REVIEW

SYNTHESIS AND PROPERTIES OF BINARY AND TERNARY INTERPOLYELECTROLYTE COMPLEXES OF CHITOSAN

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Abstract

This review provides a comprehensive summary of the results of research, published from 2002 to 2023, on the synthesis, physicochemical properties and medical applications of binary and ternary interpolyelectrolyte complexes (IPECs) based on chitosan, carboxymethylcellulose and collagen. The application of IPECs in the medical field mainly refers to their use in medical therapy and bone regeneration. In addition, we describe our original studies on IPECs based on chitosan and collagen.

Keywords: *chitosan, sodium carboxymethylcellulose, collagen, binary and ternary interpolyelectrolyte complexes, polyanion and polyampholyte*

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Abbreviations

ALg-Na	– sodium alginate
BPE	– block polyelectrolyte
BSSE	– basis set superposition error
CAS	– cellulose acetate sulfate
CMC	– carboxymethylcellulose
CMCS	– carboxymethylchitosan
Coll	– collagen
CPLG	– colloidal polylactide glycolide
CS	– chitosan
FTIR	– Fourier-transform infrared
IL-2	– invitrogen (2 μg/ml biotinylated anti–IL-2)
IPEC	– interpolyelectrolyte complex
LPE	– lyophilised polyelectrolyte
MW	– mesoporous wollastonite
Na-CMC	– sodium carboxymethylcellulose
NISPEC	– non–stoichiometric interpolyelectrolyte complex
NPs	– nanoparticles
PEC	– polyelectrolyte complex
PF	– phosphate fibronectin
PLG	– polylactide glycolide
P(LLA-CL)	– poly(L-lactide-co-ε-caprolactone)
RT-PCR	– real-time polymerase chain reaction
SEC	– sulfoethylcellulose
TPP-Na	– sodium tripolyphosphate
ΔE_{inter}	– interaction energy

1. Introduction

Interpolyelectrolyte complexes (IPECs) are formed through the interaction of polycations and polyanions/polyampholytes. Polymer–polymer complexes have ordered and disordered structures. The actual structure of IPECs is between an ordered and a disordered structure [1]. IPECs are formed in aqueous dispersions, through spontaneous association of oppositely charged polyelectrolytes due to strong but reversible electrostatic interactions. Depending on the main characteristics of the chosen polymers, IPECs exhibit special physicochemical properties due to intermolecular electrostatic interactions and flexibility. For example, when mixing two or more aqueous solutions of polyelectrolytes in a stoichiometric ratio, the resulting IPECs are insoluble and precipitate, often in the form of a colloid [2].

IPECs are formed via hydrogen bonds and electrostatic interactions between oppositely charged functional groups, as well as changes in entropy during the release of counterions that were bound to free polyions. In stoichiometric IPECs, phase separation can be avoided if at least one of the polyelectrolytes contains non-ionic hydrophilic groups. This is the case in block ionomer complexes that form 'core-shell' structures with a PEC core stabilised by the hydrophilic shell of a non-ionic moiety [3]. The uniqueness of the IPEC formation method is associated with the simplicity of its technological design, the low cost of the process, low energy consumption and high efficiency. Non-stoichiometric complexes containing an excess of one polyion have increased sorption activity with respect to drugs, dyes and proteins, because they have a net charge [4].

An excess of polyanion complexation leads to the formation of non-stoichiometric IPECs (NIPECs), which are virtually block copolymers with hydrophilic areas represented by free anionic units and hydrophobic fragments of mutually neutralised anionic and cationic units. The negative charge confers colloidal stability on NIPECs in aqueous solutions, facilitating their binding to d-metal ions or positive dispersion particles [5]. At present, according to special terminology, an NIPEC comprises a lyophilising polyelectrolyte (LPE) and a counter-charged block polyelectrolyte (BPE). In IPECs, there is an excess of LPE relative to BPE units – that is, the [BPE]/[LPE] ratio is < 1. Water-soluble IPECs can be obtained under varying conditions from oppositely charged synthetic and natural polyelectrolytes [6].

Given that CS can be modified in various ways to produce numerous complexes – in particular, binary and ternary IPECs – it is essential to study their physicochemical properties and application prospects. This review summarises the research on the preparation and use of IPECs in bone therapy and its engineering.

2. Preparation and Physicochemical Characteristics of Interpolyelectrolyte Complexes of Chitosan and Anionic Polyelectrolytes

Many researchers have prepared and characterised IPECs based on a cationic polyelectrolyte, chitosan (CS), and different anionic polyelectrolytes. Table 1 and the following text summarise the preparation methods and physicochemical properties of some IPECs.

Cerchiara et al. [7] obtained PECs based on CS and carboxymethylcellulose (CMC) for the delivery of vancomycin to the colon. They prepared various batches of PECs, using three different CS/CMC weight ratios (3:1 to 1:3) and collected them as microparticles by spray-drying. They also investigated microparticle water uptake and vancomycin release as well as its protection against gastric pepsin degradation. They selected the best formulation, CS/CMC 1:3, based on the encapsulation efficiency, water uptake and the drug-release rate [7].

In another study, the authors assessed the influence of the CS/CMC mixing ratio (1:0 to 0:1), temperature (25–85°C) and pH (3–4.5) during the preparation of macro- and micro-PEC. A mixing ratio of 1:2 and a temperature of 25°C maximised the interaction between the biopolymers and the formation of macro-PECs regardless of pH. Micro-PECs had a uniform appearance, while macro-PECs were porous mesh structures interspersed with vacuoles of different sizes. Macro-PECs were amorphous, which is optimal for encapsulating technologically important compounds [8].

CS/CMC complexes with a porous structure were obtained at a mass ratio of 1:1, after which the solution was poured into a glass beaker (1 mm thick) and the process continued in a water bath at 70°C for 12 h. The sample was lyophilised for 48 h [9].

IPECs based on CS and Na-CMC were synthesised and cross-linked with glutaraldehyde at pH 5.5 and a 1:1 mass ratio. Based on the zeta potential of the samples, the polymers interacted electrostatically [10].

CS/Na-CMC complexes were obtained by mixing a 1% solution of Na-CMC and a solution of CS in acetic acid by varying their ratios. Na-CMC and CS powders were added gradually to the solution with constant magnetic stirring for 1 h at 22°C to prevent gel formation. After complete dissolution, the studied solutions were titrated with hydrochloric acid (HCl) to pH 1 and then incubated in a cabinet for 12 h [11].

According to Zhao et al. [12], a CS/Na-CMC PEC was formed in solutions with the addition of 0.01 M CS and 0.01 M Na-CMC based on ionic interactions between

oppositely charged functional groups. The authors dried the PEC in vacuum at 60°C for 1 day and stored it in a nitrogen atmosphere.

A nanofibre based on a CS/Na-CMC ratio of 4:6 (w/w) was stable. The nanofibre made of a CS/Na-CMC ratio of 4:6 (w/w) presented the weakest fibroblast adhesion [13].

Using the method of sublimation drying, a biocomposite sponge was obtained containing CS/CMC with different concentrations of mesoporous wollastonite (MW) particles. Adding 0.5% MW to the CS/CMC sponge significantly improved the biomineralisation properties and adsorption of protein particles. The CS/CMC/MW sponge exhibited effective cytological properties against human osteoblasts, which is recommended for bone engineering. The osteogenic potential of the CS/CMC/MW scaffold was confirmed by calcium deposition and the enhanced expression of an osteoblast-specific microRNA, namely pre-mir-15b [14].

CS/CMC IPECs in a stoichiometric ratio of 1:1 (w/w) were obtained by titration to obtain thin films with antibacterial properties for biomedical use [15].

The thermal properties of CS/CMC IPECs were studied when glycerol or formaldehyde was added. The decomposition temperature of the initial CS was 120°C. The sample obtained by cross-linking with 0.2% formaldehyde exhibited high thermal stability at 220°C. Moreover, IPECs obtained with the addition of 0.4% formaldehyde in a mixture of CS/CMC (99:1) and glycerol (2%) were stable at 150°C [16].

At a CMC/CS ratio of 75:1, there was no blurring when CMC was added to the CS solution. When the CMC solution was added to the CS solution, the interaction between the polymers improved and a cloudy suspension was formed. When CMC was added to CS at a ratio of 75:1, agglomeration was observed under the microscope, and when CS was added to CMC, visible particles were formed [17].

Hydrogels based on CS and Na-CMC containing 1% hydrocortisone were prepared by mixing solid and liquid components. The solid components were hydrocortisone, Na-CMC and CS, and the liquid component was polyethylene glycol, obtained by mixing with paraffin, glycerine and distilled water [18]. In another study, researchers obtained CS/Na-CMC hydrogels [19], as presented schematically on Figure 1.

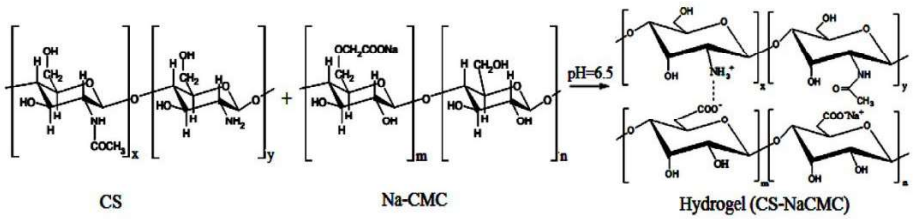


Figure 1. A scheme for the preparation of a hydrogel based on chitosan (CS) and sodium carboxymethylcellulose (Na-CMC).

The presence of intense absorption band at about 1600 cm^{-1} in the Fourier-transform infrared (FTIR) spectrum of a hydrogel showed that CS interacts with Na-CMC via positively charged $^{+}NH_3$ and negatively charged $–COO^{–}$ groups [20].

In another study, researchers prepared ionic gel microcomposites based on CS/CMC by adding various concentrations of the chelating agent sodium tripolyphosphate (TPP-Na) – 0.2%, 0.4% and 0.6% – dropwise to a 0.4% CS solution. Then, a 0.4% CMC solution was prepared and added dropwise to the CS solution chelated with TPP-Na.

They stirred the solution for 2 h and then isolated the target product by centrifugation for 15 min. The authors freeze-dried the microcarriers prepared with TPP-Na and named them CS/CMC-T1, CS/CMC-T2 and CS/CMC-T3 [21].

Inphonlek et al. [22] obtained colloidal polylactide-glycolide (PLG) particles and stabilised them by oppositely charged CS/Na-CMC complexes. They pre-emulsified dichloromethane containing dissolved PLG in the aqueous phase in mixtures of CS and Na-CMC. Evaporation of dichloromethane from the resulting emulsion forms PLG particles coated with CS/Na-CMC. The zeta potential varied from +54 to –50 mV, and the pH was 3–10.

CS and Na-CMC, as well as Egyptian clay, were used to prepare a composite hydrogel. A specific amount of clay (0–10 wt%) was added slowly to a 1 wt% CS solution, stirring for 1 day and then poured into a bottle. At 40°C, the membranes were incubated in a 1% Na-CMC solution for 24 h, repeatedly washed with distilled water and dried in a vacuum oven at 40°C [23].

In another study, the researchers obtained hydrogels with the solid components Na-CMC; methylcellulose (MC); and 3% Carbopol 934 P with 1% hydrocortisone and 1% CS. The liquid component was a hydrophilic agent (1,2-propylene glycol) mixed with dimethylacetamide and distilled water. They also obtained polymer hyaluronic acid gels containing various medicinal substances [24].

PECs based on CS and CMC were prepared by 'dry' thermomechanical mixing. The resulting films were stable and structurally intact (27% expansion and 94% weight increase after 1 day of hydration). The resulting films swelled sharply, increasing in volume by 138% and in weight by 913%, and were also brittle. The authors reported calculations for the formation of a PEC containing CS and CMC, the difference in the high hydrolytic stability of CS/CMC films with their high surface hydrophilicity and the contribution of CMC [25].

Researchers evaluated a two-layer polysaccharide coating containing CMC and CS regarding its effectiveness in preserving the post-harvest quality of tangerine and orange varieties. This CMC/CS coating was effective as a commercial polyethylene wax to improve the presentation of the fruit. Moreover, this CMC/CS coating did not negatively affect the taste of the fruit [26].

Researchers obtained a polymer-polymer mixture based on CS and Na-CMC with the trade name 'UZXITAN', which is used to encapsulate agricultural crop seeds. It is non-toxic, environmentally safe, helps suppress phytopathogens and stimulates plant growth and development [27].

In another study, researchers evaluated the effect of pH on the formation of CS/CMC IPECs and the viscosity and sorption–diffusion properties of the resulting films based on them. The IPEC films formed in an acidic medium (0.05 N) contained 2–2.5 times less water than films of the same composition obtained in an acetate-buffer medium [28].

According to Baklagina et al. [29], a PEC is formed due to the interaction of charged functional groups of CS and sulfoethylcellulose (SEC):

$$C\text{-}O\text{-}(CH_2)_2\text{-}SO_3^-Na^+ + Ac\text{-}NH_3^+\text{-}CS \rightarrow C\text{-}O\text{-}(CH_2)_2\text{-}SO_3^-NH_3^+\text{-}CS + Na^+ Ac^-.$$

To obtain a stoichiometric insoluble complex, the authors mixed 2% solutions of polyions (CS and SEC) in an equimolar ratio. They stirred the solutions for 30 min, separated the PEC from the insoluble solvent, washed it, vacuum dried it and heated it at 100°C for 1 h [29].

An IPEC containing cellulose acetate sulfate (CAS) with a CS/CAS ratio of 1:1 (mol/mol) at pH 4.3 was obtained by mixing 0.1 M acetate buffer solutions of the starting components. The authors demonstrated that it was an effective additive for increasing the biovalue of chicken eggs and broiler meat during flocculation purification [30, 31].

Researchers studied the colloidal optical properties of IPECs containing CS and CAS (at a molar ratio of 1:1.5). In buffers, the particle size increased compared with acetic acid, and their concentration was reduced. The authors found that the addition of low-molecular-weight electrolytes to excess CS as well as an ionic strength of 0.2–0.3 led to the dissolution of IPECs. Complete dissolution occurred at an ionic strength of 1.5–2 [32].

In another study, the authors evaluated the thermal properties of an IPEC prepared with a CS/CAS molar ratio of 2:1. The derivative thermogravimetry (DTG) curve had a peak at 490 K – between 473 K (for CAS) and 563 K (for CS) – and the differential scanning calorimetry (DSC) curve was not exothermic at 566 K. Thermal studies showed that the formation of a new compound based on cadmium (Cd) and CAS reduced the influence on the DTG and DSC curves [33].

Researchers prepared IPECs containing CS and melanin prepared with ratios from 10:1 to 1:1. During thermal analysis of the initial components and complexes, there was mass change with the release of water for the range of 25–100°C [34].

The gelling properties of IPECs based on CS and sodium alginate (ALg-Na) were studied. Changes in the binding between ALg-Na and CS significantly affected the formation of the internal part of the cryogels. The densest gel, with ALg-Na/CS = 1.5 mol, had the most stable mesopore [35]. Based on scanning electron microscopy, the particles were 40–120 μm in size [36, 37]. The FTIR spectrum showed a CS absorption band at 1651 cm^{-1} (δ-NH_2), characteristic of the NH_2 group, and ALg absorption bands at 1603 and 1412 cm^{-1}, characteristic of the carboxylate ion group. These results indicate electrostatic interactions between them that underlie IPEC formation [38].

When preparing IPECs, complexes tend to aggregate due to charge neutralisation. Therefore, to avoid aggregation and to control the size of nanoparticles, at least two conditions are mandatory: the PEC solutions must be diluted, and one of the polyions must be in excess to maintain the inequality $(n^+/n^-) \neq 1$ (Figure 2) [39]. If the charge ratio is greater or less than unity, then the resulting nanoparticles have the same charge as the polyion in excess. Experiments have shown that at $n^+/n^- = 1$, particles are uncharged and large aggregates are formed as a result of agglomeration.

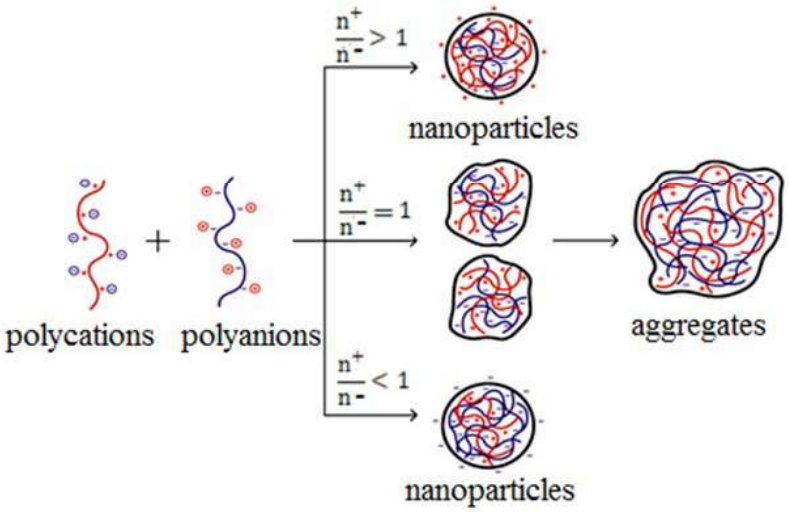


Figure 2. The influence of the charge ratio of polyelectrolytes on the size and charge of the formed polyelectrolyte complexes [39].

Researchers mixed solutions of CS-hyaluronic acid (CS molecular mass = 30 × 10^3; CS 0.1–8 wt%) and pectin (molecular mass = 26 × 10^3; 0.1–2 wt%) in phosphate buffer at pH 7.4 and 22°C to obtain IPEC hydrogels. The gel was formed due to the interaction between CS (-NH_3^+) and pectin (-COO^-) in solution [40]. The turbidimetric titration revealed that the CS/hyaluronic acid IPEC was stable at 573 nm. When mixing acetate-buffered solutions of the starting components, there was precipitation in the pH range of 3.6–6.5 [41].

Finally, researchers prepared CS/heparin complexes with mass ratios of 2:1 and 3:1. The particles were relatively small, with an average diameter of 176–192 nm. The most compact particles were obtained from a CS/heparin ratio of 2:1; they had low polydispersity and were stable for 72 h. These properties may be due to the strongest complexation of macromolecules [42].

3. Preparation of Interpolyelectrolyte Complexes Containing a Cationic Polyelectrolyte, Chitosan and Collagen

Researchers mixed 0.5% solutions of CS and collagen in different proportions (0:100 to 100:0) for 1 h, and degassed the mixture under a vacuum and then pipetted it into each cell of a 24-cell structure. They were freeze-dried by vacuum lyophilisation at –40°C for 24 h and created a porous structure and [43, 44].

CS and collagen were dissolved in 2% acetic acid at a concentration of 3 g/dl, stirred at room temperature for 6 h, poured into a mould and dried at –70°C. The resulting collagen/CS sponge was neutralised with a solution containing 0.5 N sodium hydroxide (NaOH) and ethanol, washed with a solution containing water and ethanol, and lyophilised [45].

In another study, the authors studied the morphology of cellular structures obtained from fibres based on CS/collagen complexes. Cells growing at the submicron level, especially films of CS and collagen fibres, had a greater length, up to 100 nm, compared with the control obtained on glass substrates [48].

Thin films were obtained based on solutions of CS, collagen and hyaluronic acid. First, the researchers obtained CS/hyaluronic acid complexes with three ratios (80:20, 50:50 and 20:80 wt%). Then, they added the CS/hyaluronic acid complex to the collagen solution in various ratios (10–90 wt%). The average thickness of the resulting films was 30 μm [49].

Researchers obtained IPECs with CS and gelatine. The interaction only occurred at a pH > 4.7 but < 6.2. Natural polymers are involved in the healing of living tissues and skin restoration. Hydrogels obtained based on cross-linked natural polymers and PECs can be used for the prevention and treatment of trophic wounds and burns [50, 51].

The porous structure of CS-coated collagen membranes was characterised using scanning electron microscopy. The pore size was 20–50 μm [56].

In one study, researchers evaluated IPECs containing collagen and CS at a ratio of 7:3 and with or without 0.5% or 1% β-glycerol phosphate. The maximum strength of sponges was 8–10 MPa. The addition of β-glycerol phosphate to the collagen/CS complex increased the strength of the sponge [57].

The physicochemical and dielectric properties of collagen/CS films (100:0 to 0:100 wt%) were studied. The highest dielectric constant of the CS film was at 300 K, which increased the electrical conductivity from 2.4 × 10^{-19} to 3.4 × 10^{-17} m^{-1}, and the activation energy ranged from 0.38 to 0.77 eV. When CS was added to the collagen film, the electrical conductivity of the collagen increased [58].

The catalytic activity of collagenase molecules available for binding, obtained from Clostridium histolyticum, under the influence of ultraviolet radiation

(151–6040 J/m^2) showed greater modulation than in the case of immobilisation in a CS matrix. After ultraviolet irradiation of the enzyme solution at 3020 J/m^2, the activity of the biocatalyst solution decreased by ~9%. However, when collagenase was immobilised in an acid-soluble CS substrate, the efficiency of the enzyme at all studied doses did not change [60].

Table 1. Preparation and properties of interpolyelectrolyte complexes of chitosan (CS) and collagen and chitosan/gallic acid and collagen.

Preparation and composition of interpolyelectrolyte complexes	Properties	Ref.
Chitosan/collagen		
Collagen/CS matrices (weight ratio from 1:0 to 0:1) were obtained from 1% biopolymer gels by lyophilisation.	The porosity of the membranes was characterised by fractal geometry from scanning electron microscopy. It was dependent on the CS content.	[46]
Composite collagen/CS hydrogels (weight ratio from 0:1.0 to 1:0) were prepared by mixing 0.1 M CS solution in hydrochloric acid; they also contained fish collagen and disodium β-glycerophosphate.	Mechanical properties were studied by rotational rheometry. The presence of fish collagen negatively affected the mechanical strength of the obtained scaffolds. Only the lowest collagen concentration (0.25 g per 1 g of CS) improved mechanical properties.	[47]
Collagen/CS membranes were produced via the solvent evaporation method and exposed to gamma irradiation.	The water absorption of CS, collagen and CS/collagen membranes with and without gamma irradiation initially increased steadily and then reached a plateau.	[52]
Porous scaffolds for skin tissue engineering were prepared by freeze-drying a mixture of collagen and CS solutions treated with glutaraldehyde.	The presence of CS improved the biostability of the collagen/CS scaffold under glutaraldehyde treatment. Collagen/CS scaffold cross-linked by glutaraldehyde could be a potential candidate for dermal equivalent with enhanced biostability and good biocompatibility.	[53]
CS and collagen/CS (1:1, w/w) porous scaffolds were produced by freeze-drying and evaluated as potential skin substitutes.	The CS and CS/collagen samples were characterised with several methods. A miscible blend with intermediate thermal degradation properties was obtained. Collagen/CS scaffold was cytocompatible and showed the potential for skin tissue engineering.	[54, 55]

Genipin-cross-linked collagen/CS biodegradable porous scaffolds were prepared.	The CS and genipin concentrations influenced the physicochemical properties of the scaffolds.	[55]
Microspheres of CS and gallic acid were obtained by emulsification phase separation.	The product mimicked extracellular matrix and could be used as a topical healing agent for chronic wounds.	[59]
A collagen/CS complex was obtained by adding collagen solution in sodium hydroxide to a CS solution in acetic acid and the appropriate amount of sodium tripolyphosphate as a cross-linking agent (pH 6.5).	The complex was characterised and showed potential as an orally available protein-based drug to enhance antioxidant properties and to inhibit melanin synthesis.	[61]
Chitosan/gallic acid/collagen		
Microspheres of CS and gallic acid were obtained by emulsification phase separation and cross-linking with glutaraldehyde. CS/gallic acid microspheres incorporated collagen were prepared by collagen addition to cross-linked CS/gallic acid microspheres.	Gallic acid release from CS/gallic acid and CS/gallic acid/collagen microspheres was analysed. CS/gallic acid microspheres incorporated collagen matrix mimic biomaterials as a topical healing agent for chronic wounds.	[59]

Collagen/CS complex nanoparticles were obtained using electrospinning. A mixture of 1,1,1,3,3,3-hexafluoride-2-propanol/trifluoroacetic acid (90:10, vol%) was found to be suitable for electrospinning [62].

CS/collagen sponges of 1:1 and 1:2 mass ratio were obtained. Cell growth in porous microstructures and sponges showed that porous sponges with a size of 80–100 μm were formed [63].

Collagen and CS were dissolved in 0.5 M acetic acid, transferred to a special flask (23 × 18 cm) and frozen for 3 h at -20°C. Collagen fibres bended significantly when immersed in acid and were cross-linked by glutaraldehyde via covalent bond formation [64].

Analysis of the FTIR spectra of gelatine/CS PECs revealed the formation of hydrogen bonds within and between polymer chains and electrostatic interactions between the $–COO^-$ group of gelatine and the $–NH_3^+$ group of CS [65, 66].

Researchers prepared solutions of CS/collagen = 1:1, genipin and 25% glutaraldehyde and mixed all components in 2.5% acetic acid for 4 days. After this, they freeze-dried the aldehyde and genipin cross-linked scaffolds in a lyophilic chamber in liquid nitrogen (–196°C) [67].

The interaction between collagen and CS occurs due to the charged electron-donating functional groups of macromolecules. In addition, at pH < 6.5, most of the amino groups of CS are protonated, which contributes to the formation of electrostatic bonds between the $–NH_3^+$ groups of CS and the $–COO^-$ groups of aspartic and glutamine residues in collagen [54, 68].

Collagen/CS hydrogel scaffolds were prepared to model soft tissue. A mathematical model was developed to determine the mechanical properties of the scaffold obtained under various conditions. The elastic modulus of the collagen/CS scaffold (80/20, v/v%) was 3.69 kPa. The higher the CS content, the stiffer the frame became.

The elastic modulus of a scaffold consisting of 70% collagen and 30% CS was 11.6 kPa [69].

In another study, researchers used molecular dynamics simulations to describe the effect of pH and the presence of sodium and calcium cations on the stability of molecular complexes formed by type II collagen with chitin and CS oligosaccharides [70]. Based on the Gibbs free energy of binding, all the considered complexes were thermodynamically stable over the entire pH range. The affinity of CS oligosaccharide for collagen was highly dependent on pH, whereas oligomeric chitin did not have a pH-dependent effect on the stability of molecular assemblies with collagen. On the other hand, the presence of sodium and calcium cations had little effect on the affinity of chitin and CS for collagen.

Researchers summarised the modern developments in water-soluble non-stoichiometric PECs, which have such qualities as high stability and the ability to enter into competitive interpolyelectrolyte reactions. The complexes remain stable when external conditions (pH, ionic strength and temperature) vary over a wide range, but quickly respond with high sensitivity to changes in the environment outside this range by changing the phase state [71].

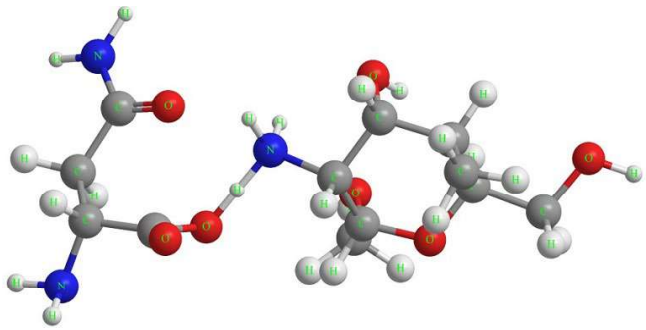
Finally, a study evaluated electrostatic complexation in an aqueous solution of cationic poly(diallyldimethylammonium chloride) with an excess of water-soluble anionic biopolymers. An NIPEC was formed: it was actually a block copolymer, with hydrophilic regions represented by free (unbound) anionic units and hydrophobic fragments of mutually neutralised anionic and cationic units [5, 72, 73].

4. Theoretical Calculations of the Formation of Interpolyelectrolyte Complexes

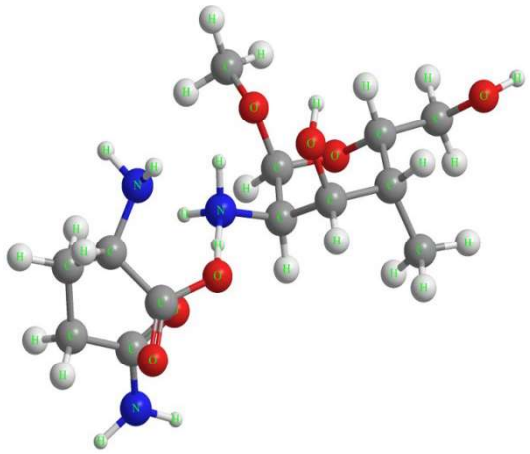
Theoretical calculations allow one to calculate possible interactions between compounds and to determine the possibility of the formation of various kinds of bonds, complexes, agglomerates, clusters and nanostructures. We have previously performed quantum-chemical density functional theory (DFT) studies of CS complexes with Na-CMC [74] or amino acids [75]. We analysed charge distribution of amino groups in CS chains of various lengths depending on the degree of deacetylation, the reactivity of acetamide and amino groups in CS, the interaction of CS with Na-CMC or CS and amino acids and the stability of their interpolymer complexes. We found that the main interaction between Na-CMC and CS changes from weak van der Waals interactions to strong electrostatic interactions, which link the two polymer chains into a compact structure. Moreover, we modelled interactions of the CS chain with Na-CMC during PEC formation [74]. The chains quickly approach each other in time, with the formation of electrostatic interactions between the polycation and the polyanion. The stability of the CS-CMC PEC is determined by the number of interactions between them, and, consequently, the chain becomes more compact.

The mechanism of PEC formation, identified using molecular dynamics simulation, is consistent with experimental data (conductometric measurements). Specifically, CS-CMC PECs are formed due to electrostatic interaction between the $–NH_3^+$ group of CS and the $–COO^–$ group of CMC. This process is affected by both the pH of the solution and the degree of deacetylation of CS [74]. The calculated group charge distribution for the tertiary hydrogen atom of the protonated amino group of CS is 1.54 e [76].

We previously performed theoretical calculations of the formation of PECs in solutions containing CS and amino acids such as asparagine and glutamine [75]. The optimised structures of these complexes are shown in Figure 3.



CS-asparagine



CS-glutamine

Figure 3. The optimised geometry of chitosan (CS) complexes with the amino acids asparagine and glutamine.

The DFT calculations revealed the presence of hydrogen bonds between the nitrogen atom of the $–NH_3^+$ group of CS and the oxygen atom of the $–COO^–$ group of the studied amino acids. According to Table 2, the distance between the H atom of the $–NH_3^+$ group of CS and the O atom of the $–COO^–$ groups of the amino acid is 1.001 Å (asparagine) and 0.999 Å (glutamine), which is typical for hydrogen bonds [77]. This indicates that CS forms hydrogen bonds with amino acids during the formation of complexes. Cationic or anionic amino acids are well known for their ability to form hydrogen bonds with oppositely charged species [78]; however, the formation of such a salt bridge in the gas phase of the complexes under consideration was not observed.

Moreover, based on interaction energy (ΔE_{inter}), the suitability of the carrier to a particular amino acid can be assessed. The calculated values of ΔE_{inter} in gas and aqueous phases are presented in Table 2. ΔE_{inter} is negative in all cases, contributing to the formation of the complexes formation. A high ΔE_{inter} for CS-glutamine may be due to the strong Coulomb force of attraction, which leads to hydrogen migration. The charge of the O atom in the $–COO^–$ group of glutamine is more negative (–0.64 e) than that of asparagine (–0.62 e).

Table 2. Hydrogen bond distance and basis set superposition error (BSSE) corrected interaction energy between chitosan (CS) and the amino acids asparagine and glutamine.

Complex	H-H bond length between N···O [Å] (aqueous phase)	ΔE_{inter} [kcal/mol] (gas phase)	ΔE_{inter} [kcal/mol] (aqueous phase)
CS-asparagine	1.001	-89.51	-18.96
CS-glutamine	0.999	-105.49	-23.1

Note. ΔE_{inter}, interaction energy.

CS/collagen complexes were formed in the aqueous phase. As seen in Table 2, the aqueous phase primarily affects the interaction energy of these systems. There is a progressive destabilisation of CS complexes with amino acids. The results show that molecules with opposite charges are indeed more separated and more stable than complexes in water, leading to a decrease in ΔE_{inter}. This can also be explained by the fact that in polar environments, the interaction with the environment (solvation) is probably more important than the electrostatic interaction between the two interacting molecules, leading to their preferential stabilisation [75].

The results indicate a strong interaction between CS and amino acids in a non-polar environment and a gradual weakening of the interaction in the aqueous phase. These results are significant for modelling the complex penetration process through a cell membrane that is non-polar in nature. The notably high ΔE_{inter} in the gas phase and very low ΔE_{inter} in the aqueous phase for the complexes indicate their suitability for use in biomedicine.

5. Application of Interpolyelectrolyte Complexes

CS-based PECs have found applications in drug delivery, wound healing, controlled-release systems, as enzyme and cell supports and bone scaffolds and tissue reconstruction, among others. Table 3 lists some examples of application of IPECs based on CS and collagen.

In an *in vivo* study using a rat model of spinal cord injury, the researchers transplanted a collagen/CS matrix immediately after a partial spinal cord injury. This transplantation restored sensory functions of the spine, maintained balance and prevented pathological physical activity [79].

There have been a wide range of experiments undertaken to evaluate CS/collagen IPECs. In one study, the researchers evaluated the biocompatibility of CS/collagen complex in composite vascular tissue engineering and performed real-time polymerase chain reaction (RT-PCR) in an effort to improve the morphogenesis, attachment, proliferation and phenotype of porcine iliac artery endothelial cells [98].

Poly(L-lactide-co-ε-caprolactone) [P(LLA-CL)] nanofibrils and collagen/CS complex were prepared using nanofibrillar electrospinning. The mechanical properties of collagen/CS complex nanofibrils varied depending on the collagen content of the complex. Biodegradation of P-nanofibrils (LLA-CL) occurred faster in collagen nanofibrils than in their membranes, and there was faster growth of P-nanofibrils in smooth muscle cells (LLA-CL) [99].

In another study, the researchers evaluated the effect of collagen and CS on the immune system. They analysed the number of T cells and their activation, wound antigen uptake and the cytokine profile in wound tissue and spleen cells. Animals treated with collagen/CS

had decreased invitrogen (2 μg/ml biotinylated anti–IL-2) (IL-2) secretion and increased IL-10 secretion in wound adhesive tissue and liver, respectively [102].

To prepare a three-dimensional nanoparticle gene delivery system based on collagen/CS sponges, body mesenchymal cells were transplanted in a mixture with plasmid-transformed β-1(PT-β1)/calcium phosphate fibronectin (PF). The glycosaminoglycan content of the three dimensional nanoparticle gene delivery system was comparable to that of those receiving PT-β1 as well as PT-β1 and dexamethasone, and was significantly higher than that of those receiving free plasmid and Lipofectamine 2000 [103].

Table 3. Application of interpolyelectrolyte complexes based on chitosan (CS) and collagen.

Material characteristics and drug used	**Application area**	**Reference**
Material tested on Wistar rats weighing 200–250 g	Wound coverings	[80, 81]
Bioeffective sponge containing Collahit-Bol and chitosan	Wound dressings for type IIIa and IIIb thermal skin burns	[82–85]
Sponges made of CS/collagen (50:50, weight ratio)	Used for osteoplasty in bone therapy	[86]
CS/collagen films (6:4, mass ratio)	Films for implants; adsorbed serum proteins on the surface the films evaluated by proteomics and bioinformatics	[68]
CS/collagen films (4:1, weight ratio) containing PLGA microspheres for VCR delivery (drug used in chemotherapy)	Implant films for the delivery of drugs in chemotherapy	[87]
CS/collagen 97.5:2.5, 95:5 and 90:10 (weight ratios)	Treatment of external epithelial damage	[88]
CS/collagen hydrogels	Treatment of myocardial infarction	[89]
CS/collagen sponges (50:50, mass ratio)	Targeted transport of medicinal substances	[90]
CS/collagen sponges (3:3, mass ratio)	Treatment of chronic wounds	[91, 92]
CS/collagen sponges (5 × 5 mm)	Wound coverings	[93]
Pills for drug, Collahit-G and Collahit-bol delivery	Treatment of various types of burns	[94, 95]
CS/collagen scaffolds (1:9, mass ratio)	Bone regeneration	[96]
CS/collagen membranes (1:1 to 1:4, mass ratio)	Bone regeneration	[97]

Note. PLGA, polylactic-co-glycolic acid.

Researchers developed a biomaterial consisting of a thin layer of fish collagen, one side of which was impregnated with a layer of CS. They used it as an artificial layer of skin for burn patients [104].

It was reported that porous collagen/CS sponge was used as a neurological indicator to restore nerve function. This transplantation contributed to change the parameters of neurological stress and improving the therapeutic effects of mesenchymal stem cells [105].

For osteochondral treatment, researchers used a mixture of collagen/CS (70:30, wt%) doped with magnesium hydroxyapatite (MgHA/collagen+CS) in the form of a three-dimensional scaffold [106]. Composite films CS collagen (a ratio of 1:0 to 0:1) accelerated the proliferation, differentiation and mineralisation of osteoblasts in MC3T3-E1 cells, the transcriptional activity of Runx2, and were recommended as a biomaterial for bone tissue engineering in bone diseases [107].

Sponges based on collagen/CS (a ratio of 1:9 to 9:1) and lauric acid were tested for use in skin tissue engineering for burns [108]. Collagen/CS hydrogels (a ratio of 1:1 to 50:1, wt %) increased the number of endothelial cells as well as angiogenesis when used in tissue engineering of the cardiovascular system [109].

Because of its cationic charge in solution, CS is prone to exhibit valuable physicochemical and biologically active properties. Despite its wide range of applications, due to lack of technology, CS is not very popular in the textile industry [110].

6. Preparation of Ternary Interpolyelectrolyte Complexes

Researchers studied the effect of the drying temperature (25 and 45°C) on the physical properties of gelatine/CMC/CS composite films (a ratio of 80:20:0 to 60:30:10). The 60:30:10 ratio was most suitable for food packaging due to its high vapour permeability and biodegradation rate [111].

Researchers obtained ternary IPECs by sequentially mixing a 0.25% solution of pectin and CS and 1% trypsin in a mass ratio of 2:1:2. They used it to carry biologically active substances. It prolonged the effects of these substances [112].

In another study, researchers reacted solutions of carboxymethylchitosan (CMCS), CMC, collagen and trans-glutaminase (T-Gase) in various mass ratios (40:40:20 to 25:25:50). They obtained anti-adhesion CMCS/CMC/collagen membranes [113].

Synthetic sponges based on collagen/Na-CMC/HA IPECS at a weight ratio of 2:1:8 wt% have been widely used in bone tissue engineering. Such sponges must have sufficient porosity to allow bone cells to grow and develop so that they can be used to treat bone defects and fractures [114–120].

IPEC nanoparticles based on CS, collagen and ALg-Na were obtained by electrospinning. A 4:1:1 mass ratio produced porous, bioresorbable three-dimensional matrices [121, 122]. In another study, the authors recommended collagen/CS/Na-hyaluronate IPEC as a suitable material for corneal tissue engineering [123].

Ternary IPECs are mainly used in medical practice. For example, biodegradable polymer CMCS/CMC/collagen membranes (40:40:20, 35:35:30 and 25:25:50) have been proposed as physical barriers to prevent peritoneal adhesions [116], and biomaterials have been obtained for bone engineering and regeneration. The effects of CS/collagen/glutaraldehyde [106, 114, 118, 124, 125] and CS/collagen/gelatine membranes on the activity of C3A cells was studied in an attempt to control cell growth and to improve growth rate *in vitro* for tissue engineering applications [126].

Three-dimensional scaffolds containing CS; collagen; and L-arginine, L-glutamic acid or L-lysine are used as biomaterials for tissue engineering and wound healing.

Their limitations include the toxicity of synthetic cross-linkers, poor mechanical stability, low biocompatibility and rapid biodegradation [127]. For skin regeneration, a CS/collagen/CMC complex was obtained, in which stem cells loaded into the studied scaffolds have proven their potential effect in improving skin healing and regeneration [128].

The collagen/CS/glycerol/hydroxypropyl methylcellulose complex as an artificial cornea was synthesised and characterised (functional cluster, cytotoxicity, morphological and antibacterial tests). All samples exerted minimal cytotoxicity (> 85% live cells) [129].

In another study, the authors evaluated the mechanical and physical effects of gelatine/CMC/CS composite films resulting from the addition of sorbitol. With glycerine as a plasticiser, solutions of gelatine/CMC/CS composite films containing graduated concentrations of sorbitol (0–30%). The results showed that an increase in the composite content resulted in a corresponding decrease in melting temperature and tensile strength. However, films with 25% sorbitol had increased tensile strength due to anti-plasticisation [130].

A hydrogel based on CS, collagen, hydroxypropyl-γ-cyclodextrin and polyethylene glycol was developed and characterised. Incorporation of nanohydroxyapatite and pre-encapsulated hydrophobic/hydrophilic model drugs reduced the porosity of the hydrogel from 81.62% ± 2.25% to 69.98% ± 3.07% [131].

7. Conclusions

Based on this review, we can draw the following conclusions:

1. Binary and ternary IPECs have indisputable utility: they are widely used for pharmaceutical and medical applications and in other areas.
2. Despite the huge amount of research, some fundamental aspects regarding how to obtain stable binary and ternary IPECs require additional research on regulating the charge of the surface layer of the complex and, accordingly, searching for areas of application of the synthesised complexes based on CS.
3. At present, the dependence of the initial ratios of polyions and the hydrodynamic characteristics of binary complexes CS/collagen and three-component IPECs with CS/collagen/Na-CMC has not been fully studied.

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