via self-assembly through electrostatic interaction. These properties make pectin an efficient wall material to be used in pharmaceutical and food formulations through different kinds of pectin-based nanocarriers (Lin et al., 2015; Neufeld & Bianco-peled, 2017; Noreen et al., 2017; Rehman et al., 2019).

#### 2.2.4. Dextran

Dextran is a complex polysaccharide synthesized from sucrose by a different strain of bacteria such as *Leuconostoc, Streptococcus*, and *Acetobacter* species. It consists of monomeric glucose units with varying lengths approximately from 3 to 2000 kDa, linked with a linear 1,6-glycosidic bond with branching at 1,3-linkage. Dextran is well-known for its degradability by dextranase, biocompatibility, and non-toxicity. In addition, dextran-based nanoparticles can enhance water solubility, have a high drug-loading capacity and intrinsic viscosity, and are very stable. As a result, dextran can be used as a coating material to protect and improve the biocompatibility of bioactive molecules. For example, ovalbumin-dextran nanogels were fabricated via the Maillard reaction followed by a heat-gelation process to enhance curcumin bioavailability. The system increased the bioaccessibility and enhanced the chemical stability of curcumin (Debele et al., 2016; Jin Feng, Wu, Wang & Liu, 2016; Jin et al., 2016).

#### 2.2.5. Chitosan

Chitosan is commercially produced by chitin deacetylation through alkaline hydrolysis at high temperatures (100–160 °C) to remove the N-acetyl groups. Chitin is the second most abundant polysaccharide found in nature and is in the outer shell of crustaceans and insect exoskeleton. In addition to being productive because of its biological activities and physicochemical properties, chitosan is a very attractive biopolymer with a great prospect of encapsulating bioactive compounds. The amino groups of chitosan (pKa 6.5) provide solubility in a mildly acidic solution to the polymer and positive charges, promoting electrostatic interactions with polymers or polyanions in aqueous environments. Furthermore, chitosan is a nontoxic, biodegradable, biocompatible, and mucoadhesive biopolymer. For instance, it has been reported chitosan-based nanogels exhibit low toxicity and good biocompatibility (Barclay et al., 2019; Devi et al., 2017; Mohan, Oluwafemi, Kalarikkal, Thomas & Songca, 2016; Rahaiee, Shojaosadati, Hashemi, Moini & Razavi, 2015).

#### 2.2.6. Other polysaccharides

A range of food-grade polysaccharides of different sources is also convenient for manufacturing nanoparticles for encapsulation and delivery of bioactive compounds. Natural polysaccharide gums, such as guar, xanthan, carrageenan, and Arabic gums, can produce highly viscous aqueous solutions and form gels at low concentrations. They can create three-dimensional networks by intermolecular interactions between two different polymer chains, protecting and delivering bioactive compounds and other substances. For instance, protein-polysaccharide hydrogels have been reported based on attractive electrostatic interactions between xanthan gum and $\beta$-lactoglobulin. Also, gum Arabic, carboxymethylcellulose, carrageenan, and alginate were applied to prepare nanogels with egg yolk lipoprotein through nanospray dryer technique to deliver nutrients. It was suggested that hydrogen bonds, electrostatic and hydrophobic interactions were the main forces involved to fabricate nanogels (Barclay et al., 2019; Le & Turgeon, 2015; Zhou et al., 2016).

## 3. Biopolymer-based nanogels

Among the distinct food-grade materials, proteins and polysaccharides, or their combination, are the most attractive and promising biopolymers to form food-grade colloidal encapsulating and delivery systems due to their high abundance in nature, economical processing, biodegradability, biocompatibility, bioactivity, low toxicity, and good solubility. Additionally, proteins have particular interest due to their ability to form different structures, amphiphilic nature, high nutritional value, antioxidant activity, and functional properties, such as gelation,

foaming, and emulsification. Furthermore, proteins and polysaccharides have a variety of reactive functional groups on their backbone which can be easily manipulated to efficiently interact with many substances (e.g., bioactive compounds, drugs, and nutraceuticals) (Debele et al., 2016; Mohammadian et al., 2020; Wei & Huang, 2019).

Highly ordered structures of proteins and polysaccharides are obtained when they are linked with each other. The novel protein-polysaccharide complexes offer different functional and promising properties compared to single biopolymers, such as may enhance the formed complexes' solubility, gelling, foaming and emulsifying properties, surface activity, and conformational stability. These enhanced technological functionalities are due to the synergistic combination of both proteins and polysaccharides, dependent on the structural and conformational characteristics of various biopolymers. As a result of these multi-functional characteristics, biopolymer-based nanogels have been widely studied to protect and increase the bioavailability and the physical and chemical stabilities of the encapsulated compounds in food and non-food products (Mohammadian et al., 2020; Zhang et al., 2021).

### 3.1. Nanogel

Nanogels are defined as nanosized hydrogel particles structured by chemically or physically crosslinked hydrophilic or amphiphilic polymer networks. They have the exceptional swelling ability and can retain a large volume of water without losing their three-dimensional network architecture. The term "hydrogels" is used when the solvent utilized is water (Bourbon, Cerqueira & Vicente, 2016; H. Zhang et al., 2016).

Hydrogels can be classified based on their origin (e.g., natural, synthetic, and hybrid hydrogel), synthesis methods (e.g., homopolymer, copolymer, multipolymers, and interpenetrating polymeric network hydrogel), electric charge (e.g., neutral, cationic, anionic, ampholytic, and hydrophobically modified hydrogel), pore size (e.g., nonporous, microporous and super porous hydrogel), physical properties (e.g., conventional and environmentally sensitive "smart" hydrogel), configuration (e.g., amorphous, crystalline and semi-crystalline hydrogel), degradability (e.g., biodegradable and non-biodegradable), and crosslinking (e.g., physical and chemical crosslinking hydrogel). These systems have been used for food and pharmaceutical applications due to the fact they can entrap in their nanogel network substances and bioactive compounds and effectively enhance bioaccessibility, physical and chemical stabilities, and the controlled release properties (Abreu, Oliveira, Paula & Paula, 2012; Debele et al., 2016; Singhal & Gupta, 2015).

Nanogels can be formed by an extensive variety of different methods (e.g., coacervation, thermal-denaturation, emulsification, injection or extrusion, shearing, spray chilling, etc.). In general, novel approaches for the preparation of biopolymer nanogels are based on the self-assembly between proteins and polysaccharides through the non-covalent interactions (such as the electrostatic interactions, hydrophobic interactions, hydrogen bonding, and steric exclusion) or covalent interactions (enzymatic crosslinking, chemical crosslinking, and Maillard reaction). Typically, covalent crosslinking agents, such as genipin, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), and glutaraldehyde, are commonly used to enhance the stability between proteins and polysaccharides in the formation of nanogels, which may cause unwanted toxic effects and damage the entrapped delicate substances, such as cells, proteins, etc. On the other hand, physically self-assembly nanogels of proteins and polysaccharides, formed by thermal-denaturation gelation methods, are highly attractive for the assembly of nanostructure-based delivery systems, mainly due to the absence of toxic and hazardous crosslinking agents (Fig. 1). Furthermore, this approach is an environmentally friendly, cost-effective, and convenient technique (Neamtu et al., 2017; Wei & Huang, 2019; Zhang et al., 2021, 2015).

## 4. Application of nanogels in nanoencapsulation

### 4.1. Food-grade protein-polysaccharide nanogels fabricated via self-assembly

Nanogels are promising applications since they are ideal for encapsulating a variety of different bioactive substances (e.g., phenolic compounds, antioxidants, vitamins, anticancer drugs). However, the successful use of nanogels in food applications depends on food-grade and generally recognized as safe (GRAS) biopolymers rather than synthetic polymers or chemically-modified biopolymers and on the consumers' acceptance of functional foods enriched with nanostructures (Fasolin et al., 2019; Zhang et al., 2015). Thus, the fabrication of physically self-assembly nanogels of proteins and polysaccharides without toxic and hazardous crosslinking agents is fundamental to pursue human acceptability and security.

A wide range of colloidal delivery systems for pharmaceutical and food applications can be obtained from food-grade biopolymers, including polysaccharides and proteins, or their combination, to form biopolymers-based complexes through self-assembly methods, such as nanogels. In a self-assembly system, the molecules spontaneously organize in higher-order structures by noncovalent binding, mainly hydrogen bonding and hydrophobic and electrostatic interactions. Many studies have recently reported that globular proteins can form nanogels when heated above their thermal denaturation temperature under controlled solutions parameters, such as biopolymer concentration, pH, ionic strength, and holding temperature/time (Mohammadian et al., 2020). After the initial heat denaturation, proteins bind to the exposed non-polar groups through hydrophobic interaction. Protein-polysaccharide-based nanogels can be formed when the attractive electrostatic force is greater than the repulsive electrostatic interactions but not strong enough to precipitate the complexes and conjugates of biopolymers. Due to their high stability at a broad range of pH and salt, they are usually used to encapsulate hydrophobic bioactive molecules in physiological conditions that are thermally unstable. The globular proteins and ionic polysaccharides have been widely used to formulate nanogels via self-assembly through electrostatic interaction after heat treatment (Zhang et al., 2015). The resulting nanogels usually present a protein core with polysaccharides around it. In fact, in the molecular self-assembly of biopolymers, these nanogels can be formed either by heating a solution of globular proteins above their thermal denaturation temperature in a particular pH followed by complexing with an anionic polysaccharide or by directly heating the protein-polysaccharide complexes solution above the thermal denaturation temperature of the protein in a particular pH (Mohammadian et al., 2020; Zhang et al., 2015).

### 4.2. Nanoencapsulation of vitamins and antioxidant compounds

Novel nano-hydrogels were developed using food-grade and natural biopolymers of bovine serum albumin and citrus peel pectin through the self-assembly method. The nano-hydrogel was used as a delivery system to load the functional ingredient vitamin C. The results indicated that vitamin C encapsulation efficiency was about 65.31%, and the in vitro release mechanisms involved diffusion, swelling, and erosion. Furthermore, the stability of the nano-hydrogel systems was 73.95% after ten weeks of storage. Based on the results of this study, this novel self-assembly hydrogel system can be used as a potential carrier to improve the stability and bioavailability of functional agents (Peng et al., 2016).

In another study, it was fabricated a nano-hydrogel made of bovine serum albumin (BSA) using vitamin B6 bearing pullulan (VBBP) as bio-crosslinkers in the presence of zinc ions (Tsuchido, Sasaki, Sawada & Akiyoshi, 2015). As the result, the zinc ions enhanced the affinity between the protein and the bio-crosslinker and the ability of VBBP to form nanogels by crosslinking with the anionic BSA despite the electro-

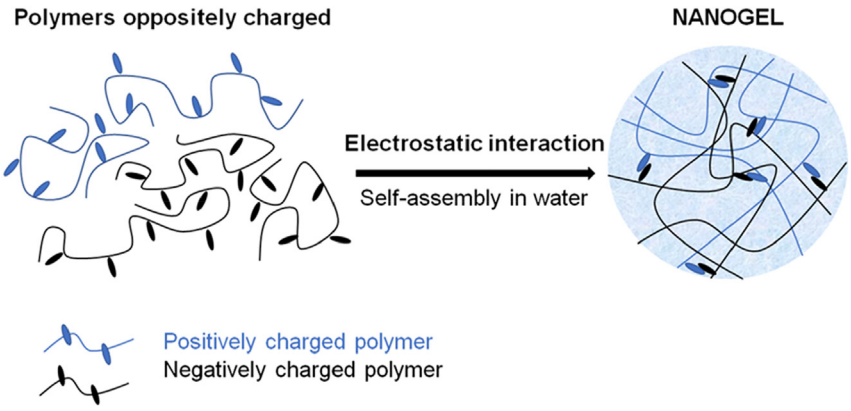


**Fig. 1.** Physically crosslinked nanogel in aqueous media. Schematic structures of nanogels fabricated via self-assembly method through electrostatic interaction between oppositely charged polymers.

static repulsion between the two chemical structures. Authors reported that the formation of hybrid nanogels via coordinated complexation of Schiff bases and zinc ions might be potentially employed in biomedical applications. For instance, they could be used for cytosolic protein delivery (Tsuchido et al., 2015).

Vitamin B2 was nanoencapsulated using dextran and thermally denatured soy protein through a self-assembling process (Jin et al., 2016). The nanogels were spherical with ~40 nm of diameter at pH 6. The encapsulation efficiency was 65.9% and riboflavin release was slower in the simulated gastric compartment compared to the intestinal one. The study demonstrated that nanogels prepared with dextran and thermally denatured soy protein exhibited nanoscale sizes and low distribution, a fair encapsulation efficiency with the delivery of riboflavin in its intact form (Jin et al., 2016).

A nanogel preparation was done to encapsulate folic acid since this compound could be degraded depending on pH, temperature, and light (Gazzali et al., 2016). Soy proteins and soy polysaccharides were self-assembled at pH 4 with folic acid being nanoencapsulated. The nanogels with encapsulated folic acid could be used for intestinal release since in neutral pH, folic acid was gradually released. Moreover, folic acid was not degraded at lower nor higher pH than 4, and the nanoencapsulated compound was also stable at high temperature, high incidence of light, and high oxygen pressures, being possible to use the nanogels in diverse beverages and foods (Ding & Yao, 2013).

Low-density lipoprotein (LDL) and pectin were used to prepare nanogels via a pH- and heat-induced facile process. The resulting nanogels were loaded with curcumin as a model compound with antioxidant and anti-inflammatory properties and potential anticancer activities. The optimal nanogels exhibited spherical with a diameter of 58 nm, smooth surface, homogeneous size distribution, and negative surface charge. The nanogels showed high encapsulation efficiency (up to 100%) and excellent stability in simulated gastric and intestinal conditions due to the protective effects of pectin coating that protected against the enzymatic digestion of the protein. The release behavior of curcumin from nanogels was investigated in three different pH (2.0, 4.0, and 7.5), and the results indicated a pH-dependent release behavior for the nanogels. The overall results revealed that the obtained nanogels had a great prospect as potential oral delivery systems for lipophilic bioactive compounds (Zhou et al., 2016).

### *4.3. Nanoencapsulation of anticancer compounds*

A simple green self-assembly method was also used to prepare nanogels based on bovine serum albumin and chitosan to entrap doxorubicin hydrochloride as an anticancer drug commonly used against a broad spectrum of tumors. The results revealed that the prepared nanogels showed a size range below 350 nm with a drug entrapment efficiency of 46.3%. They found that the doxorubicin reduced its cytotoxicity after encapsulation by the nanogels, and the loaded drug also showed a slow and sustained release within 24 h. More importantly, according to the results of cellular uptake assays, it was found that the doxorubicin-loaded into nano-hydrogels diffused faster into the cancer cells (76.4%) compared with free drug (54.6%) after 12h-incubation. Authors further concluded that food-derived biopolymers-based nanogels could be effective for encapsulation and controlled release not only of drugs but also other bioactive compounds (Wang et al., 2016).

Nanogels were also developed combining lysozyme (Ly) and sodium carboxymethyl cellulose (CMC) through self-assembly. The prepared nanogels were loaded with 5-fluorouracil as a model drug, which is clinically used to treat cancer, and their release ability was investigated (Zhu et al., 2013). The nanogels of the smallest size (241 nm) and highest encapsulation efficiency (30.48%) and loading capacity (10.16%) were obtained after heating 80 ºC for 60 min with a weight ratio of Ly:CMC = 2:1. In vitro release experiments showed that the release in the simulated gastric fluid was more slowly (33.9%) compared with that in simulated intestinal fluid (58.6%), which can ensure the site-specific delivery of 5-fluorouracil in the intestine (Zhu et al., 2013).

Self-assembled lysozyme/carboxymethylcellulose nanogels were also prepared by a green and sample method. A hydrophobic drug named methotrexate was adopted as a model compound to evaluate the drug encapsulation properties of the nanogels (Li, Xu, Zhang, Chen & Li, 2015). The resulting drug-loaded nanogels were of regular spherical shape, had a diameter of about 123 nm, and drug loading efficiency of 14.2%. The nanogels increased the bioavailability and anticancer activity of methotrexate. The nanogels made of lysozyme and carboxymethylcellulose can effectively load and slowly release the loaded drug, which may be favorable for chemotherapy. These findings might be used to extend the applications of nanogels for the delivery of drugs and other functional agents (Li et al., 2015).

A flexible nanogel formed by self-assembly of lysozyme and pectin was also successfully used to deliver the antitumor compound methotrexate (Lin et al., 2015). The resulting nanogels presented a loading efficiency of 17.58%. The nanogels revealed a pH-dependent release behavior and an accelerated release of drug in the intracellular acid conditions. The methotrexate-loaded nanogels possessed a higher anticancer activity in comparison with the free methotrexate. The obtained nanogels had good biocompatibility and low toxicity. In this regard, the authors reported the potential of lysozyme-pectin nanogels as excellent biocompatibility, biodegradable, and pH-sensitive delivery systems for enhanced anticancer efficacy.

## 5. Release mechanisms of active ingredients

The mechanism of controlled and targeted release of bioactive compounds from hydrogel particles plays an essential role in achieving better bioavailability. The release/controlled release process depends mainly on the substance-specific location over a sustained period (sustained-release) and environmental interactions with the hy-

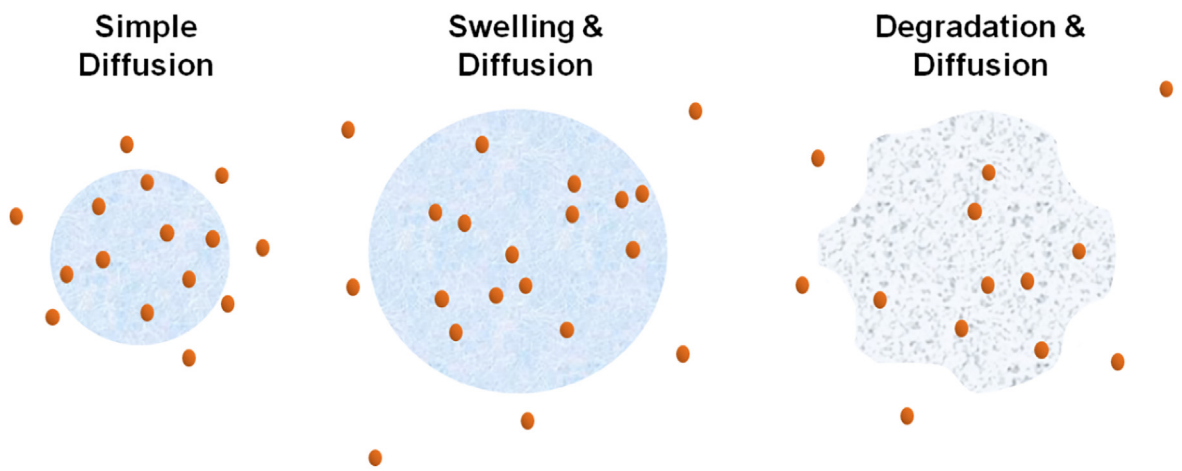


**Fig. 2.** Controlled substances release from biopolymer-based nanogels. Schematic illustrations of diffusion, swelling, and degradation release mechanisms of nanogels.

drogel and the physiochemical characteristics and compatibility of the hydrogel. In general, the release procedure of active ingredients from hydrogel delivery systems can be categorized as diffusion-controlled; swelling-controlled; and chemically-controlled (Fig. 2) (Zhang et al., 2016, 2015).

### 5.1. Diffusion

Diffusion is the most common mechanism of substance released from many types of hydrogel particles. The release property is primally dependent on the mesh sizes within the matrix of nanogel and the medium. For instance, entrapped small active ingredients can quickly diffuse from the mesh, whereas large molecules will have a sustained release. The release rate of both small-molecule and macromolecule should decrease as the medium viscosity increases (Fredenberg, Wahlgren, Reslow & Axelsson, 2011; Zhang et al., 2016).

### 5.2. Swelling

Swelling is another release mechanism of the nanogels in an aqueous environment, with an increase in volume. The physical dimensions of a hydrogel particle are established by the balance between the osmotic pressure and the polymer elasticity. For instance, when the functional groups of pH-responsive nanogels generated by polyelectrolyte networks are ionized, the osmotic pressure inside the nanogel increases due to entrapped counterions, which results in the swelling of the nanogels. The process of swelling is controlled by structural features of the nanogel such as chemical composition, the hydrophilicity of crosslinkers, and the degree of crosslinking, or by environmental parameters (Soni, Desale & Bronich, 2015).

### 5.3. Degradation

Degradation of a hydrogel particle can be triggered by surface erosion, bulk erosion, or fragmentation processes, resulting in the subsequent release of active compounds. The degradation of the nanogels may involve many physical and chemical phenomena, including diffusion of molecules into or out the particles, biopolymer degradation, and crosslink disruption. In summary, the physicochemical characteristics of natural polymers can be used to fabricate “intelligent nanogels” that will break under specific environmental stimuli, such as enzyme-induced, electrostatic-induced, hydrophobic-induced, and temperature-induced degradations. In this way, the control release properties of drugs and bioactive compounds under specific environmental conditions can be achieved by using different monomers to prepare nanogel particles with specific targeting and release properties (Zhang et al., 2016, 2015).

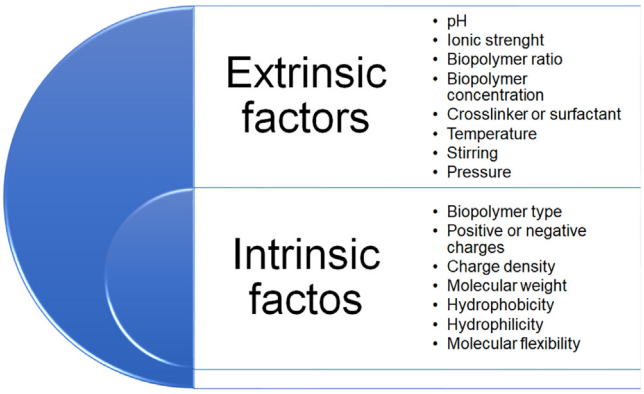


**Fig. 3.** Extrinsic and intrinsic factors and the formation of self-assembly nanostructures. Possible parameters that may affect the formation mechanisms of physically crosslinked biopolymer-based nanogels.

## 6. Factors affecting the formation of biopolymer-based nanogels

Interactions between proteins and polysaccharides play a significant role in controlling the structure and contribute to the quality attributes in food matrixes, including appearance, color, texture, flavor, taste, and stability of nanogels and the entrapped compounds. Protein-polysaccharide nanogels formed via self-assembly depend on the nature of these biopolymers and the environmental conditions. The dependence of both biopolymer’s characteristics with the environmental conditions resembles in innumerous combinations, but only with some of those combinations returning satisfactory methodologies for the optimal formation of nanogels. Thus, understanding the influencing parameters is essential for the fabrication of environmental nanogels with unique properties to be used in different areas (Abaee et al., 2017; Wei & Huang, 2019; Zhang et al., 2021). The influencing parameters can be divided into extrinsic factors and intrinsic factors regarding external physic-chemical parameters (e.g., pH, ionic strength, biopolymers ratio and concentration, presence of crosslinkers or surfactants, temperature, stirring, pressure) and physic-chemical parameters of the biopolymers (e.g., biopolymer type, charges, molecular weight and flexibility, hydrophobicity, hydrophilicity), respectively (Fig. 3).

### 6.1. pH

The main force involved in the formation process of physically crosslinked nanostructures is the electrostatic force. For the self-assembly mechanism of proteins and polysaccharides to occur, these

biopolymers must have opposite charges. The pH is an extreme modeling factor as it is primarily responsible for adjusting the degree of ionization of the functional groups of proteins (amino group) and polysaccharides (carboxyl group). In general, self-assembly between protein-polysaccharide complexes occurs when the pH of the mix solution is adjusted between the isoelectric point (pI) of the protein and the pKa of the polysaccharide leading to protonation of amino groups and deprotonation of carboxyl groups, respectively. As a consequence, it increases the electrostatic interaction due to increased charge difference between the reacting of a cationic protein with an anionic polysaccharide molecule (Zhang et al., 2015). Natural nanogels have successfully been fabricated via the self-assembly method through electrostatic interaction, for example between the positively charged chains of lysozyme and the negatively charged chains of pectin. The pI of lysozyme is around 10.7, while the pKa of pectin is approximately 2.9–3.2. At neutral pH, the two biopolymers are oppositely charged and attract each other due to the ionized amino groups of lysozyme ($NH^{+3}$) and the ionized carboxylic acid groups ($COO^{-}$) of pectin, leading to the creation of a new, more complex, and ordered structure (Lin et al., 2015; Neufeld & Bianco-peled, 2017).

### 6.2. Ionic strength

The ionic strength is another factor of great relevance in forming protein-polysaccharide gels and is generally influenced by the concentration of salts in the system. In high salt concentration, saline ions interact with the ionic groups available on the surface of these biopolymers, decreasing the electrostatic interaction between them and, consequently, reducing or preventing the formation of a new, more complex structure of greater magnitude. In addition to concentration, the class of ions used is also crucial for the gelling process. In general, studies have investigated the influence of ionic strength by the addition of mono (NaCl) and divalent ($CaCl_2$) cations (Abaee et al., 2017). In an experiment with the $\beta$-lactoglobulin protein and the xanthan gum, self-assembly was enhanced when the concentration of NaCl was small (<20 mM). In contrast, the electrostatic gel properties were suppressed at higher NaCl concentrations (>50 mM). In another study, this time with whey protein isolate and flaxseed gum, the formation of cold gels was evaluated by adding NaCl and $CaCl_2$ at a concentration of 150 mM. The authors observed that the properties of cold-set gels with whey protein isolate-flaxseed gum generated by $CaCl_2$ addition were stable, stronger, and capable of retaining more water plus lower deformability in comparison with NaCl (Kuhn, Cavallieri & da Cunha, 2011; Le, Rioux & Turgeon, 2017).

### 6.3. Mixing ratio and concentration

Another critical parameter that affects the electrostatic process is the mixing ratio and the final concentration of biopolymers in the medium in which they are suspended. The ratio of biopolymers in the system is a critical factor in controlling the charge balance and the interaction between these biomaterials available in the system. For the maximum interaction between protein-polysaccharide to occur, it is necessary to know the ideal proportion of each biopolymer for which the electrostatic interactions reach a balance between associative and repulsive interactions to allow gel formation with more excellent resistance at a specific pH. For example, the optimal fabrication conditions of novel nanogels prepared from lysozyme/sodium carboxymethyl cellulose, lysozyme/carboxymethyl cellulose, lysozyme/high-methoxyl pectin, and low-density lipoprotein/pectin were observed at the mass ratio of 2:1, 10:3, 1:1, and 4:1, respectively (Li et al., 2015; Lin et al., 2015; Zhou et al., 2016; Zhu et al., 2013). The total concentration of biopolymers in the system is also essential and must be considered in the gelation process from the interaction between protein-polysaccharide pairs. It has been described that the stability of the hydrogel generally increases as the concentration of the gelator or mixture of gelators (biopolymers precursor) increases. This is due to their functional properties such as gelling, solubility, and water-binding capacity, allowing the formation of an ordered gelled structure (hydrogels) with solid networks to enhance mechanical strength and regulate active agents' release behavior. For instance, physically cross-linked hydrogels between pectin-chitosan prepared at a constant total polymer concentration of 0.5% m/v, 1% m/v, or 2% m/v, as encapsulating matrices of three different compounds, demonstrated that as polymer concentration increases, the mesh size of the hydrogel decreases, leading to slower solution diffusion and slower release rates under simulated gastric conditions (Karoyo & Wilson, 2017; Neufeld & Bianco-peled, 2017; Warnakulasuriya & Nickerson, 2018).

### 6.4. Charge density and biopolymer-type

The protein-polysaccharide charge density and distribution, and polysaccharide-type is another critical parameter. It can determine the optimum pH and molar ratio to form a new biopolymer and influence the gelling property. In a study carried out with xanthan gum (>1000 kDa) and $\lambda$-carrageenan (300–600 kDa) polysaccharides, in interaction with different protein sources, a higher concentration of biopolymers for $\lambda$-carrageenan compared to xanthan gum was necessary due to their chemical composition. This observation can explain the higher charge density of $\lambda$-carrageenan (3 sulfate groups per disaccharide). Thus, although $\lambda$-carrageenan has a lower molar mass than xanthan gum, it has more reactive sites per unit length that can promote stronger electrostatic interactions with other macromolecules (Le et al., 2017). In addition to the charge density, the biopolymer nature and characteristics significantly influence intermolecular electrostatic interaction between oppositely charged partners. Flexible proteins (e.g., gelatin, casein) have more flexibility (or conformational entropy) to rearrange and to associate through hydrophobic interactions with other proteins and polysaccharides when compared to globular proteins. It is noteworthy that the protein conformation can change under different pH environments. Polysaccharides can have different side-chains depending on the composition of the monosaccharide units, which may cause steric hindrance thus decreasing interactions with proteins and the polysaccharide during physically crosslinked polymeric networks (de Souza Simões et al., 2017; Warnakulasuriya & Nickerson, 2018).

### 6.5. Other extrinsic factors

Other extrinsic factors, including temperature and stirring, may also influence the physically crosslinked interaction between biopolymers. Natural globular proteins are generally unable to form gels, as most reactive groups (mainly hydrophobic groups) are enclosed toward the interior. In contrast, hydrophilic groups are exposed outside of the molecule. Temperature is one of the main factors responsible for modifying the native structure of proteins by affecting the covalent and non-covalent interactions (hydrophobic interactions, hydrogen bonds, and electrostatic interactions). Lower temperatures favor hydrogen bonding, whereas higher temperatures favor hydrophobic interactions as they can promote the breaking and weakening of sulfhydryl (-SH) and disulfide bonds (S-S), leaving more reactive groups available to interact intermolecularly. For instance, a study investigated the influences of three kinds of polysaccharides ($\kappa$-Carrageenan, konjac glucomannan, and inulin) on lysozyme protein´s structure, activity, and stability under heated and unheated treatments. They reported that after heat treatment, $\kappa$-Carrageenan and konjac glucomannan improved the stability of complex systems even at a temperature higher than 70 °C, showing that the protein's structure and activity could be regulated by the interaction with polysaccharide at optimum temperature. Furthermore, a gentle stirring can contribute to better intermolecular interaction between macromolecule (Abaee et al., 2017; Nazir, Asghar & Maan, 2017; Xu et al., 2018).

## 7. Characterization techniques

Biopolymer-based nanostructures can be characterized by using most of the techniques used to identify polymers and biopolymers. Several properties can be determined through some characterization techniques such as size, polydispersity, degree of association, conformation, and interactions. Selected examples of interest are briefly presented to obtain a better understanding of the design parameters for processing and determination of the biopolymers' performance characteristics to be used in a wide range of applications due to their distinguishing properties (Mohan et al., 2016).

### 7.1. Particle size

The structural and morphological properties of biopolymer-based nanostructures such as sizes, polydispersity indexes, and surface charge differ significantly depending on the materials and preparation conditions used in their fabrication. The small size and surface area of the particles play a crucial role because they influence optical clarity, physical stability, physicochemical properties, encapsulation efficiency, controlled release characteristics, and biological activity of nanoparticle systems in drug delivery (Joye et al., 2014). Dynamic Light Scattering (DLS) is a traditional technique commonly applied for studying the diffusion behavior of macromolecules in solution in the range of a single nanometer to a few micrometers. The method is non-invasive, requires small sample volumes, and provides fast, precise (reproducible) particle characterization (Stetefeld, Mckenna & Patel, 2016). The polydispersity index (PDI) is dimensionless and used to describe the degree of non-uniformity of the size distribution of particles. The PDI value ranges from 0 to 1, where 0 refers to homogenous (monodisperse), i.e., non-interacting particles of perfectly uniform size, and 1 refers to highly heterogeneous (polydisperse), i.e., extensive particle size distribution. Particles with PDI values up to 0.05 are considered highly monodisperse. However, particles with PDI values greater than 0.7 indicate that the sample is highly heterogeneous, and the DLS technique may not be appropriate for analyzing polydisperse samples. In biopolymer-based systems, values of 0.2 PDI and below are deemed acceptable and indicate a monodisperse population of nanoparticle materials (Danaei et al., 2018).

### 7.2. Zeta potential

The zeta potential is the electrokinetic potential at the slipping plane or the shear plane of any particle in suspension, macromolecule, and colloidal systems (e.g., aerosols, foams, emulsions, colloidal suspensions, and colloidal dispersions) moving under an electric field. This method is used to determine the surface charge of the nanoparticles measured by a zeta potential analyzer. Measures of the zeta potential are of great importance to indicate the particle stability and propensity to aggregate. Typically, the zeta potential of colloidal systems more positive than +30 mV or more negative than −30 mV is considered strongly cationic and strongly anionic, respectively. Generally, colloids with high zeta potential in modulus (positive or negative) provide good stability. The existence of higher electrical charges on the surface of particles within the sample will repel each other and avoid aggregation. A zeta potential value minimum of ± 30 mV is desired for the excellent stability of electrostatically stabilized colloidal nanostructures in delivery systems. Several factors can influence the zeta potential measurements of suspensions, such as pH, additive concentration, and ionic strength, which impact their stability, especially in aqueous dispersions. Thus, a zeta potential titration curve of the particles in dispersion as a function of the pH is often used to determine the isoelectric point (or point of zero charges) and the surface charge under different conditions. At pH values close to the isoelectric point, colloidal systems lose stability and agglomerate or flocculate (Bhattacharjee, 2016; Clogston & Patri, 2011; Honary & Zahir, 2013).

### 7.3. Infrared (IR) spectroscopy

Infrared spectroscopy is an advantageous technique for providing a structural characterization of gels related to the intermolecular interactions present in biopolymers. Fourier Transform Infrared Spectroscopy (FTIR) reveals the composition of the various functional groups which can be detected by analyzing the full spectrum of samples. Furthermore, the FTIR technique can also be used to explore the intra- and intermolecular interactions among the molecules of proteins, polysaccharides, and biopolymers-based nanogels enriched with bioactive compounds (Karoyo & Wilson, 2017; Mohan et al., 2016). For example, the O–H stretching vibration peak in pure zein sample from 3230 $cm^{-1}$ to a higher wavenumber of 3311 $cm^{-1}$ for curcumin-loaded based on zein and propylene glycol alginate was studied, suggesting an increase in the average number of hydrogen bonding interactions during the formation of the complex particles. Additionally, the potential electrostatic attraction formed during the encapsulation process exhibited a significant change in the amide II peak from 1533 $cm^{-1}$ for zein to 1514 $cm^{-1}$ for the curcumin-loaded complex (Dai et al., 2018).

### 7.4. Microscopy methods

The morphological analysis of nanoparticles, in general, is related to external characteristics, such as pore size distribution, shape, and morphology of aggregation of the hydrogels. Two essential techniques in this area are scanning electron microscopy (SEM) and transmission electron microscopy (TEM). Depending on the resolving power of the SEM (high-resolution ≥ 0.4 nm), its environment, and the sample, it is possible to measure the size and size distribution of nanoparticles starting at 1 nm size with excellent visualization of simple structures (Vladár & Hodoroaba, 2020). TEM has been considered the golden standard for general nanoparticle characterization. TEM is a flexible technique that generates images in the range size from 1 to 100 nm allowing to determine the physical properties of nanoparticles (size, shape, surface topology, crystal structure, and general morphology), and also give insights into the morphology of aggregation and chemical composition of a great variety of samples (Mast, Verleysen, Hodoroaba & Kaegi, 2020).

### 7.5. Thermal analysis

Differential Scanning Calorimeter (DSC) allows investigating the thermal behavior and the physical properties of pure samples, their mixtures, and interactions with other substances as a function of temperature and/or time. Fundamentally, DSC allows the measurement of energy absorbed (the endothermic process) or released (the exothermic process) by a sample after heating, cooling, or keeping isothermally at a constant temperature. DSC has been widely used for polymeric materials as it can easily measure the thermal phase transition of the material. Furthermore, DSC can be used to measure several properties such as glass transition temperature, melting temperature, denaturation temperature, decomposition temperature, the enthalpy of unfolding in biomolecules, among others. DSC is a suitable indicator for any interaction that may occur between samples due to the process of polymerization (Neufeld & Bianco-peled, 2017; Rosales, da Silva, Lourenço, Hassimotto & Fabi, 2021; Ullah et al., 2019).

### 7.6. Other techniques

A variety of different other techniques are also available that can be used to study the physicochemical properties and stability of protein-polysaccharide complex nanostructures, including nuclear magnetic resonance (NMR), confocal laser scanning microscope (CLSM), atomic force microscope (AFM), X-ray diffraction (XRD), Isothermal Titration Calorimetry (ITC), rheology (e.g., ball drop method and viscosity), and computational methods. These methods provide a structural characterization of nanostructures in terms of the morphological features such

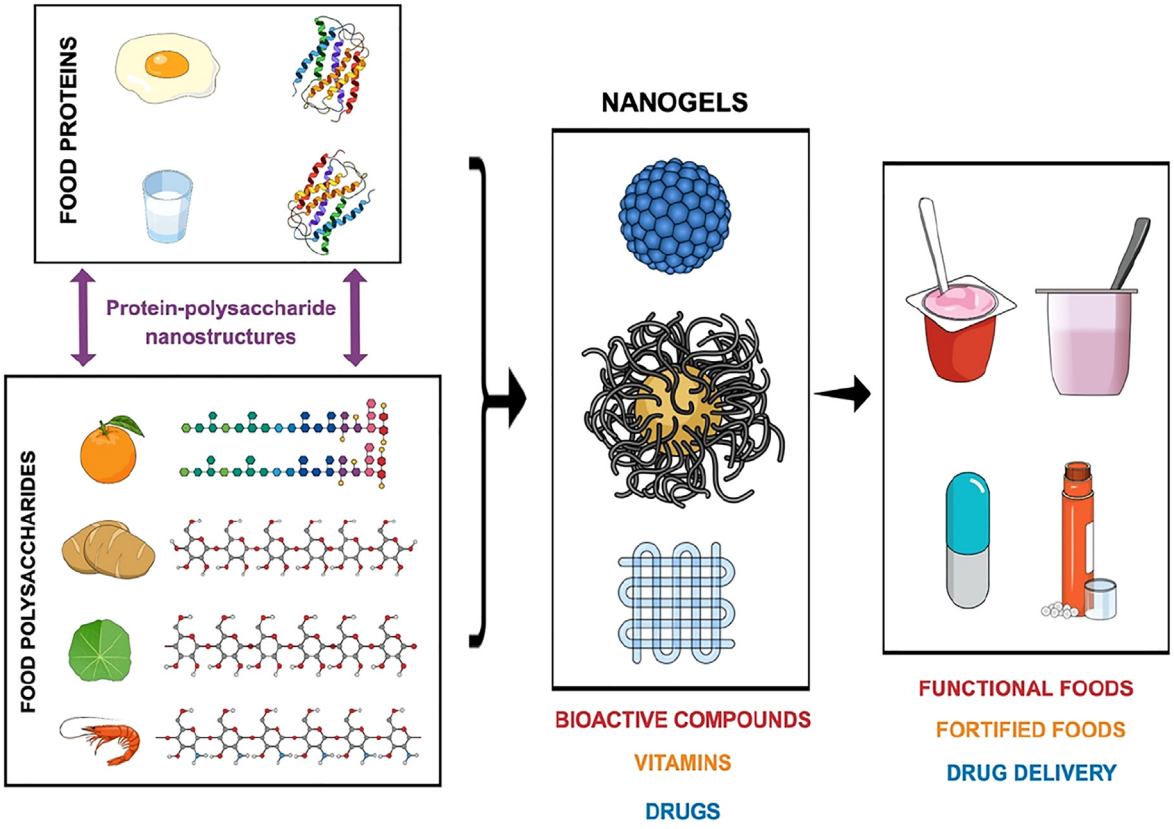


**Fig. 4.** Food-derived proteins and polysaccharides can be assembled in nanogels for the encapsulation, protection, and controlled delivery of many different food bioactive compounds, nutraceuticals, and pharmaceuticals compounds to create new functional foods, fortified foods and drug delivery. The figure was created with Mind the Graph (https://mindthegraph.com).

as surface and topography, visual appearance, structural characterization, elasticity and deformation, particle size and distribution, encapsulation efficiency, release mechanism, storage stability, intermolecular interactions, thermal properties, among other parameters (Karoyo & Wilson, 2017; Zhang et al., 2021).

## 8. Challenges and futures perspectives

Although biopolymers-based nanostructures are ideal for entrapment, protection, and controlled delivery of many different bioactive compounds, nutraceuticals, and drugs, there are still challenges in nanotechnology that are unique to nanostructures and nanomaterials. Efforts are needed to enhance the incorporation of bioactive compounds, increase their application in food products, and optimize their production processes. In this way, the successful use of biopolymer-based nanogels for nanoencapsulation and release behavior of bioactive agents depends on the selected suitable food-derived proteins and polysaccharides as carrier systems to form nanogels with carefully considering the overall structural characteristics and properties of the biopolymers and the specific needs of bioactive delivery. For instance, structurally designed nanogels prepared by the combination of food-derived proteins and polysaccharides with hydrophobic cavities are more appropriate to enhance bioavailability, stability, and control release behavior of hydrophobic bioactive compounds. Compared to other polysaccharides, chitosan can readily adhere to and infiltrate the intestinal mucus layer. Thus, chitosan-based nanogels have emerged as carrier systems to increase intestinal permeability and regulate substances release (Li et al., 2020; Wei & Huang, 2019).

Another concern is using some toxic and hazardous materials as crosslinkers between polysaccharides and proteins. Genipin, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC), and glutaraldehyde, are commonly used as crosslinkers in biopolymers-based nanostructures and control the burst release of bioactive substances. However, they may cause unwanted toxic effects and damage human cells. For food industry applications, nanogels must be produced from GRAS materials and, therefore, they need to be secure, non-immunogenic, and fully/semi biodegradable. In this regard, food biopolymer-based nanogels prepared by solvent-free, environment-friendly, cost-effective, and convenient techniques, provide additional challenges. Many materials commonly used to prepare biopolymer-based nanogels have been profoundly studied in recent years. Due to the lack of inexpensive, robust, and scalable methods, most of these biopolymer-based nanostructures are still in academic laboratories. Future works are still required in developing or implementing new processes to fabricate appropriate carrier systems that are economical and environmentally feasible for production. In addition, nanogels are being prepared to protect bioactive substances from being degraded or deactivated at some regions along the gastrointestinal tract but then release them at specific sites of action. The suitable combination of proteins and polysaccharides should be achieved as wall materials to entrap the active agents for targeted delivery. For example, pectin is stable at low pH and resistant to proteases and amylases in the upper gastrointestinal tract, but it is easily processed (fermented) by the colonic microflora. Pectin-based nanogels can be applied as colon-specific drug delivery due to prolonged retention in the gastrointestinal tract. Additionally, clinical studies and in vitro tests regarding the effect of these nanostructured biopolymers loaded with bioactive compounds should be carried out to fill the gap existing in efficiency, safety, and reproducibility of these delivery systems, regarding the physicochemical and sensory properties for developing enriched, healthy foods and functional products (Minhas, Ahmad, Anwar & Khan, 2016; Mohammadian et al., 2020; Wei & Huang, 2019).

## 9. Conclusion

Food-derived biopolymers-based nanogels are promising applications since they are ideal for the encapsulation, protection, and controlled delivery of many different food bioactive compounds, nutraceuticals, and pharmaceuticals compounds (Fig. 4). Nanogels made of protein-polysaccharide via self-assembly through electrostatic interaction, followed by the heat gelation process, may be fabricated from several natural biopolymers. These nanostructures can encapsulate hydrophilic and hydrophobic food bioactive compounds. Biopolymers-based nanogels offer advantages, including high nutritional value and distinct physicochemical and biological properties that can lead to novel material functionalities. Due to their versatile functional attributes, they can enhance dispersion, preserve chemical activity, improve bioaccessibility and bioavailability, and even control the release properties of bioactive compounds. Several factors may affect the assembly of protein-polysaccharide complexes and their functionality, such as types of biopolymers, concentration, the molar ratio between biopolymers, pH, and salt. It should be mentioned that the selection of the right biopolymers for the biologically active substances as well as the encapsulation techniques are essential features for a more comprehensive understanding of biopolymers-based complexes (e.g., nanogels) in developing a new encapsulated product. In summary, these nanostructures are ideal materials for the encapsulation and delivery of an extensive range of bioactive substances.

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Studies in humans and animals

The manuscript did not use studies in humans nor in animals.

## Conflicts of Interest

The authors declare no conflict of interest.

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