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Polyelectrolyte complexes of chitosan: formation, properties and applications

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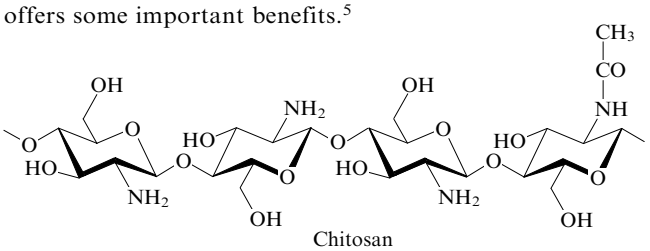
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Abstract. The results of studies of polyelectrolyte complexes of chitosan are summarised and described systematically. Chitosan complexes with biopolyelectrolytes, with modified natural polyelectrolytes and with synthetic polyanions are considered. Medical and biotechnological applications of chitosan complexes are discussed. The bibliography includes 156 references.

I. Introduction

In recent years, several publications reviewed data on complexes of the polysaccharide chitosan (Ch) with anionic polyelectrolytes (mainly of natural origin) and considered the potential applications of chitosan compositions in biomedicine, pharmacy, food industry, *etc*.[1–4] The present review summarises literature data on the synthesis, studies and applications of polyelectrolyte complexes of chitosan with natural, natural modified and synthetic polyanions.

Chitosan is a partially deacetylated derivative of the natural polysaccharide chitin (poly[(1 → 4)-*N*-acetyl-2-amino-2-deoxy-β-D-glucopyranose]), which has a number of attractive properties for many fields of science, biotechnology and medicine. Due to the diverse, recyclable and unlimited sources of the raw material, this biopolymer offers some important benefits.[5]



Chitosan

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Chitosan has unique physicochemical and biological properties such as biocompatibility, antimicrobial activity, the ability to activate macrophages and enhance fibroblast proliferation, *etc*. One of the attractive characteristics of chitosan is its ability to form non-covalent complexes with other polyelectrolytes. These complexes possess potentially useful specific properties.[2] Complexation is greatly affected by the conformation and molecular mass of chitosan macromolecules, degree of polydispersity, degree of deacetylation (DD), dissociation constant (pK_a) of the protonated amino groups of chitosan and the distribution of functional (amino and acetamido) groups along the polymer chain. The degree of deacetylation of chitosan determines the value of pK_a, which can vary from 6.46 to 7.32.[6] The conformation state and size of chitosan macromolecules in solution also depend on the DD and solution ionic strength.

For DD > 80%, the Coulomb repulsion of the protonated amino groups of chitosan dominates within a certain range of ionic strength. For 50% < DD < 80%, hydrogen bonds formed with participation of acetamide groups of chitosan make an additional contribution to intermolecular interactions. The bulky acetamide residues and the hydrogen bond network create steric hindrance and decelerate the rotation of the pyranose units around the β-glycosidic bond, increasing the rigidity of the polysaccharide macromolecule. As DD decreases, these effects become more important, and hydrophobic interactions increase; all this leads to increased self-aggregation of chitosan molecules in solutions, which also depends on the polymer concentration.[4]

Thus, the ability of chitosan to form polyelectrolyte complexes depends on the concentration, degree of ionisation and molar ratio of polyelectrolytes in a complex, charge density and distribution along polymer chains, *etc*.

II. General concept of the formation of polyelectrolyte complexes

The complex systems formed by macromolecules (interpolymer complexes) can be classified according to the dominant type of intermolecular interaction.

1. Complexes formed by the van der Waals interactions.
2. Complexes formed by hydrogen bonding between macromolecules.
3. Coordination complexes.

4. Covalent complexes.

5. Polyelectrolyte complexes stabilised by intermolecular ionic bonds.

6. Sterically matching ('key – lock' type) complexes.

In addition, there are combined types of interpolymer complexes, which owe their stability to cooperative interactions involving the above-mentioned types of bonding.

The overwhelming majority of publications are devoted to polyelectrolyte interactions at the level of linear, dendritic, protein, *etc*. macromolecules. Moreover, complexation of polyelectrolytes with oppositely charged surfaces (*e.g*., latex particles, monolamellar liposomes)[7,8] and macroscopic, weakly cross-linked hydrogels[9,10] was described. Several recent publications dealt with the properties of systems formed by the interaction of oppositely charged polyelectrolyte networks (microgel particles)[11] and nanosized particles.[12]

The ionogenic groups of the macromolecular chain of polyelectrolytes dissociate in polar media into charged units on the polymer chain and low-molecular-mass counterions. The electrostatic attraction of oppositely charged polymer units leads to the formation of polyion pairs, namely, polyelectrolyte complexes (PEC) sometimes called interpolyelectrolyte complexes (see, *e.g*., Ref. 13). In this review, we adhere to the term PEC. The pioneering works by V A Kabanov and A B Zezin laid the foundations of the theory of PEC formation and organisation.[13–18]

Polyelectrolyte complexes have attracted the attention of researchers because of their unique properties and ease of preparation. Due to their ability to change their phase state after insignificant changes in the external factors (pH, ionic strength, temperature, *etc*.), PEC solutions can be regarded as smart polymer systems.[1,19]

Some of the possible approaches to the classification of PEC are as follows:

— according to the nature of polyions involved in the formation of PEC, one can distinguish complexes based on polymers of only natural or synthetic origin and PEC of mixed type, involving synthetic polymers and biomacromolecules;

— complexes can be formed by strong or weak polyelectrolytes;

— according to their composition, PEC may be stoichiometric and non-stoichiometric;

— soluble and insoluble PEC can be formed depending on the conditions.

A distinction of the interactions of polyelectrolytes (compared with processes involving their low-molecular-mass analogues) is the cooperative character of bonds formed between the electrostatically complementary anionogenic and cationogenic macromolecules involved in complexation. This type of bond imparts high stability to PEC within wide ranges of pH and ionic strengths of the medium.

The formation of PEC and variation of its composition (*i.e*., the polyelectrolyte reaction) is a reversible process controlled by many factors such as the nature of polyelectrolytes, their molecular mass and molecular mass distribution, ionic strength, pH of polyelectrolyte solutions, *etc*. The same factors and the sequence in which the components are mixed regulate the formation of soluble and insoluble as well as stoichiometric and non-stoichiometric PEC.

There are several procedures for the preparation of PEC. The simplest method is mixing of the aqueous solutions of polycations and polyanions. Another way is polymerisation of ionic monomers on oppositely charged polyionic matrices.[13]

Upon mixing of solutions containing oppositely charged polyions, PEC are formed by an ion-exchange reaction, occurring at a high rate even in highly dilute starting solutions.

If complexation involves weak polyelectrolytes [for example, polyacrylic acid and poly(dimethylaminoethyl methacrylate)], the extent of reaction (conversion of ionogenic groups to the so-called interchain 'salt' bonds between two polyions) can be varied by changing the pH of the reaction system. In this case, the measure of the free energy of complexation (ΔG_{cs}) is ΔpH, which is the function of ionicity (α) and conversion (θ) of polyelectrolytes that defines the multicentre cooperative interaction between the oppositely charged polyion units for $\theta = \alpha$ (*i.e*., if each ionogenic group of co-polyelectrolytes of PEC is assumed to form, after being charged, an ionic bond with the corresponding oppositely charged group).[13]

$$\Delta\mathrm{pH}(\alpha,\theta) = \frac{\Delta G(\alpha) - \Delta G(\theta)}{2.3\,RT} = \frac{\Delta G_{cs}}{2.3\,RT},$$

where $\Delta G(\alpha)$ and $\Delta G(\theta)$ are the free energies of ionisation of a weak polyelectrolyte in the absence and the presence of an oppositely charged polyion for the given α and θ.

If a PEC is formed upon matrix polymerisation of charged monomers on the oppositely charged polyion (matrix), the change in the free energy (motive force of complexation) is described by the equation

$$\Delta G_{tp} = \Delta G_{p} + \Delta G_{cs},$$

where ΔG_{p} is the free energy of polymerisation in the absence of a matrix. When monomer polymerisation is thermodynamically forbidden ($\Delta G_{p} > 0$), but ΔG_{cs} is negative and $|\Delta G_{cs}| > |\Delta G_{p}|$, the matrix polyion shifts the equilibrium toward the oppositely charged polyion. Note that the same factor is responsible for clusterisation of protein globules with oppositely charged polyions to form protein polyelectrolyte complexes.

When the ratio of the numbers of ionised groups ($Z = m:n$) of the starting polyelectrolytes is 1 and $\theta \to 1$, stoichiometric PEC are formed in the system, which are insoluble in any solvents, but can swell in water to a certain extent. These insoluble PEC resemble cross-linked hydrogels in their properties. They have found wide use as binding agents to prevent wind and water erosion of soil in agriculture; as effective coagulants of colloidal dispersions in industry; as sorbents and matrices for enzyme immobilisation and bio/hemocompatible coatings for medical materials contacting with blood and other biological media in medicine and biotechnology.[1,2,5,13]

Despite the long history of PEC studies, the structure and properties of insoluble PEC are much less defined than the structure and properties of soluble PEC. In earlier papers (see, *e.g*., Ref. 20), a hypothesis was formulated that in insoluble PEC, more or less extended regions with interchain ion pairs co-exist with separate loops and 'tails', which can be regarded as structural defects. The higher the conversion θ the larger the number of 'paired' units in PEC. The free hydrophilic units localised in loops and 'tails' determine the degree of PEC swelling in solvents.[18,20]

If $Z = 1$ but $\theta < 1$ (incomplete reaction), water-soluble PEC are formed as equilibrium products of complexation.

The units of the starting polyelectrolytes not involved in the formation of interchain ionic bonds play the role of lyophilic (hydrophilic) fragments of macromolecules due to which PEC particles are held in solution. When $\theta \to 1$, water-soluble PEC are formed if the molar ratio between the units (φ) of the polyelectrolyte taken in deficiency (blocking polyelectrolyte, BPE) and polyelectrolyte taken in excess (lyophilising polyelectrolyte, LPE) in PEC is smaller than 1, *i.e.*, if the LPE is present in excess in the system. In the last works by Kabanov (see, *e.g.*, Ref. 19), these pair-forming polyelectrolytes are called guest polyelectrolyte (GPE) and host polyelectrolyte (HPE), respectively. Below we use these abbreviations.

As a rule, reactions of polyelectrolytes can form equilibrium systems in which soluble and insoluble PEC co-exist. The equilibrium can be shifted toward the soluble PEC by itroducing an excess of HPE as the lyophilising high-molecular-mass component in the system.

If the degree of polymerisation of HPE is not lower than that of GPE, soluble products are formed. The critical value (φ_c) is the limiting degree of occupation of GPE chains in HPE above which the hydrophilicity of the excess single-stranded segments is not sufficient for PEC particles to be held in solution; as a consequence, phase separation occurs in the system.[16,18] For the majority of defined systems, φ_c is 0.2–0.6 and depends on the chemical nature of polyelectrolytes. Soluble equilibrium PEC with $0 < \varphi < \varphi_c$ behave in dilute solutions as if they were individual polyelectrolytes with different charge densities.

The behaviour of non-stoichiometric PEC formed by the linear polyions of HPE with a high degree of polymerisation and small charged GPE particles (cationic dendrimers, globular proteins, *etc.*) in solution is similar to the behaviour of non-stoichiometric PEC consisting of linear polyelectrolytes. The non-stoichiometric PEC containing hydrophilic single-chain segments of HPE and the relatively hydrophobic GPE–HPE double-chain domains are regarded as some kind of block copolymers.[21]

Yet another family of soluble PEC are the so-called block ionomer complexes (BIC), including a block co-polymer of ionic and non-ionic blocks as one of the components.[22–26] These complexes remain soluble even when $Z = \varphi = 1$. For example, a BIC based on the diblock co-polymer of polymethacrylate–block polyethylene oxide and polyvinylpyrrolidone (PVP) is known.[24] For $Z = \varphi = 1$, the BIC unimer contains a hydrophobic domain of the stoichiometric polymethacrylate–PVP complex covalently bound with the hydrophilic block of polyethylene oxide and is thus an amphiphilic macromolecule. The behaviour of these compounds is similar to that of surfactants. As in the latter case, polymer micelles are formed at concentrations higher than the critical micelle concentration. A micelle has a water-insoluble nucleus (PEC block) and a hydrophilic 'crown' (polyethylene oxide block), due to which it is soluble in water and has aggregation stability. Unlike classical polymer micelles, they disaggregate when low-molecular-mass salts are added because of nucleus decomposition caused by the electrostatic screening of the charge.

The above tendencies in the formation and behaviour of PEC are characteristic of systems in which the Coulomb interactions are dominant. More complex behaviour is shown by PEC where electrostatic interactions co-exist with hydrophobic, donor–acceptor and dipole–dipole interactions and hydrogen bonding. The regularities of complexation of these multifactor polyelectrolyte systems are not discussed here. Major attention is paid to structure organisation, physicochemical properties and applications of chitosan PEC.

III. Polyelectrolyte complexes of chitosan with biopolyelectrolytes and modified natural polyanions

1. Polyelectrolyte complexes of chitosan with polysaccharides

The majority of publications are devoted to studies of chitosan polycomplexes with other polysaccharides. All polysaccharides are conventionally divided into four groups: phytopolysaccharides, zoopolysaccharides, polysaccharides of microorganisms and synthetic polysaccharides. The typical representatives of phytopolysaccharides are cellulose, hemicelluloses, xylans, glucomannans, galactans, pectins, starch, and algal polysaccharides (agar, carrageenan, fucan, galactans, *etc.*); zoopolysaccharides include chitin, glycogen, and glycosaminoglycans of connective tissue (heparin, hyaluronic acid, chondroitin sulfates and keratan sulfates); the well-known polysaccharides of microorganisms include dextrans and mannans, hyaluronic acid from streptococci A and C, *etc.*[27]

Polysaccharides exhibit high biological activities; at the same time, they are low-toxic, biocompatible and biodegradable. Due to this combination of properties, they have many useful scientific and practical applications, in particular, they are used for constructing various biomaterials by complexation with oppositely charged biomolecules.

The best studied chitosan PEC include complexes with zoopolysaccharides, including those with glycosaminoglycans (GAG). As is known, GAG play an important role in living organisms. Thus the mucopolysaccharide keratan sulfate is part of the proteoglycan complex, which can form complexes with hyaluronic acid and is involved in the formation of blood vessel walls.[28,29] Heparin can accelerate multiplication of some cells and inhibit growth of others; it can stabilise the growth factor; moreover, it possesses anticoagulant, antisclerotic and anti-inflammatory properties and is one of the most widely used medicines for treatment and prevention of cardiovascular diseases. Chitosan, in turn, is a biocompatible and bioresorptive polymer; it is cytocompatible with keratinocytes and fibroblasts the adhesion and proliferation of which on chitosan depends on DD (the higher the DD the higher the cell adhesion). Fibroblasts adhere better than keratinocytes, but proliferation of the latter is faster when the DD of chitosan is higher.[30] *In vivo*, chitosan can be bound to GAG (in particular, to chondroitin sulfate and hyaluronic acid)[28] to form stable PEC.

The PEC of chitosan with hyaluronic acid, chondroitin-4-sulfate, chondroitin 6-sulfate, heparin and sulfated cellulose were described.[31–34] It was found that the stoichiometric PEC of glycol chitosan with hyaluronic acid[32] could be obtained at pH 6.0. In the structure of chitosan, there is one weakly basic amino group per pyranose ring; in the structure of hyaluronic acid, there is one weakly acidic carboxyl group per two pyranose rings (*i.e.*, the number of amino groups is not equivalent to the number of carboxyl groups). In addition, there is steric hindrance for the rotation of pyranose rings, which rationalises the deviation from the stoichiometric composition at low pH values. In contrast to hyaluronic acid, the heparin molecule has two strongly acidic (sulfate) and one weakly acidic (carboxyl) groups

per two pyranose rings; therefore, in the case of the chitosan PEC formed at low pH values, the calculated and experimental data were in agreement.[32]

Complexation of heparin, chondroitin sulfate A, chondroitin sulfate C with *N*-acylated chitosan derivatives (*N*-benzoylchitosan, *N*-myristoylchitosan, *N*-propanoylchitosan, *etc*.) with different degrees of substitution is reported.[35] It was noted that maximum turbidity of the system increased with the degree of acylation of chitosan and decreased to minimum because of PEC precipitation after the system had been stored for 24 h. It was shown that at pH 2.8 and 4.5 stoichiometric PEC of chondroitin sulfates A and C with chitosan and *N*-benzoylchitosan could be formed.

The physicochemical aspects of the interactions of highly deacetylated samples of chitosan (DD 98%) with chondroitin 4-sulfates (55% and 70% sulfo groups, molecular mass 45.5 kDa), chondroitin 6-sulfate (90% sulfo groups, 59 kDa) and hyaluronic acid were considered.[33, 34] As could be expected, the degree of electrostatic binding depended on the degree of ionisation of polyelectrolytes, the nature of ionogenic groups and charge density.

For chitosan PEC with chondroitin 4- and 6-sulfate, the result was independent of the position of the sulfo group in the polysaccharide units. When the salt form (sodium) of chondroitin sulfate was mixed with chitosan, maximum precipitation was observed at a molar ratio (ρ) of 1 between the glucosamine residues and the residues of the sulfo and carboxylic groups. The results agreed with previously published data.[31, 32] For the acidic form of chondroitin sulfate, the equivalence point corresponded to $\rho = 0.75$; in this case, the NH_3^+ groups of chitosan formed ionic bonds with all sulfo groups of chondroitin sulfate (which completely dissociated) and only half of all carboxylic groups. Complexation of chitosan with both salt and acid forms of hyaluronic acid led to the formation of a 1 : 1 PEC.[33] The effect of the DD of chitosan on the stability of the resulting complexes with hyaluronate showed that as the DD (charge density) increased, the stability of PEC, as well as the cooperativity of the electrostaic interactions, increased.[34] In the course of the formation of chitosan PEC with acid forms of GAG, the carboxylic residues of polyelectrolytes were deprotonated. Depending on the pK_a value, deprotonation was either partial (for chondroitin sulfate) or complete (for hyaluronic acid, whose pK_a is lower).[33] The order of the addition of polyelectrolytes did not affect the complexes formed. PEC characterised by high stability over wide ranges of pH and ionic strength were obtained by the interaction of chitosan with GAG in both acid and salt forms.

Materials based on biodegradable polymers such as chitosan and GAG possess useful biological properties. They are cytocompatible and can stimulate wound healing processes and induce cell proliferation for the recovery of cartilage, skin tissue, *etc*. Hydrolysis of complexes with specific enzymes (chondroitinase, hyaluronidase) and the ability of biomaterials based on chitosan and GAG to be cytocompatible with keratinocytes and chondrocytes were studied.[28, 36] It was shown that chitosan in PEC acted as a protector against hydrolytic cleavage of GAG. However, chitosan showed better results in experiments on cell adhesion and proliferation and in the series of *in vivo* experiments, which demonstrated its higher efficiency as a wound healing agent compared with the efficiency of a PEC of chitosan with GAG.

Films of chitosan – hyaluronic acid PEC in NaCl solutions can reversibly change their form under the action of an electric field;[37] the bending angle can be adjusted by varying the ionic strength of the salt solution and the electric field strength. These 'electrosensitive' PEC are promising for use as the components of artificial organs (*e.g*., as contractive structures mimicking the muscle work), sensors, on-off switches, *etc*.

Porous constructions[38] (membranes, blocks, tubes, *etc*.) with controllable (by varying the freezing – drying conditions) pore sizes (1 – 250 μm) were obtained on the basis of chitosan. These structures were later used for the formation of chitosan – GAG (heparin) PEC. Complexation did not violate the inner microstructure of pores.

Glycosaminoglycans such as heparin and heparane sulfate are capable of binding the growth factors and cytokines and also initiate or enhance their synthesis. Thus, constructions containing chitosan – GAG complexes can be used as systems capable of retaining and accumulating the necessary factors secreted by the colonising cells and even growth factors from liquid around tissues. These constructions are suggested as potential implants in tissue engineering.[38 – 40]

Chitosan PEC with carrageenans [anionic linear sulfated polysaccharides containing D-galactopyranose residues linked by regularly alternating α(1→3) and β(1→4) bonds] have been considered.[41 – 46] The ι- and κ-carrageenan molecules include 3,6-anhydro-D-galactopyranose residues and have a double helix conformation in aqueous solutions, which determines their gel-forming ability. λ-Carrageenan has a high content of sulfo groups and no anhydrogalactose residues; in addition, it cannot form gels. Carrageenans are non-toxic and biocompatible and can therefore be useful in food and pharmacological industries.

When chitosan forms complexes with κ-, ι-, and λ-carrageenans, the type of the resulting PEC is dictated by the charge density on polysaccharide molecules, their concentration, and conformation (globular or helical) of carrageenan macromolecules.[46] Chitosan PEC with ι-, κ-, and λ-carrageenans[42] for medical and biological applications were prepared at the Institute of Chemistry, Far-Eastern Branch of the Russsian Academy of Sciences. It was noted that at concentrations higher than 0.1% for aqueous chitosan and higher than 0.3% for λ-carrageenan, PEC were formed as gels in the bulk; insoluble PEC were formed at lower polymer concentrations. Complexes can be obtained from concentrated (4%) and highly concentrated (20%) polysaccharide systems, their mixing generally led to the precipitation of complexes.[43 – 46]

Structural organisation of gels and consequently their mechanical and rheological properties depended on the chitosan content in PEC and on the nature of carrageenans.[42] λ-Carrageenan did not substantially affect the properties of PEC; it played an auxiliary role of a co-polyelectrolyte required for the preparation of PEC with chitosan. ι- And κ-carrageenans were more important for the complexation; their gels with chitosan were thermally sensitive, which was explained by the thermally reversible helix – knot conformation transition of carrageenan molecules, occurring as a sol – gel transition at low temperatures.

The least viscous gels were prepared by using λ-carrageenan. Static viscosity increased substantially on passing to ι- and κ-carrageenans. As a result, chitosan gels with κ-carrageenan were more rigid. The reason for these distinctions is possibly the formation of additional cross-links,

which formed in systems with ι- and κ-carrageenans, but were absent in the case of λ-carrageenan.

pH-Sensitive polyelectrolyte complex hydrogel systems of chitosan with carboxymethylcellulose (CMC) and κ-carrageenan were described.[41] The homogeneity and swelling ability of these hydrogels depended on the chitosan : carrageenan : CMC ratio and the content of low-molecular-mass salts in polymer solutions. The threshold polyelectrolyte to salt ratios were determined at which homogeneous or heterogeneous gels formed or not. The degree of swelling of chitosan – carrageenan – CMC tripolymer gels decreased proportionally to the decreasing content of carrageenan in PEC because of the decrease in the osmotic pressure and reached maximum at pH 11 – 12; a similar dependence was also observed for chitosan – carrageenan bipolymer PEC.

Polyelectrolyte complexes of chitosan with phytopolysaccharides (alginic acid, pectin, carrageenan) are extensively used for the creation of systems with controllable release of drugs.[47 – 58] Films,[54] microcapsules,[55] and pellets[49, 56, 57] of chitosan – carrageenan complexes were prepared for the starting concentrations of carrageenan of 0.1% – 4.0% in solutions. One of the most important characteristics of these systems is the swelling ability, which determines the degree of release of drugs. The swelling ability of drug matrices based on chitosan – alginate and chitosan – carrageenan PEC was analysed using the Hopfenberg model[49] for evaluating the contributions of various physicochemical processes (diffusion, relaxation, sorption – penetration, *etc.*). Because of the better water-binding ability of carrageenans (*e.g.*, compared with the same ability of hydroxypropylmethylcellulose[57] or alginate[49] for the same polyanion content in PEC), the pelletised forms were liable to undergo fast (in less than 30 s) disintegration after the swelling stage.

Thus, chitosan – carrageenan systems proved less favourable matrices for the preparation of drugs with a prolonged activity than chitosan – alginate PEC.[49]

Immobilised indometacin was obtained from genipin-cross-linked chitosan – sodium alginate PEC.[58] The swelling ability of these systems decreased with pH and alginate concentration in the gel-forming solution. Moreover, this parameter also depended on the degree of cross-linking. As could be expected, the degree of cross-linking decreased with the increasing degree of protonation of the amino groups of chitosan because the nucleophilic attack of the dihydropyran ring of genipin was inhibited. As a result, the rate of indometacin release from PEC particles with low degrees of cross-linking (formed at lower pH) was higher.

PEC of chitosan with its sulfated derivative (its biological properties are similar to those of heparin), polystyrene sulfonate[59] and alginic acid[60, 61] were obtained.[59 – 61] The effects of the DD and molecular mass of chitosan on the stability of the complexes formed were studied. The higher the DD and the lower the molecuar mass, the more stable the complexes. Yan *et al.*[61] studied the PEC of sodium alginate (100 kDa) with chitosan samples (DD 95%) with low (low-molecular-mass chitosan, LMCh, 130 kDa), medium (530 kDa), and high (HMCh, 1000 kDa) molecular mass. The HMCh complex with alginate formed large gel-like coacervates, while the LMCh – PAA polyelectrolyte complexes were more finely dispersed. The most homogeneous films with the highest water permeability in neutral media were obtained from PEC of this type.

Sodium alginate – chitosan PEC (3.5 and 29 kDa) and PEC of sodium alginate with the graft copolymer of chitosan and vinyl alcohol (which is readily soluble at physiological values of pH 6.5 – 7.2) were synthesised. Methods for the preparation of microcapsules based on these PEC were developed and optimised;[62] microcapsules with a semi-permeable polyelectrolyte membrane, which are stable on storage and insensitive to changes in the ionic strength of a medium, were obtained for immobilisation of tumour cell; in these 'microcontainers', prolonged *in vitro* culturing afforded multicell tumour spheroids. The microcapsules are a new *in vitro* model of small solid tumours suggested for use in studies of the new types of antitumour therapy and the mechanisms of activity of the new forms of cytostatics.

Interactions in chitosan – pectin systems and the properties of the PEC obtained were studied in many works.[63 – 68] For example, nanoparticles of PEC of chitosan with pectin from lemon with different ratios of the starting components and polymer concentrations were obtained[66] under the action of precipitant and sonication. Membranes and fungistatic laminated films based on chitosan – pectin PEC[67, 68] possessed pervaporative properties. The swelling ability of the films of these PEC[65] can be controlled by varying the composition and pH of the medium; the highest values were obtained at pH < 2 and > 7.

A study of chitosan PEC with sodium dextran sulfate (SDS) was reported.[44, 69 – 74] The PEC obtained were sensitive to changes in pH and the solution ionic strength.[44] At pH 10.5, the swelling was maximum and decreased when the solution ionic strength increased. Variation of pH, molecular mass and the rate and order of mixing of polyelectrolytes led to PEC with a molar ratio of Ch : SDS from 0.8 to 2.5.[69, 70] Two types of polyelectrolyte complexes[70] with different molecular structures and properties could be formed; In the first series (A), PEC were obtained by mixing solutions of chitosan and SDS of the same concentration (1 g litre^{-1}) in 2% acetic acid at volume ratios of 4 : 1 and 1 : 4; in the second series (B), equal volumes of polymer solutions of the same concentration were mixed in 1% hydrochloric acid. As a result, PEC of the series A were characterised by a greater number of salt bonds between $-NH_3^+$ and $-OSO_3^-$ groups than PEC of the series B. Moreover, hematologic tests showed that PEC of the series A had anticoagulant (antithrombogenic) properties, while PEC of the series B enhanced blood coagulation. Complexes with low-molecular-mass SDS inhibited hemocoagulation to a greater extent than PEC with high-molecular-mass SDS.[69] The order of mixing also produced a certain effect on the anticoagulant properties of PEC; polyelectrolyte complexes formed by the addition of chitosan to SDS had a more pronounced thrombogenic activity than complexes obtained by mixing the components in the reverse order.

Kikuchi and Takewayashi[72] studied insoluble PEC of chitosan and carboxymethyldextran, their antithrombogenic properties depended on the pH of the polymer solutions; at pH 5.5 less thrombogenic products were obtained than at pH 3.5. These effects were explained by differences in the molecular structures of the PEC and the presence of residual carboxylic groups in them.

Soluble non-stoichiometric PEC of chitosan and SDS were prepared by mixing the solutions of chitosan hydrochloride with a molar excess of SDS in the presence of urea.[73, 74] To obtain an equilibrium system with minimum

turbidity, the authors used a chitosan solution with pH 6.5 (50% ionised amino groups). At pH 5 (*i.e.*, at higher degrees of protonation of amino groups), the turbidity of the PEC solution increased because of the non-equilibrium character of the system, which was confirmed by the presence of a hysteresis loop during back titration of the solution. Variation of the ratio of the starting components led to changes in the composition of PEC and their hydrodynamic characteristics; as the fraction of chitosan in PEC increased, the intrinsic viscosity of solutions decreased and the chain flexibility parameter increased. The PEC formed particles of the size 60–130 nm depending on the solution ionic strength and the nature of the low-molecular-mass counterion. EPR studies of the PEC solutions of SDS and spin-labelled chitosan showed that the intramolecular mobility of the aminopolysaccharide decreased as the fraction of chitosan in PEC increased. When a 2 M NaCl solution was added to the system, the intermolecular bonds were broken, and the local mobility of chitosan macromolecules was restored.

In the case of compexation of chitosan with low-molecular-mass heparin, the segmental mobility of the former significantly decreased, but its dependence on the composition of PEC retained. The decreased mobility of chitosan units in the PEC with heparin was explained[73,74] by the increased rigidity of heparin macromolecules compared with that of high-molecular-mass SDS.

The ability of the obtained PEC to bind lipoproteins and their anticoagulant activity were studied; a non-linear dependence of these parameters on the PEC composition was found. The Ch–SDS polyelectrolyte complexes possess selective complexing ability with respect to low-density lipoproteins (LDL); the sorption selectivity decreased as the PEC : LDL ratio increased. The effects of the PEC concentration and PEC : heparin ratio on the interaction of PEC with heparin were as follows. At PEC concentrations smaller than 10 μg ml^{-1} and the same or lower content of heparin in blood plasma, the activity of heparin was enhanced; when the PEC concentration increased to 100 μg ml^{-1}, the antiheparin effect was observed.

With the optimum ratio of the components, the Ch–SDS complex showed higher anticoagulant and anticholesterol activities compared with those of the individual SDS. The effect of higher activity of SDS in its complex with chitosan was explained[73,74] by the optimisation of its physicochemical parameters (when SDS macromolecules were chemically modified with chitosan), in particular, by the decreased density of the negative charge and the flexibility of SDS macromolecules.

Thus, the efficiency of modification of anionic polysaccharides with chitosan through non-stoichiometric complexation and the possibility of controlling certain biological properties of PEC were shown.[73,74]

Highly selective membranes were obtained from films of the PEC of chitosan (550 kDa, DD 87%) with carboxymethylchitin (CMCh, 60 kDa, 1.17 carboxymethyl groups per unit) possessing a specific structure stabilised by a system of ionic and hydrogen bonds; the membranes could be used for separating water–organic mixtures and for hemodialysis.[75] The composition of Ch–CMCh PEC used for casting of films varied from 0.5 to 4.0 (Ch : CMCh); this determined the conditions of additional hydrothermal treatment and the basic physicochemical characteristics of films such as swelling, sorption capacity, mechanical and transport properties, *etc*. At higher chitosan contents in films, the degree of swelling in water increased drastically. Films with a stoichiometric composition had the lowest degree of swelling. Thermal treatment of films led to dehydration of interchain salt bonds and resulted in covalent cross-links (amide bonds). The resulting partially cross-linked PEC films were characterised by poorer swelling in all solvents and lost solubility in acidic solvents (in contrast to thermally non-processed PEC).

The system of ionic and hydrogen bonds in Ch–CMCh polyelectrolyte complexes ensured the formation of a more rigid (compared with the structure of chitosan), strained and fragile structure. When the composition of the PEC film approached stoichiometric, their sorption capacity decreased, while the stability and rigidity slightly increased. Thermally treated stoichiometric PEC having greater numbers of salt and covalent cross-links than other non-stoichiometric PEC demonstrated the highest selectivity in the pervaporative separation of water–organic mixtures and high permeability. It was noted, however, that Ch–CMCh PEC films are inferior to Ch–PAA PEC films in ability to separate water-enriched water–propan-2-ol mixtures.[76,77] This effect was explained[75] by the presence of two bulky substituents in the CMCh macromolecule because of which the structure of Ch–CMCh PEC probably had lower packing density than the structure of Ch–PAA PEC, despite the higher steric complementarity of co-polyelectrolytes in the case of chitosan and CMCh.

Stoichiometric complexes of chitosan with carboxymethylcellulose, alginic and polygalacturonic acids were obtained at equimolar ratios of components;[78–81] the highest degree of swelling of PEC was observed at pH 4.0–5.8 (Ref. 81).

Complexation of chitosan (230 kDa, DD 80%) with polygalacturonic acid (PGA, 102 kDa, 87.5% galacturonic acid) was studied by conductometry.[82] The conductivity of the system was measured during titration of sodium polygalacturonate with solutions of chitosan hydrochloride; the change in the slope of the conductometric curve suggested complete PEC formation. A stoichiometric PEC formed at the equivalence point (Ch : PGA = 0.98 ± 0.04), which was confirmed by several independent methods (viscosimetry, potentiometry, turbidimetry and gravimetry); the position of the equivalence point was independent of the order of mixing of polyelectrolytes. The conversion of polyelectrolytes (degree of complexation) was determined, which depended on the molar ratio of the reactants. The dependences found suggested that under the chosen conditions complexation in the Ch–PGA system was cooperative.

Chitosan (370 kDa, DD 82%) PEC with carboxymethylated cashew gum (*Anacardium occidentale*)[83] and carboxymethylglucomannan extracted from tubers of *Amorphophallus konjac* were described.[84] The highest product yield was achieved at a ratio of chitosan : cashew gum of 25 : 75.[83] Thermogravimetric and IR spectrometric analyses of the PEC were performed and the activation energies of thermal destruction and the degradation temperatures of polysaccharides and their complexes were determined. It was found that the thermal stability of PEC decreased relative to that of the starting polysaccharides. Based on chitosan–carboxymethylglucomannan PEC,[84] nano- and microparticles were obtained, their size varied from 50 nm to 1.2 μm depending on the initial concentrations of the polysaccharides, pH and ionic strength of solutions. It was shown that nanoparticles based on chitosan–carboxy-

methylglucomannan PEC could effectively be used as systems for encapsulating water-soluble drugs.

PEC were suggested for immobilisation of enzymes. For example, chitosan–alginate PEC with immobilised urease [85] and ternary chitosan–polyanion—glucoamylase PEC [86] were described. SDS, polyvinyl sulfate and heparin [86] were used as polyanions. Insoluble PEC were prepared under certain conditions by co-precipitation of enzymes by mixing chitosan solutions with polyanions. The presence of an enzyme did not affect the composition of the resulting PEC. The enzyme activity depended on the type of PEC. Thus, in insoluble chitosan–SDS PEC, glucoamylase exhibited 100% activity, while in chitosan–polyvinyl sulfate and chitosan–heparin PEC, the activity decreased by 16% and 54%, respectively.[86] Immobilisation of urease in the chitosan–alginate PEC led to increased thermal stability of the enzyme and stability on storage; after 70 days, the catalytic activity was 48% of the initial level, while native urease was deactivated by 50% in 7 days and by 100% in 20 days. The observed effects were explained by the appearance of a multipoint ionic interaction between the immobilised enzyme and PEC, which is the scaffold that imparts high conformation stability to the urease macromolecule.[85]

Yet another interesting type of chitosan complex is PEC with lipopolysaccharides (LPS), which are the components of the outer membrane of gram-negative bacteria. Lipopolysaccharides consist of regions differing in structure: lipid A having low structural variability, a highly variable O-specific polysaccharide chain and a less variable oligosaccharide segment (core). In rough mutants (R forms), there are no polysaccharide chains. Lipid A is the most conservative part of polysaccharides, which is invariable in structure in the majority of microorganisms studied. Lipopolysaccharides play an important role in the physiological functioning of bacteria; they are involved in transmembrane transport of various compounds and are endotoxins, antigens (the so-called O-antigens) and bacteriophage receptors, they are the primary target of the attack by antibacterial drugs and immunoglubulins. The polysaccharide chain of LPS is oriented toward the environment, and its fine structure is responsible for the recognition of bacteria and the specificity of immune reponse in higher animals and humans. Lipid A, which is closely associated with proteins, ensures the entity and stability of the cell membrane by decreasing its permeability and increasing its stability against hydrophobic agents; it is also responsible for the toxic properties of lipopolysaccharides.[4,87] The death of gram-negative bacteria as a result of inflammation, antibiotic activity, *etc*. liberates large amount of endotoxin. As is known, endotoxins (LPS, in particular, lipid A) have a pyrogenic action on the infected living organism; in particular, they result in severe intoxication (the so-called endotoxemy) and endotoxic shock.[88] Therefore, of particular interest is seeking the substances capable of reducing the toxicity of LPS.

LPS molecules bearing the negative charge on the surface (due to the presence of phosphate, pyrophosphate and carboxyl groups) interact with polycations (*e.g*., chitosan macromolecules) to form low-toxic chitosan–LPS complexes with different stoichiometry, which are promising as immunostimulants.[89–93] The mechanism of this complexation was studied by various techniques. It was found that the binding of chitosan to an endotoxin is a complicated process, largely depending on the macromolecular organisation of endotoxins, the molecular mass and the DD of chitosan, reactant concentration and reaction temperature and time.[89–92] The complex is formed by means of not only ionic binding, but also other types of interaction. Stable chitosan–LPS complexes were obtained only after preliminary incubation of the components at 37 °C. Presumably, the complexation was accompanied by additional dissociation of LPS aggregates and formation of a chitosan complex, which was quite stable over prolonged periods of time in solutions with high ionic strength.[89–91] An electron microscopic study of the complexes revealed a considerable effect of chitosan on the morphology of LPS. This effect manifested itself as the loss of the ultrastructural organisation of the endotoxin and dissociation of aggregates formed by the granular PEC particles. It was noted that morphological inhomogeneity depended on the concentrations and ratio of the starting components and was probably related to the concentration dependence of the supramolecular structure of the LPS, which in turn affected its binding to chitosan.[93]

It was found that chitosan specimens with lower molecular mass and degree of acetylation had higher affinity for LPS, which could be explained by the higher flexibility of chitosan macromolecules with a lower molecular mass. As a result, steric hindrance to the binding of high-molecular ligands diminished and hence the sites for binding became more accessible. The affinity for chitosan increased with the increase in the length of the O-specific polysaccharide chains of LPS; this enhanced the interaction between the components (increased the binding constants), but decreased the number of binding sites; this resulted in a decreased amount of bound LPS. As the LPS concentration in a solution decreased, the amount of the endotoxin capable of binding to chitosan increased. The stoichiometry of the complexes depended strongly on the LPS concentration in a solution.[90–92]

In a series of biological experiments, it was shown that complexation of LPS with chitosan effectively decreased the acute toxicity of endotoxins.[90,91]

2. Polyelectrolyte complexes of chitosan with DNA

One of the promising directions of research in biotechnology is the creation of non-viral vectors for transferring the specific genetic information to cells. These artifial viruses can successfully be used in gene therapy for the treatment of diseases caused by gene malfunction. The curing action is achieved by the expression of the gene vector, which replaces the defective gene or, *vice versa*, inhibits the excess activity, *e.g*., of the transforming gene or introduces a new gene for vaccination purposes.

To incorporate DNA into a cell, one can use [94] viral vectors, varieties of liposomal transport, specially designed protein or peptide vectors, including those addressed by the ligand–receptor or antigen–antibody interactions to certain cell lines, and vectors with positively charged HIV–TAT type peptides.

One of the methods [95] for increasing the efficiency of the supply of the gene material to the cell uses soluble PEC of DNA with polycations. The introduction of DNA molecules in a PEC increases their compaction and stability against cleavage by nucleases, enhances the interaction with the cell membrane and increases the transforming activity with respect to procaryotic and animal cells. Modification of polycations with selective ligands capable of specific binding and endocytosis provides cell capture of the given

gene construction and addressed supply of the information included in the PEC to the target cells.

Polycations used for the creation of avirulent gene constructions include polyethyleneimine, polyphosphazene, polylysine, polybrene, polyamidoamine dendrimers and other synthetic polymers. They are readily synthesised and have low immunogenicity. However, they are toxic and possess low biocompatibility, which restricts their applications in practice; moreover, the efficiency of the supply of gene material to cells is low.[96]

To obtain effective non-toxic and biocompatible DNA carriers, new systems are being developed that use polymers of natural origin, in particular, chitosan and its derivatives. In the resulting PEC, the positive charge of the amino groups of chitosan is neutralised by the negative charge on DNA; this leads to DNA compaction, protects the molecule from cleavage by endonucleases, and ensures gene penetration through cell membrane. Recent studies [97] on models of the membrane bilayer showed that chitosan could penetrate into the cell breaking the double lipid layer of the cell membrane and could also induce the formation of transport channels in the latter, thus prolonging the supply of DNA and drugs to the cell.

Transfection of the chitosan–luciferase plasmid (pGL3-Luc) complex was studied by laser microscopy using FITC-labelled plasmid (FITC is fluorescein isothiocyanate) and Texas Red-labelled chitosan (Texas Red is sulforhodamine 101).[98] The transfection activity and efficiency of incorporation in the cell were found to be affected by the molecular mass of chitosan, the stoichiometry of complexes and pH of the medium. The highest level of transfection of the chitosan–plasmid complex was observed when using chitosan with a molecular mass of 40 and 84 kDa, the ratio of chitosan nitrogen to DNA phosphorus (N : P) equal to 5, the serum content in the tranfection medium 10% and pH 7. The study of the transfection mechanism showed that the chitosan–plasmid complex formed aggregates of the size 5–8 μm, which were adsorbed on the surface of the cell, penetrated in it as a result of endocytosis and were further liberated from endosomes under the action of the lysosomal hydrolytic enzymes. Moreover, Ch–DNA complexes in the form of nanosized particles [38 ± 4 nm (Ref. 99), 200–400 nm (Ref. 100)] were proposed for the use in gene therapy. It was noted [100] that using chitosan with a molecular mass of 10 kDa formed large particles of the size 600–1000 nm. When the molecular mass of chitosan increased to 40–150 kDa, PEC particles of the size 200–400 nm could be obtained; in this range of molecular masses, the DD and the N : P ratio did not appreciably affect the size of the aggregates.

Chitosan and polygalactosamine were used as non-viral vectors for the transfection of luciferase plasmid pGL3 in tumour cells.[101] Chitosan was several times more effective than polygalactosamine in supplying the plasmid complex to the target cells. The efficiency of the transfection depended on the stoichiometry of the chitosan–plasmid complex, pH of the culture medium and the molecular mass of chitosan. The optimum parameters were the chitosan : plasmid ratio 5 : 1 and pH 6.9. The luciferase activity and gene expression efficiency for systems containing chitosan with molecular masses of 15 and 52 kDa were higher than for systems with chitosan with a molecular mass of $\geqslant$100 kDa. The chitosan heptamer (1.3 kDa) did not show any gene expression.

Studies on DNA complexation with *N*-alkylated and non-modified chitosan (50 kDa, DD 99%) showed (see, *e.g.*, Ref. 102) that Ch–DNA PEC formed at a charge ratio of 1 : 1, while the hydrophobically modified Ch–DNA complex formed at a ratio of 1 : 4. The introduction of an alkyl substituent (C_4–C16) favoured easier penetration of DNA through the cell membrane due to hydrophobic interactions and easier 'unpacking' of DNA from the carrier as a result of a weakening of electrostatic interactions between hydrophobically modified chitosan and DNA. The efficiency of transfection increased with the length of the hydrocarbon fragment in the range C_4–C_8, whereafter its level changed insignificantly. While facilitating the penetration of plasmid into the cell, the hydrophic core of the resulting plasmid complex also performed the protecting function, namely, it protected the DNA molecule from the action of deoxyribonucleases.

A methodological approach is now being developed that uses modification of chitosan with low-molecular-mass specific ligands to achieve the specific supply of DNA complexes to the target cells. Thus, artificial viruses become 'recognisable' by definite cells. For example, low-molecular-mass chitosan was modified [103] with galactose residues to make the vector 'recognised' by the liver cells having specific receptors for galactose. Galactosylated chitosan was used *in vitro* for the transfer of the reporter β-galactosidase in gene pSV to human hepatocarcinoma cells (HepG2, SMMC-7721) and hepatocytes of the L-02 line. The complex obtained possessed low cytotoxicity and high selectivity for hepatocytes, while the efficiency of gene transfection increased with the degree of chitosan galactosylation.

It is worthy to note the studies on the properties of liquid-crystalline complexes formed by chitosan with linear double-stranded molecules of nucleic acids (NA) of the B and A families and with synthetic double-stranded polyribonucleotides (PRN).[96, 104–106] In the reaction with NA molecules, chitosan converts the system into the liquid-crystalline state. Its form depends on the molecular mass of chitosan and the values of DD, pH and ionic strength of solutions.

The Ch–NA complex was obtained by adding aqueous solutions of low-molecular-mass fractions of chitosan (2–31 kDa) with different contents of amino groups (46%–85%) to the aqueous salt solutions of NA with stirring. Spectroscopy detected the formation of PEC dispersions. Circular dichroism (CD) studies of the dispersions showed that the interaction of chitosan with DNA resulted in the formation of liquid-crystalline dispersions with a characteristic helically twisted (cholesteric) spatial structure. The classical cholesterics formed by the phase exclusion of the double-stranded DNA molecules of the form B exhibited an abnormal negative band in the CD spectrum, which pointed to the levorotary form of the spatial cholesteric structure. The CD spectra of dispersions of the Ch–DNA PEC showed an abnormal positive band, which corresponded to the cholesteric dextrorotary structure. PEC of chitosan and double-stranded DNA molecules of the form A, as well as 'pure' DNA molecules, formed a cholesteric dispersion with a levorotary spatial structure.

Studies of the formation conditions of Ch–NA PEC liquid-crystalline dispersions showed that there existed a definite value of the molecular mass of chitosan, starting from which the PEC formed in a cooperative way. The abnormal optical activity of Ch–DNA and Ch–PRN PEC

dispersions depended differently on the molecular mass of chitosan. At pH ~ 6.8 (corresponding to the pK_a of chitosan in aqueous salt solutions), the optimum cholesteric packing of the Ch–DNA PEC formed. Under the given conditions, the degree of ionisation of chitosan was 50%. At pH < 7 and pH > 7, two types of Ch–DNA liquid-crystalline dispersions formed, which differed in the molecular packing; dispersions of the first type were characterised by an abnormal positive band in their CD spectra, while dispersions of the second type did not show any changes in the CD spectra. For each chitosan sample with a definite DD, there exists a definite range of ionic strength values, within which the chitosan–DNA PEC with an abnormal band in the CD spectra can form. The liquid-crystalline dispersion was not formed outside these limits. Moreover, abnormal optical properties of the liquid crystals in question depended on the distance between the amino groups in chitosan molecules and the solution ionic strength; varying these two parameters affords liquid-crystalline dispersions that showed a negative or positive band in the CD spectra or did not show any abnormal optical activity at all.

To evaluate the steric and energy characteristics of chitosan complexation with DNA in solutions with variable ionic strength, a theoretical approach was developed, which allowed the construction of a mathematical model of binding of these polyelectrolytes that adequately described the experimental data.[106] The binding constant was calculated within this approach and was shown to decrease when the solution ionic strength increased.

Note that treatment of the chitosan–DNA PEC dispersion with daunomycin (DAU), the molecules of which intercalate between the DNA base pairs, led to the formation of a cholesteric dispersion, in which the initial dextrorotary spatial structure changed to the levorotary structure. However, despite the reversal of the sign of the abnormal band in the CD spectrum, the X-ray parameters of the Ch–DNA and Ch–DAU–DNA PEC phases coincided; *i.e.*, the introduction of DAU in PEC did not affect the distances between DNA molecules in the resulting dispersion.

The dependence of the optical properties of Ch–DNA PEC liquid-crystalline dispersions on a number of parameters makes it possible to use the optical properties of these PEC as quality testers for chitosan samples employed in medicine and biotechnology. Studies of the mechanism of the transiton of plasmid DNA to the liquid-crystalline state upon complexation with chitosan and the properties of the resulting PEC with different twist of the cholesteric structure for their future use for gene transfection are in progress.

3. Polyelectrolyte complexes of chitosan with proteins

Association of protein and polysaccharide molecules through Coulomb and non-Coulomb interactions leads to the formation of PEC.[107] Under certain conditions, proteins (natural polyampholites) can show the properties of both polyacids and polybases; for complexation with chitosan, the protein should act as an acid.

The PEC of completely deacetylated chitosan with bovine atelocollagen were obtained.[108,109] The structure of PEC in which co-polyelectrolytes were bound through electrostatic interactions was proved by various methods. It was noted that under the conditions chosen, gel formation of collagen competed with collagen complexation with chitosan. It was impossible to choose the conditions that provided exclusively the ionic complexation mechanism of the interaction of collagen with chitosan. At elevated temperatures at which protein denaturing was induced, it was possible to approach the synthesis of a stoichiometric polycation–polyanion complex. When chitosan was present in a large excess, the complexation was dominant, but there was hydrogen bonding between the protein and polysaccharide in addition to the electrostatic interaction. Biocompatible membranes were prepared from nanofibres of the chitosan PEC with collagen[110] cross-linked with glutaraldehyde and exceeded the widely used collagen sponges in their wound-healing properties.

Chitosan PEC with legumin of fodder beans and of its partial trypsinolysis product, legumin-T, were described.[111,112] It was shown that the functional properties of plant proteins could be regulated by complexation with charged polysaccharides; complexation imparted solubility to the precipitated protein molecules near their isoelectric point, which allowed control over the solubility of precipitated globulins in weakly acidic and weakly basic media. Moreover, the formation of chitosan PEC with legumins considerably increased the stability of protein emulsions.

The chitosan–ovalbumin complexes obtained as coacervates in water within narrow range of conditions [low ionic strength (0.005–0.03 M NaCl), pH 5.8–6.4 and mass fraction of chitosan 0.05–0.15 in a mixture] were designed for use as effecive encapsulants of lipophilic compounds.[113]

Porous mono- and bilayer biomimetic structures were developed on the basis of chitosan PEC with gelatin; due to their properties, they are recommended for use in tissue engineering.[114,115] Tubular sandwich structures based on chitosan–gelatin (4 : 1 by mass) PEC with a wall thickness of 1 mm and porosity of 81.2% were designed for tissue reconstruction of blood vessels.[116] It was shown that vascular smooth muscle cells of blood vessels spread and grew effectively on these polyelectrolyte structures. Tanabe *et al.*[117] prepared chitosan–keratin PEC films having enhanced mechanical properties (flexibility, elasticity and stability) compared with the properties of films from the starting polyelectrolytes and swelling in acid, neutral, and alkaline media (this was shown for pH 4.0, 6.3 and 8.9) due to the properties of the biopolymers of the PEC, namely, the acidic protein keratin having pI 4.9–6.1 and precipitating in acidic media and the basic polysaccharide chitosan having pK 6.3 and precipitating in alkaline media. Chitosan–keratin films have an antibacterial activity inherent in chitosan films, stimulate the attachment and proliferation of fibroblasts and serve as substrates for mammalian cells.

For fluorescent polarisation studies of the molecular mechanism of the interactions of chitosan with mucins, FITC-labelled chitosan (FITC-Ch) was synthesised.[118] Mucins are representatives of a special subclass of high-molecular-mass glycoproteins with high carbohydrate content (up to 50%–80% by mass). They possess branched oligosaccharide chains linked by the O-glycosidic bonds with the protein moiety of the macromolecule. Mucins are characterised by high content of such amino acids as serine, threonine and proline and such monosaccharides as D-galactose, L-fucose, *N*-acetylglucosamine and *N*-acetylgalactosamine as the main carbohydrates and sialic acids. The monosaccharide residues form oligosaccharide chains linked to the protein by the O-glycosidic bond of *N*-acetylgalactosamine and the hydroxyl group of the side chain of serine or threonine.[119]

The effects of the molecular mass of chitosan (70, 750, 2000 kDa), pH (2.9–5.0) and ionic strength (0.01–1.0 M NaCl) of a medium on the complexation with bovine submaxillary mucin (400 kDa) containing 12% sialic acid residues were studied by fluorescence polarisation.[118] Dextran sulfate or albumin were used as reference co-polyelectrolytes instead of mucin. For FITC-Ch complexes with mucin, the degree of polarisation increased with the mucin : chitosan molar ratio and the solution ionic strength. For example, at a ratio of mucin : FITC-Ch = 19 : 1 (750 kDa) and pH 2.9 (0.1 M acetic acid), the degree of polarisation increased by 61%. In addition, it was found that the increased molecular mass of chitosan, as well as the increased mucin content in the complex, also led to the increased degree of polarisation. Thus, the number of sites capable of adding mucin increased with the molecular mass of chitosan. It was assumed that the chitosan molecule might have more than one mucin molecule bound to it, while binding of chitosan to dextran sulfate occurred according to the 'one-to-one' principle, and complexation of chitosan with albumin did not occur at all under these conditions. The maximum change in the degree of polarisation in the interaction of chitosan with mucin was observed at lower pH (to pH 2.9 where the degree of ionisation of chitosan was almost 100% and the degree of ionisation of sialic acid residues was the least, 20%), which points to the dominant effect of chitosan on the interaction with mucin.

These studies allowed the researchers to conclude that the binding of two polymeric molecules involved not only electrostatic interaction between the D-glucosamine residues of chitosan and the sialic acid residues of mucin, but also hydrogen bonds and hydrophobic interactions.

Qaqish and Amiji[118] intended to use chitosan–mucin complexes for the creation of pH-sensitive drugs for localised suppy of drugs to the alimentary canal.

IV. Polyelectrolyte complexes of chitosan with synthetic polyanions

In the field of chitosan PEC with synthetic polyacid anions, the largest number of publications were devoted to chitosan PEC with polyacrylic acid (PAA).[76, 77, 120–133]

Insoluble PEC of chitosan (220 kDa) with PAA (202 kDa) of different compositions obtained at pH 3–6 were studied.[121] It was found that the solution ionic strength in the range 0.025–0.3 did not affect the formation of PEC. At pH < 3, insoluble chitosan–PAA PEC were not formed. The pH value affected the ionisation of the reactant (charge density distribution along the chain) and hence the composition of PEC. The content of polysaccharide in the insoluble fractions of PEC increased with pH. Thus, PEC with controllable composition and properties could be prepared with consideration of the tendencies found for complexation.

The formation and structure of chitosan PEC (190 kDa, DD 80%) with PAA (500 kDa) was reported.[122] The dependences of the degree of complexation and the composition of PEC on pH were studied by potentiometric titration. It was shown that insoluble PEC enriched in PAA formed at low conversion. As the reaction proceeded further, PEC particles became enriched in chitosan; in neutral media, stoichiometric PEC with quantitative amounts of both polyelectrolytes were formed. In alkaline media, the complex decomposed with liberation of polyacrylate anions from PEC particles and isolation of chitosan-enriched insoluble PEC fraction.

The behaviour of the PEC of water-soluble chitosan (DD 50%) with PAA was described.[123] Hydrodynamic studies showed that an increase in the chitosan fraction in PEC led to compaction of complex particles and hence to a decrease in the intrinsic viscosity of solutions; the minimum viscosity corresponded to the composition [Ch] : ([Ch] + [PAA]) = 1 : 2. In acidic media (at pH < 2), PEC completely dissociated into the starting polyelectrolytes; in basic solutions (at pH > 8), optical transmission decreased from 100% to 70% because of partial precipitation of chitosan, which is characteristic of the behaviour of water-soluble chitosan PEC. It was emphasised that hydrogen bonding also made a considerable contribution to complex stabilisation along with electrostatic interactions, which played the major role in the formation of PEC.

One of the major applications of Ch–PAA PEC is the preparation of semi-permeable membranes, which are widely used in food industry, medicine (in blood, plasma and blood substitute purification), pharmaceutical industry, waste treatment, oil products purification, *etc*. Semi-permeable membranes possess high separating ability (selectivity), high permeability, stability against the action of the medium being separated and sufficient mechanical stability. Note that semi-permeable membranes can serve as models of biological membranes characterised by high biological and chemical selectivity; therefore, their studies are also important for scientific purposes.

The structure of Ch–PAA PEC films and their mechanical and physicochemical properties, as well as the potential applications as membranes for pervaporation, were described.[76, 77, 124–126] Studies of the mechanical properties of Ch–PAA films showed [125] that tensile strength increased, while the relative lengthening during sample destruction decreased with respect to those of the starting polymer films. This result is explained by ionic cross-linking between the macromolecular chains in PEC. The lability of the PEC obtained at different polyelectrolyte ratios (depending on the pH of the medium) was evaluated from the change in the degree of swelling of films and IR spectroscopic data. Thus for Ch : PAA = 1 : 1 PEC, swelling increased at pH 3 and 9 and was minimum at pH 7. In the IR spectra of films after swelling at pH 3, the characteristic absorption band of the carboxyl group of PAA (1610 cm^{-1}) vanished; at pH 9, the band corresponding to the vibrations of the carboxylate ion of PAA was observed at 1590 cm^{-1}; and at pH 7, the absorption peak characteristic of the C=O stretching vibrations shifted because of the formation of the Ch–PAA complex. The pervaporation properties of the obtained membranes were studied using various aqueous-organic mixtures; it was shown that the efficiency of pervaporation depended on the PEC composition.[77, 125]

The sample films of chitosan (190 kDa, DD 80%) PEC with PAA (200 kDa) of different compositions (Ch : PAA ratio 0.25–0.67) were obtained by casting from formic-acid solitions of polymers (pH 2). The properties of pervaporation membranes prepared, which were designed for separating water–organic mixtures were studied.[77] The mechanical stability of films was found to increase as PEC was enriched in rigid chains of chitosan. In contrast to PEC based on flexible-chain synthetic polymers, the films retained their stability in neutral and weakly alkaline media and demonstrated high transport and sorption characteristics. Materials with these properties are extensively

used for the design of systems with controllable release of drugs in pharmacology, for the creation of constructions with definite chemomechanical properties, *etc*.[107,127,133]

Wang *et al*.[126] obtained PEC by mixing equimolar amounts of an acetic-acid solution of chitosan (300 kDa, DD 86%) with aqueous solution of PAA (230 kDa). After covalent cross-linking with glutaraldehyde, pH-sensitive membranes were obtained from PEC. A series of tests showed that the starting Ch–PAA complex dissociated at pH < 2 and manifested high swelling at pH > 7. At the same time, the degree of swelling of Ch–PAA membranes was high at pH < 2, decreased drastically as pH increased to 8 and then increased to a maximum at pH 11. Swelling of membranes in salt solutions increased with the ionic strength and depended on the valence of cations at constant values of the ionic strength; *i.e*., it was maximum in solutions of trivalent metal salts (M = Al) and gradually decreased on passing to divalent (M = Ca, Mg, *etc*.) and univalent (M = K, Na) metals. Note that the mechanical properties of membranes became reversible when they were treated alternately with KCl and $CaCl_2$ solutions at equal ionic strengths; when membranes were treated with a $CaCl_2$ solution, their size increased, but diminished after treatment with KCl solution. This behaviour is determined by the ion-exchange properties of PAA.

Of particular note are works where PEC were obtained by the free-radical polymerisation of ionised monomers on a polymer matrix.[129–132] For example, self-polymerising Ch–PAA membranes were prepared using free-radical polymerisation of acrylic acid on a chitosan matrix at moderate temperatures (37 °C).[132] The structure of polyelectrolyte membranes was confirmed by IR spectroscopy. The degree of swelling of membranes was found to depend on pH and composition; it increased with pH (from 5 to 10) and the PAA content in PEC. Thermogravimetric studies showed that interchain amide bonds formed in membranes at high temperatures (starting from 100 °C). Subsequent thermal treatment at elevated temperatures led to further dehydration of ionic bonds between the chitosan and PAA macromolecules in PEC, mass loss and eventually PEC degradation.

Other synthetic polyanions such as polyvinyl sulfate,[86] polystyrene sulfonate,[59,134] and poly-γ-glutamic acid[135] were also introduced in complexes (as mentioned in the previous section). Interpolymer complexes of chitosan with poly(metacryloylamidobenzoic acid) were described.[136] They were obtained by mixing equimolar amounts of polyelectrolytes with subsequent evaporation of solvents. Further thermal treatment of these complexes at 120 °C led to the formation of covalent interchain amide bonds. The complexes had physicochemical properties typical of cross-linked gels, in particular, their degree of sweeling correlated with the degree of dehydration of the system in the course of thermal treatment.

Complexation of chitosan with tripolyphosphate is used for immobilisation of enzymes such as lipases.[137,138] Lipases are widely employed in biotechnology for separation of enantiomers, hydrolysis of fats to fatty acids, catalysis of cleavage of glycerol esters at the lipid–water interface, *etc*. The applications of these enzymes are limited by their instability and narrow range of the conditions (pH and temperature) of their biocatalytic activity. Lipases can destroy the outer layer of the microbial cell membranes. However, their shell contains waxlike lipids, and hence phagocytosis of bacteria cannot be effected by macrophages. One of the solutions to this problem is immobilisation of lipases on polymer carriers capable of penetrating through the cell barrier. For this purpose, ionic gelation of chitosan solutions with tripolyphosphate was performed to obtain microcapsules based on the chitosan PEC used for immobilisation of lipase from *Candida rugosa*. It appeared that the enzymatic activity of the lipase enclosed in chitosane microcapsules was fivetimes higher than the activity of the enzyme in alginate microcapsules. The molecular mass of the chitosan samples used for microencapsulation did not substantially affect the enzymatic activity of the enzyme. For maximum efficiency of lipase liberation from capsules, the DD of chitosan should be >90%.

The PEC of chitosan and alkylene oxide (ethylene oxide or copolymer of ethylene oxide with propylene oxide) copolymers with maleic acid[139,140] were used to obtain pH- and thermally labile films (on-off switches) for selective transport of drugs. These films swell at low pH and temperatures and are capable of reversibly and rapidly changing the degree of swelling in response to the change in the ambient pH and temperature. The introduction of model drugs (salicylic acid, phenol and glucose) into films and their isolation from the latter depending on the pH (3.8, 6.2, 7.2) and temperature (25 and 50 °C), as well as the molecular mass of a drug, were examined. Upon increase in temperatures, decrease in pH from 7.2 to 3.8, and decrease in molecular mass of model substances, the rate of their penetration in films increased, while the rate of release of the drugs increased with pH and temperature. This effect was explained by an increase (with pH) in the electrostatic repulsion between the carboxylic groups of the copolymers of alkylene oxide with maleic acid and the anionic groups of the model compounds (phenol or salicylic acid), and also by an increase in the diffusion coefficient. Moreover, the rate of liberation of active substances depended on the microscopic structure of films and the degree of hydrophobicity of alkylene oxide copolymer samples.

The conditions of synthesis, as well as the properties and structure of chitosan PEC with polyanionic co-polymers based on maleic acid were studied by Samoilova *et al*.[141–145] *N*-Vinylpyrrolidone, styrene and ethylene were used as comonomers of maleic acid. The complex was also modified by introducing a modified polymer derivative, namely, the copolymer of *N*-vinylpyrrolidone with monomaleamide containing an amino acid residue of lysine. New approaches were suggested to the preparation of biospecific polymeric materials (thromboresistive coatings for devices contacting blood, affinity sorbents for the isolation of wheat germ lectin).

The choice of maleic acid copolymers as co-polyelectrolytes of chitosan for the preparation of PEC was dictated by the regular structure of these polymers (strictly alternating monomer units) and the possibility of transforming maleic acid residues into reactive anhydride groups, which react (under mild conditions) with amino- and hydroxyl-containing ligands, thus providing easy modification of the polyacid before or after complexation. The above properties are not inherent in the chitosan co-polyelectrolytes described in the literature. Moreover, it is certainly of interest to study the formation of these PEC at the molecular (polymer unit) level, taking into account interatomic distances in reactant groups and the conformations of the polymers used.[142]

V. Conclusions

This review convincingly demonstrates that chitosan-based PEC are of great practical value and widely used in scientific research, medicine, biotechnology, food and textile industries, *etc*. Table 1 summarises the major medical and biotechnological applications of these PEC.

Chitosan PEC with a wide range of polyanions of natural and synthetic origin have been studied. It was found that complexation between oppositely charged polyelectrolytes is a complicated process, which depends on many parameters. It should be emphasised that chitosan PEC generally have different types of binding, *e.g*., hydrogen bonding and intermolecular hydrophobic interactions in addition to electrostatic interactions.

The structure and properties of chitosan PEC depend on the nature and stucture of co-polyelectrolytes (molecular masses, conformations of macromolecules, charge density and distribuiton of ionised groups along the polymeric chain), and also on the conditions of PEC formation (pH and solution ionic strength, temperature, concentration, and polymer ratio). Soluble or insoluble and stoichiometric or non-stoichiometric PEC are formed at certain combinations of the above factors.

By varying the type of co-polyelectrolyte and the conditions of the polyelectrolyte reaction, one can obtain chitosan PEC in the form of gels, nano- and microparticles, films, membranes, porous structures and liquid-crystalline dispersions. PEC with natural anionic polyelectrolytes are best studied, but PEC with synthetic polyanions, including block copolymers, are also of great interest because they expand the possibilities for selectively adjusting the properties of the obtained PEC.

Table 1. Use of chitosan PEC in medicine and biotechnology.

Application	Co-polyelectrolyte of chitosan in PEC	Ref.
Specific (bio)sorbents	copolymers of maleic acid with *N*-vinylpyrrolidone, styrene, or ethylene	141, 143 – 145
Immobilisation of BAC according to the polyelectrolyte interaction principle	antigenes	146, 147
Films and pervaporation membranes	poly(4-styrene sulfonate)	59, 134
	sodium alginate	61, 148 – 150
	pectin	65 – 68
	carboxymethylchitin	74 – 76
	polyacrylic acid	76, 77, 121 – 126
	carrageenan	54
Hemocompatible and thromboresistant materials	copolymer of *N*-vinylpyrrolidone with N_{α}-lysine monomaleamide	143 – 145
	carboxymethyldextran	72, 150
	carboxymethylcellulose	151
	dextran sulfate	70, 73, 74
	heparin	152
Substrates for BAC immobilisation and drugs with selective and prolonged transport of BAC	dextran sulfate	86
	polyphosphoric acid	137, 138
	alginic acid	47, 49 – 51, 53, 58, 61, 85
	pectin	48, 52, 53
	carrageenan	49, 55 – 57
	carboxymethylglucomannan	84
	mucin	118
	alkylene oxide – maleic acid copolymer	139, 140
	polyvinyl sulfate	86
	heparin	86
Bioconstraction materials for replacement of coverlets, blood vessels, and bone tissue	glycosaminoglycans	28, 31 – 34, 36, 37, 39, 40, 153 – 155
	gelatin	114 – 116
	keratin	117
Non-viral vectors of genetic information transfer	DNA	98 – 103, 156
Complexation to reduce toxicity	endotoxin	89 – 93

Note. BAC are biologically active compounds.

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