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Interpolyelectrolyte complexes: advances and prospects of application

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Advances in the development of water-soluble nonstoichiometric polyelectrolyte complexes, which are characterized by high stability and can be involved in competitive interpolyelectrolyte reactions, are summarized and analyzed. The complexes remain stable over a wide range of external conditions (pH, ionic strength, temperature), but show a rapid, reversible and highly sensitive response to environmental changes outside this range by changing the phase state. The review considers methods of preparation and properties of nonstoichiometric polyelectrolyte complexes formed by interactions between oppositely charged polyelectrolytes. These reagents can be used for controlled modification of various surfaces, the preparation of soluble complexes functionalized by different molecules, the suppression and prevention of protein aggregation. The review briefly summarizes new types of soluble polyelectrolytes and polyelectrolyte complexes of different nature and with different structures, including biopolymers and dendrimers, suitable for solving problems in medicine and agricultural biotechnology. In order to evaluate the results achieved, there is a need to integrate and analyze the data on interpolyelectrolyte reactions, which are of most interest for a wide range of researchers.
The bibliography includes 118 references.

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1. Introduction

Water-soluble nonstoichiometric polyelectrolyte complexes (NPECs) based on natural and synthetic polyelectrolytes were discovered ~50 years ago. Advances in research on interpolyelectrolyte reactions and a signifcant extension in the scope of their application are associated with the availability of NPECs soluble in water and water-salt media. However, this property may lead to undesirable outcomes, for example, to a decrease in the efficiency of separation of biological mixtures and difficulties in layer-by-layer adsorption of oppositely charged polyions. The

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latter technique has been widely applied to fabricate polyelectrolyte multilayer ultrathin films and nanocapsules in recent years. The conditions for the formation of soluble NPECs, their structures and properties have attracted and continue to attract great attention of researchers. The results of studies of NPECs composed of oppositely charged synthetic polyions or those of polyions and oppositely charged natural polyelectrolytes (proteins, enzymes, nucleic acids) are discussed and systematically covered in reviews.[1–3] The properties of such complexes and the phase behaviour of NPEC solutions follow general patterns revealed in studies of cooperative interpolyelectrolyte reactions. Thus, the stabilizing effect of complexation generally becomes less pronounced and the region of existence of soluble complexes decreases with decreasing chain length of the polyelectrolyte molecule, although in some cases the charged oligomer can exhibit significantly higher efficiency than long chains. These effects are of particular importance for protein systems, in which polyelectrolytes are used for the suppression of protein (enzyme) thermal aggregation.

Currently, the research on polyelectrolytes and their complexes focuses on two main directions:

— the extension of the range of polymer counterparts by using polyions with unusual structures, *e.g.*, charged dendrimers of different generations or polymer multilayers;

— the use of accumulated experience for solving practical problems, mainly in biochemistry, nanotechnology and the environment.

This review deals with both these lines of research. The advantages of interpolyelectrolyte reactions involving soluble NPECs are demonstrated in relation to certain cases. Just before going directly to the consideration and analysis of the data on this subject published in recent years, let us mention the milestones in the research of NPECs.

Water-soluble NPECs have been known for a rather long time. Japanese researchers were the first to discover and prepare these polyelectrolyte complexes. Tsuchida *et al.*[4] attributed solubility of NPECs in aqueous media solely to the structural features of ionene polycations involved in these complexes. However, it was later found[5] that soluble NPECs can be prepared, if certain conditions are met, from oppositely charged polyelectrolytes with different chemical structures, and the suggested[4] structural chemical limitation is erroneous. This field of research has been efficiently and rapidly developed, as demonstrated in reviews.[6–8] These reviews consider the thermodynamics, kinetics and mechanisms of reactions between oppositely charged polyelectrolytes in aqueous solutions giving insoluble stoichiometric and soluble nonstoichiometric polyelectrolyte complexes. The mechanisms of interpolyelectrolyte exchange reactions were discussed. In aqueous solutions, equilibrium NPECs were shown to exhibit dual nature. Thus, on the one hand, NPECs act as traditional linear polyelectrolytes and, on the other hand, their behaviour is similar to that of solutions of amphiphilic block copolymers. The addition of low-molecular-mass electrolytes to NPEC solutions usually leads to the redistribution of electrostatically bound chains and phase separation. An analysis of such processes is decisive for the assignment of these complexes to a individual family of polymer materials. The data on the conformational state of polyions in solid interpolyelectrolyte complexes were reported and their thermomechanical properties in a swollen state were considered.

The further development of this area of research requires the extension of the range of substances by utilizing biological polyelectrolytes as their counterparts in order to reveal common features and differences in the behaviour of polyelectrolyte complexes of different chemical nature. The data on polyelectrolyte complexes formed by oppositely charged synthetic polyions and natural polyelectrolytes (proteins, enzymes, nucleic acids) are summarized and analyzed in a review.[2] The properties of such complexes and phase separation in their solutions were shown to follow general trends revealed in studies of cooperative interpolyelectrolyte reactions. The review addresses the principles of functioning of polyelectrolyte complexes, which are responsible for their self-assembly in solution and determine their ability to remain stable over a wide range of external conditions and then cause fast, reversible and very sensitive changes in the state of the systems due to changes in the molecular characteristics and(or) phase separation. The results of basic research on polyelectrolyte complexes were shown to be useful in solving topical problems in biotechnology, materials science and biomedicine.

The main results of this stage of research are summarized in the comprehensive review on NPECs.[1] These results can be rightly regarded to be of fundamental importance for the formulation of the basic principles of the functioning of cooperative polyelectrolyte systems.

Therefore, approaches to studies of NPECs were developed and basic results were obtained at the beginning of the 21st century. In recent years, great attention was paid to the development of methods for the synthesis and design of new soluble polyelectrolytes and polyelectrolyte complexes of different nature and with different structures, including biopolymers and dendrimers, suitable for solving different problems in medicine and agricultural biotechnology. This review is intended to get its readers acquainted with recent advances in this field, which to a large extent determine the appearance and investigation of the growth points of the most promising practically important lines of research.

The following designations and abbreviations are used:

CMC — carboxymethyl cellulose,

CHT — chitosan,

DAB-dendr-(NH_2)(x) — aliphatic dendrimers,

DIVEMA — divinyl ether – maleic anhydride copolymer,

ENM — engineered nanomaterials,

GAPDH — glyceraldehyde 3-phosphate dehydrogenase (the enzyme commonly used as a model protein),

GTMAC — glycidyltrimethylammonium chloride,

LbL — layer-by-layer,

mCHT — modified chitosan,

NPECs — nonstoichiometric polyelectrolyte complexes,

PAA — polyacrylic acid,

PAAC — polyallylammonium (cation),

PEVP — poly(*N*-ethyl-4-vinylpyridinium bromide),

PMA — polymethacrylate (anion),

PMA* — fluorescent-labelled polymethacrylate (anion),

PSS — polystyrene sulfonate (anion),

SEM — scanning electron microscopy,

sPP — supercharged polycations,

wPPPDs — aromatic pyridine-containing polyphenylene dendrimers.

2. Soluble polyplexes in biotechnology

The large-scale production of highly purified plasmid DNA is required for gene therapy and vaccine development.[9, 10]

The chromatographic isolation of plasmids from bacterial cell cultures generally consists of several steps, including the precipitation with solvents, salts and polymers combined with the enzymatic degradation of nucleic acids.[11, 12] Until recently, none of the methods enabled the selective binding of plasmid DNA immediately from a purified alkaline lysate. Wahlund *et al.*[13] solved this problem using the polyallylammonium cation (PAAC), which is involved in the electrostatic interaction with negatively charged plasmid DNA giving a polyelectrolyte complex, the so-called polyplex (including a insoluble stoichiometric one). This allows the efficient separation and removal of impurities, particularly RNA and proteins. This method is useful for the large-scale production of highly purified plasmids due to low cost and commercial availability of PAAC.

Methods for the isolation and purification of plasmid DNA by polyelectrolyte complexes were described in detail by Wahlund *et al.*[14] Plasmid DNA is selectively precipitated from a purified alkaline lysate with PAAC capable of binding to nucleic acids. Both nucleic acids, DNA and RNA, form insoluble polyplexes with synthetic polycations. However, in a certain concentration range of the low-molecular-mass electrolyte (NaCl), plasmid DNA is quantitatively precipitated, while RNA totally remains in solution. The precipitated DNA is redissolved upon the treatment with the concentrated saline, and the polycation is removed by gel filtration.

The factors controlling phase separation in aqueous salt mixtures of polycations and DNA were revealed. It was shown[15] that the phase separation in solutions of polyplexes follows the same trends and patterns as those found for solutions of flexible-chain vinyl polyions. However, the rigidity of the DNA double helix is apparently the key factor responsible for the phase diagram type. Thus, the region of insoluble complexes is expanded at the expense of the region of soluble polyplexes. This can substantially limit the use of polyplexes in medicine and biology, but is relevant for the development of simple and efficient procedures in biotechnology for the separation of DNA from other biological impurities.

A comparative analysis of phase separation in aqueous salt solutions of polyplexes formed by RNA and DNA was performed by Wahlund *et al.*[16] (Fig. 1). The formation of insoluble polyplexes composed of RNA and polycations was monitored by measuring the absorbance of supernatants after separation of precipitates. The polyallylammonium cation and 2,5-nonene bromide were used as polycations. The results of this study were compared with the earlier experimental data for DNA polyplexes. The cited study[16] is a part of the project aimed at developing a specific method for the separation of mixtures of DNA and RNA as one of key issues in the preparative isolation of plasmid DNA from cell extracts. As opposed to the precipitation of DNA, which is quantitatively accomplished at a particular charge-to-charge ratio $Q = [+]/[-]$, a significant fraction of RNA remains in solution at any Q under the same conditions. Moreover, the addition of sodium chloride led to an increase in the fraction of RNA that remained in the supernatant. The less efficient precipitation of RNA is attributed to the difference in the molecular structures of

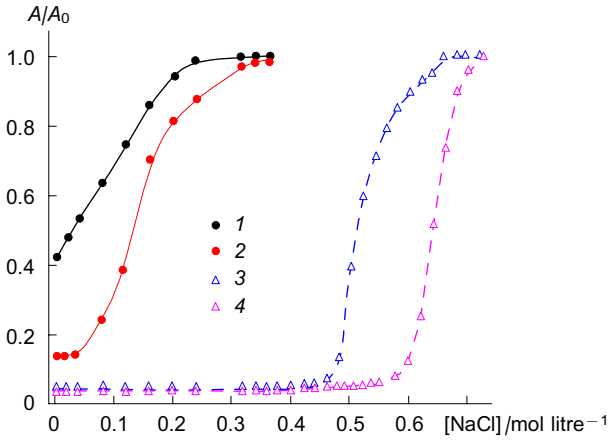


Figure 1. Plots of the relative absorbance (A/A_0) of RNA (*1*, *2*) and DNA (*3*, *4*) solutions after their precipitation with 2,5-ionene bromide (*1*, *3*) and PAAC (*2*, *4*) *versus* the NaCl concentration.[16]

nucleic acids (the DNA double helix and much less ordered RNA structure) and to the presence of a significant percentage of short chains in RNA, which cannot form a cooperative system of ion pairs with the polycation. The same situation was observed in solutions of polyplexes containing relatively short DNA double helices. The differences in the phase behaviour of solutions of DNA and RNA polyplexes may be employed for solving biotechnological problems.

3. Water-soluble dendrimers and dendriplexes

Dendrimers comprise a relatively new but well characterized family of polymers. The most distinguishing and attractive feature of dendrimers is that their targeted synthesis can be performed under strictly controlled conditions. The structure of dendrimers is determined by their generation number.[17–21] The progress in this area of research is related to the synthesis and characterization of water-soluble aliphatic and aromatic dendrimers containing ionic or ionogenic groups.[22]

A series of commercially available Astramol poly(propyleneimine) dendrimers, DAB-dendr-$(NH_2)(x)$, were used as aliphatic dendrimers. The complexation of water-soluble cationic dendrimers DAB-dendr-$(NH_2)(x)$ of the first five generations (x = 4, 8, 16, 32, 64) containing the fluorescent-labelled polymethacrylate (PMA*) anion was examined.[23] The formation and dissociation of the products was monitored by fluorescence spectroscopy based on the ability of amino groups of dendrimers to quench fluorescence of the label. In salt-free media, complexes dissociated in strongly alkaline and strongly acidic solutions regardless of the generation number of the dendrimer. Stability of the complexes to degradation by the added salt (NaCl) gradually and significantly increased with increasing parameter x. The phase separation proved to be sensitive to pH and the charge-to-charge ratio of the components but was independent of the dendrimer generation. On the contrary, in aqueous salt solutions the phase diagram essentially depends on the generation number. Thus, the increase in the parameter x leads to a significant increase in the heterogeneous region. These results provide evidence that the complexes under consideration follow general patterns

typical of NPECs composed of oppositely charged polyions. Due to the possibility of preparing negatively and positively charged complexes with controllable stability and solubility,[23] dendrimers DAB-dendr-(NH_2) hold a considerable promise for practical applications, in particular, for the construction of systems capable of self-assembly and self-adjustment. This is particularly useful in the solution of biotechnological and biomedical problems.

The next important step in research of the dendrimers DAB-dendr-(NH_2) with an extended range of anionic counterparts was made by Zhiryakova and Izumrudov.[24] Calf thymus DNA was used along with PMA*, which made it possible to monitor the formation and dissociation of complexes of dendrimers with nucleic acids, also referred to as dendriplexes, by fluorescence quenching of an intercalating dye. Even in weakly acidic media, a part of amino groups of the dendrimer cannot be involved in the formation of ion pairs with DNA phosphate groups (the process was controlled by varying pH). Stability of all dendriplexes to the added salt increased with increasing generation number, all dendriplexes being stable in solutions of physiological ionic strength. Unlike the model highly charged linear polycation of poly(*N*-ethyl-4-vinylpyridinium bromide) (PEVP) with different polymerization degrees used by the authors,[24] the cationic dendrimers formed more stable complexes with the flexible-chain PMA* anion compared to nucleic acids due probably to the fact that some amino groups of dendrimers cannot be involved in interactions with the rigid double helix. These results can serve as a platform for constructing dendriplexes with controllable stability, which are highly demanded in the design of systems for the targeted delivery of genetic material into living cells.

The synthesis of a new family of aromatic dendrimers, namely soluble cationic polyphenylpyridinium dendrimers (wPPPD) of the first four generations (D1, D2, D3 and D4, see Fig. 2) was reported.[25] The interactions of these dendrimers with plasmid DNA were investigated.

The mixing of wPPPD and DNA solutions resulted in the formation of dendriplexes (Fig. 3) that were stable in solutions of physiological ionic strength at physiological pH values. Hydrophobic interactions involving phenylene groups were found to make a considerable contribution to stabilization of dendriplexes. The results obtained by turbidimetric titration, high-speed sedimentation and ζ-potential measurements provide clear evidence that the positively charged pyridinium groups buried inside the dendrimer cannot be involved in the formation of ion pairs with phosphate groups of the rigid double helix. Water-soluble positively and negatively charged nonstoichiometric dendriplexes were prepared and the phase separation in their solutions was investigated. According to the dynamic light

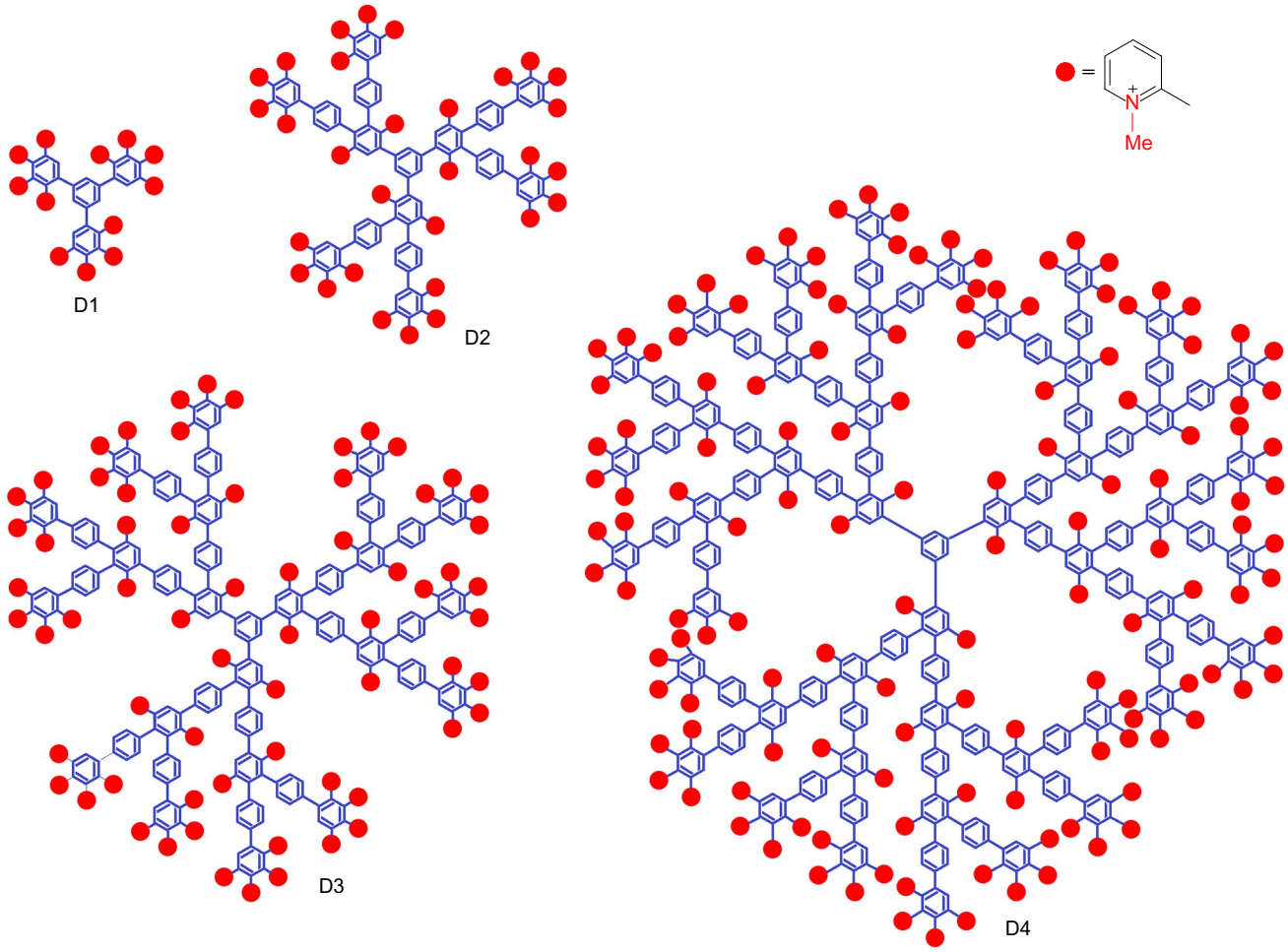


Figure 2. Structural formulas of wPPPD dendrimers of the first four generations (D1 – D4).[25]
N-Methylpyridinium cationic groups are in red; hydrophobic phenylene groups are in blue.

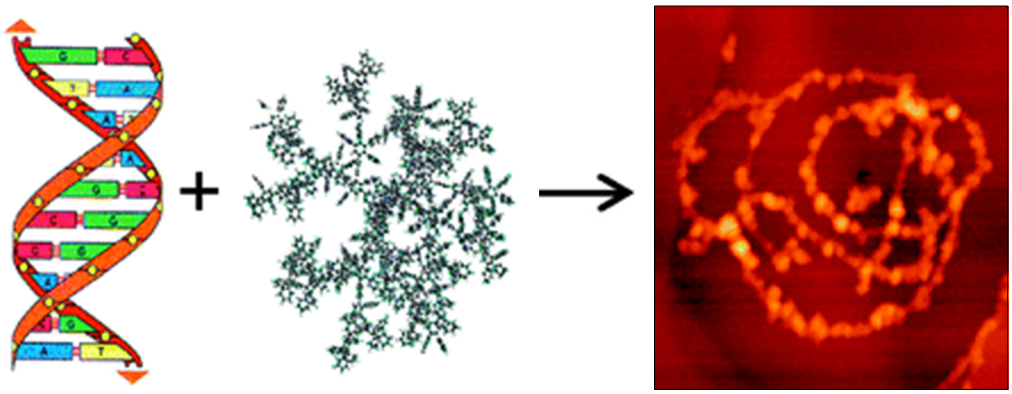


Figure 3. Schematic representation of the interaction between plasmid DNA and wPPPD dendrimers.[25]

scattering data combined with the results of sedimentation analysis, the addition of wPPPD to a DNA solution in a wide concentration range of the components (before the phase separation) leads to the coexistence of dendriplex nanoparticles and free nucleic acid. The spontaneous formation of soluble nanoparticles of cationic dendriplexes stable in solutions of physiological ionic strength at physiological pH values is of particular interest for the design of wPPPDs carriers for the target delivery of genetic material into cells.

Water-soluble aromatic dendrimers can serve as model systems for the development of dual-action drugs.[26] A comparative analysis of the binding of two anionic pyrenyl probes to highly polymerized pyridinium cations of different topology, namely PEVP and wPPPD, was performed by means of fluorescence quenching technique. The hydrophilic probe containing three sulfonate groups more efficiently interacts with the flexible highly charged polycation throughout the pH range under study. The dendrimer with a hydrophobic probe containing one carboxyl group binds more strongly to the polycation upon acidification of the solution, and this interaction predominates in weakly acidic media. The interaction of DNA with the dendrimer containing the hydrophobic probe has no effect on the formation of a dendriplex and only leads to the release of a small amount of the bound probe into solution. The model study demonstrated that it is possible to construct a dual-action dendrimer, which can simultaneously deliver the genetic materials and a hydrophobic drug to the target cells.

Competitive reactions in solutions of dendriplexes and polyelectrolyte complexes were studied[27] in relation to ternary systems consisting of DNA, the cationic wPPPD dendrimer and the quaternized cationic polysaccharide chitosan. This investigation revealed the factors responsible for the fine control of ternary systems, in which DNA molecules and a polymethacrylate anion compete for the binding to the cationic polymer. The dendrimer was displaced from the dendriplex with a polystyrene sulfonate (PSS) anion regardless of the pH value and the chain length, even in the case of short oligomer chains. On the contrary, the PMA anion was weakly active in slightly acidic solutions, whereas it not only showed activity in neutral media but the activity increased with increasing chain length of the anion. The fact that the PMA anion cannot compete with DNA in slightly acidic media even despite the pronounced stabilization of the PMA – dendrimer complex by hydrogen bonds is a clear evidence of the crucial role of Coulombic forces in the behaviour of ternary polymer systems with a mixed type of interactions. Similar patterns were observed in studies of polyplex solutions. The results of the study[27] provide approaches to the construction of efficient transfection vectors.

The sizes of polyelectrolyte complexes formed by PSS anions and rigid wPPPD dendrimers of different generations were evaluated by dynamic light scattering of water-soluble polyphenylenepyridyl dendrimers.[28] The effects of the chain length of the linear polyanion, the generation number of the dendrimer and the charge ratio on this molecular parameter were found and characterized. This study confirmed the conclusions of the theoretical analysis of interactions between the dendrimer and oppositely charged counterparts, according to which the mixing of aqueous solutions of the components leads to the spontaneous formation of nanoparticles of water-soluble polyelectrolyte complexes.

The formation of soluble complexes of wPPPD with native DNA was studied by the titration of a nucleic acid solution with a dendrimer solution.[29] The addition of the dendrimer was accompanied by the appearance and accumulation of compact dendriplexes 100 ± 10 nm in diameter The dendriplexes coexist with particles having sizes identical to those of free DNA (450 ± 50 nm). The fraction of complex particles gradually decreased and finally disappeared when the equimolar ratio of charged groups of the components was reached. The coexistence of almost free DNA and compact negatively charged particles was confirmed by sedimentation analysis. The subsequent addition of the dendrimer to a certain ratio of charged groups that is far from the unity led to phase separation. Further, in the presence of a significant excess (almost fivefold) of charged groups of the dendrimer, the system was completely transformed into a single-phase solution of compact cationic particles (110 ± 10 nm). The positive charge and the small size of the complex particles (smaller than the critical value of 150 nm) are the factors responsible for interactions between the negatively charged cell membranes and these particles and their uptake by the cell *via* endocytosis.

The ability of an oligomeric anion to penetrate into the rigid aromatic wPPPD dendrimer was demonstrated.[30] Unexpectedly, a decrease in the degree of polymerization of polyanions to the values typical of oligomers led to an increase in the efficiency of their electrostatic binding to

cationic wPPPD. Nothing like that was observed upon complexation of oppositely charged linear polyelectrolytes or pairs of polymers stabilized by hydrogen bonding. On the contrary, the replacement of a polymer counterpart in the complex by its oligomer was always accompanied by a decrease in the efficiency of interchain binding. This result is direct evidence that the oligomeric anion can penetrate into the rigid dendrimer structure, resulting in the formation of new ion pairs with pyridinium cations inaccessible to highly polymerized polyanions. The less efficient binding of dendrimers to long chains, while remaining completely accessible to oligomeric anions, confirms the theoretical predictions and may be a decisive factor for the development of practical applications.

Studies of dendrimers, including the model issue, are summarized in a review.[31] Special attention was paid to the problems with using dendrimers, in particular the ability of high-generation dendrimers to cause cell membrane damage due to an excess surface charge. This problem can probably be solved by finding ways to decrease the cell surface potential without a significant decrease in the ability of the dendrimer to be bound to nucleic acids. Another important issue is to synthesize polyplexes sensitive to changes in pH to the value, at which the release of the delivered nucleic acid definitely occurs. The cited review presents a comprehensive survey of several approaches developed for the fabrication and investigation of dendrimers as DNA and RNA carriers.

4. Soluble chitosan polyelectrolyte complexes

Chitosan (CHT) is an available cationic polysaccharide, the useful properties of which are not fully realized, its potential being retained and enlarged upon complexation with anionic polymers. Interactions of CHT with polyanions were considered in numerous original publications. The results of these studies were integrated in a number of reviews dealing with the synthesis, characterization and applications of polyelectrolyte complexes with CHT. The results of research on the binding of the polysaccharide to synthetic polanions and to modified natural polyanions, nucleic acids and proteins are surveyed in a review.[32] Peniche *et al.*[33] focused their attention on cooperative reactions of CHT with polanions and the properties of membranes produced from these reaction products. A review [34] is devoted to properties of polyelectrolytes isolated from biomass, mainly alginates and carraghenates. Chitosan microspheres are of great interest as carriers of physiologically active substances. The preparation of CHT microspheres and their properties are considered in several publications.[35 – 37] The pharmaceutical application of CHT as a biocompatible polyelectrolyte for microcapsulation was analyzed by Karteek *et al.*[37] The biomedical issue is addressed in the review [38] concerned with new chitosan micro- and nanofibres. The use of CHT as a non-viral transfection vector for gene therapy occupies a special place.[39] Chitosan microparticles are fabricated by different methods.[40] Due to mucoadhesive properties of CHT, these particles are able to moderately interact with the cell membrane or tissue. It was emphasized [41] that the incorporation of CHT in a polyelectrolyte complex imparts new useful properties to the polysaccharide without loss of biocompatibility. Hence, CHT nanoparticles, microparticles, fibres and sponges are highly promising for the targeted transport.

Recent advances in this field of research are presented in reviews.[42 – 52] For instance, a review [42] is devoted to the use of CHT and its derivatives for antimicrobial modification of textile materials. The data on sulfonate and sulfate derivatives of CHT for biomedical applications are surveyed and integrated by Dimassi *et al.*[43] Advances in the manufacture of chitosan-based hydrogels for wastewater treatment were summarized.[44, 45] The design and construction of chitosan-based materials for tissue engineering were analyzed by Ahmed *et al.*[46]

Drug delivery systems for the targeted therapeutic treatment using chitosan carriers were described in detail by Merli *et al.*[47] The multifunctional nature of hetero derivatives of chitosan as biodegradable adsorbents for heavy metal ions was discussed by Ahmad *et al.*[48] The decoration of nanoparticles of chitosan and its derivatives by folic acid for the delivery of drugs and genetic material to cancer cells was analyzed by John *et al.*[49] Chitosan-centred bionanocomposites for application in medicine are considered by Ahmad *et al.*[50]

Eroglu *et al.*[51] reviewed the data on biopolymers based on sugars (including CHT) for nanomedicine and suggested the beginning of a new era in the visualization of cancer cells and their therapy. This conclusion is confirmed by the review [52] on polysaccharide nanomaterials for the targeted delivery of anticancer drugs.

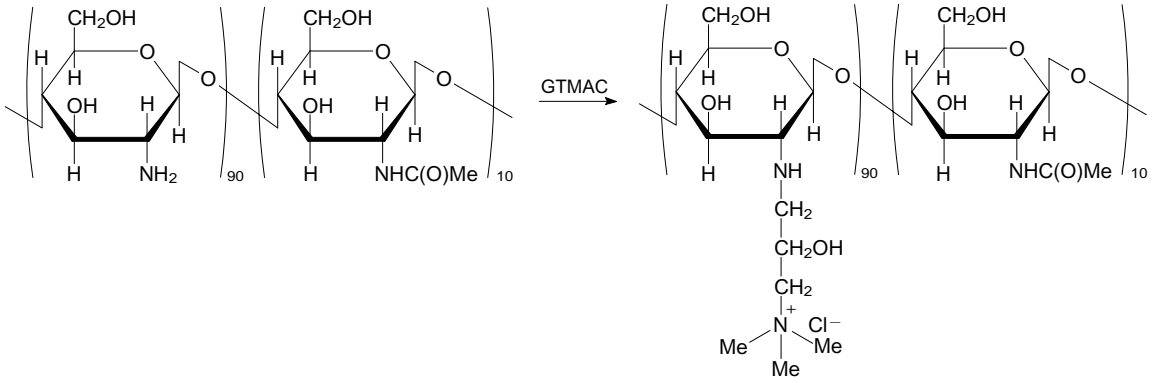
The preparation of nonstoichiometric chitosan polyelectrolyte complexes soluble in water and aqueous salt media stimulates the further development of this area and provides the basis for the construction of new biocompatible and biodegradable polymer systems.

It is worth noting that, while the advantages of the use of NPECs in basic and applied research are evident, the incorporation of CHT in such complexes has remained a long-standing problem. This is due to very low solubility of the polysaccharide in slightly acidic solutions and its insolubility in neutral and alkaline media. This drawback associated with the deprotonation of primary amino groups of the polymer and their involvement in intermolecular hydrogen bonds interferes with the use of CHT under physiological conditions (pH 7.2, 0.14 M NaCl). Numerous attempts to improve solubility of the polysaccharide by incorporating it in soluble NPECs or by means of chemical modification of CHT chains were not successful.

Both approaches were further developed [53 – 60] and promising results were obtained. Thus, the mixing of solutions of CHT and a PSS anion in strongly acidic media resulted in the preparation of soluble NPECs;[53] however, the need for performing experiments under conditions that are far from physiological significantly restricts investigation and practical application of such complexes. The problem was overcome by neutralization of the resulting acidic solutions of NPECs with an alkaline solution.[54] This sequence of treatments makes it possible to avoid phase separation, which occurs in the case of direct mixing of CHT and polyanion solutions in neutral and weakly acidic media. The prepared complexes remained stable and soluble up to pH 7.2, thereby meeting the criteria of behaviour of water-soluble NPECs.[55]

Despite advantages, this approach has some shortcomings. Not every anionic biopolymer retains functional properties in strongly acidic media, which are necessary for the

Scheme 1



formation of soluble NPECs in the first stage of their preparation. Another problem is a rather narrow pH range, in which water-soluble NPECs can exist. Even a slight alkalification of the medium (pH > 7.2) leads to cooperative destruction of a system of ion pairs due to the deprotonation of amino groups of CHT in the complex. Hence, it is important to reveal factors capable of controlling the pH-dependent dissociation profile of the complex.

The second approach is based on the use of modified chitosan (mCHT), the primary amino groups of which were selectively and exhaustively alkylated with glycidyltrimethylammonium chloride (GTMAC) (Scheme 1).[56 – 60]

The structurally homogeneous polymer contained amino groups of two types in each modified unit, namely, the quaternized amino group, which provides a positive charge and solubility of the chains throughout the pH range, and the secondary amino group.[56] The specific structure and properties of mCHT allow the preparation of soluble complexes under mild conditions by the simple mixing of a polysaccharide solution with a solution of PMA or PSS anions in neutral media. The reaction products have properties of water-soluble NPECs and are characterized by high stability.[57] The main results of model experiments were confirmed by the study of mCHT – nucleic acid polyplexes. The mixing of mCHT and nucleic acid in different ratios gave soluble, both negatively and positively charged, polyplexes stable at physiological pH and ionic strength of the solution. Soluble positively charged polyplexes are of particular interest because these complexes are electrostatically bound to the negatively charged cell membrane, thereby providing the first transfection step.

The presence of secondary amino groups in mCHT that are able to be (de)protonated upon changes in the acidity of the medium may play an important role in the delivery of the target nucleic acid *via* chitosan vector to the cell nucleus. The key factor of transfection is the timely release of the polyplex, which is taken up by the cell *via* endocytosis, into the cytosol. The ionization of secondary amino groups of mCHT ncorporated in NPECs[24] or polyplexes[58] occurs in slightly acidic media typical of endosomes and lysosomes. This is a prerequisite for the known proton sponge mechanism because the ionization of amino groups leads to an increase in the uptake of low-molecular-mass counterions (anions), resulting in a sharp increase in the osmotic pressure in endosomes and lysosomes and facilitating their destruction and escape of the polyplex into the cytoplasm.

Another important factor that needs to be taken into account in constructing efficient vectors is the presence of anionic structures in the cell. These structures may have a considerable effect on the mechanism of transfection. The interactions between positively charged polyplexes and the negatively charged cell membrane are unlikely to be solely electrostatic. Apparently, this process involves interpolyelectrolyte reactions, in which the delivered nucleic acid and anionic structures of the cell compete for the biding to the cationic carrier. Since polycarboxylic acids and poly(sulfonic acids) are present in the cell as anionic fragments, competitive reactions were carried out using model systems containing a mCHT-based polyplex and poly(methacrylic acid)[59] and(or) sodium polystyrene sulfonate.[60]

Fluorescence quenching studies using pyrenyl-labelled poly(methacrylic acid) and ethidium bromide (intercalating dye) demonstrated that the mCHT complex with polymethacrylate was much more stable in slightly acidic solutions compared to neutral media, whereas stability of the polyplex to the added salt remained virtually unchanged.[59] Despite the pronounced stabilizing effect of the slightly ionized polyacid due to an intermolecular hydrogen bonding system, the equilibrium of the competitive replacement was shifted towards the formation of the mCHT polyplex, which attests to the predominant role of electrostatic interactions in mixed-type systems stabilized by both ion pairs and hydrogen bonds. Ignoring this fact may lead to erroneous conclusions, in particular to the wrong prediction of the direction of the competitive binding in such systems, based only on the comparison of stability of the corresponding binary complexes in aqueous salt media.

The PSS anion, which is not involved in hydrogen bonding, shows a different behaviour. This anion almost completely displaces mCHT from the polyplex in the whole pH range under study even in the case of short oligomers consisting of eight styrene sulfonate units. High efficiency of binding of polysulfonate anions may be indicative of an important role of mucopolysaccharides in transfection. A contact of the negatively charged cell surface with cationic polyplex molecules can be accompanied by their interaction with heparan sulfate (or chondroitin sulfate) immobilized on the cell membrane and the competitive displacement of the delivered nucleic acid into solution. Hence, the vector should be produced in the presence of a large excess of the polycation in order to ensure stability and solubility.

Experiments in living cells[60] provided strong evidence in favour of the aforesaid. Soluble cationic polyplexes, which were prepared by the reaction of mCHT with plasmid DNA or small interfering RNAs according to the recommendations developed in studies of model systems, did exhibit

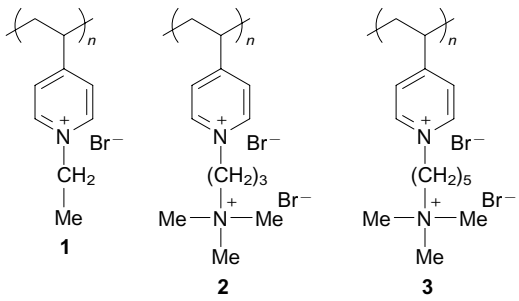
transfection activity that significantly increased with increasing chain length of mCHT. High-molecular-mass polysaccharide preparations, being less toxic, were found to be more efficient even than the commonly used transfection reagent polyethyleneimine.

Divinyl ether – maleic anhydride copolymer (DIVEMA) deserves special attention. This copolymer has intrinsic antitumour and immune-stimulating activities and is used as a drug carrier in antitumour drug delivery systems. Anionic DIVEMA preparations with different degrees of polymerization were synthesized and characterized[61] and their interaction with the PEVP cation was studied. According to potentiometric titration data, almost all carboxyl groups of the copolymer form ion pairs with the polycation. In aqueous and aqueous salt solutions of component mixtures, both soluble and insoluble polyelectrolyte complexes can be formed depending on the pH value, the charge ratio and the degree of polymerization of both polyions. Phase separation follows general principles found in studies of polycation mixtures, in particular PEVP, with polycarboxylic acids. However, a significant expansion of the heterogeneous region was observed for DIVEMA due to soluble complexes. Therefore, the polyelectrolyte complexation has obvious advantages as noncovalent modification of a polymer carrier, which makes DIVEMA able to undergo reversible transitions between the solution and the precipitate, in particular at physiological pH and ionic strength of the solution.

5. Supercharged polycations and their complexes

Highly charged polyelectrolytes having no more than one charged group per repeating unit, in particular vinyl polymers, were characterized in detail and are widely applied in practice, for example, in biotechnology for the separation of protein mixtures or for the nucleic acid delivery to the cells. Polyelectrolytes of higher charge density, *e.g.*, those bearing ionic or ionogenic groups in the repeating unit, are less well known. Meanwhile, these supercharged polyions would be expected to be efficiently bound to oppositely charged (bio)polymers giving polyelectrolyte complexes.

Structures 1 – 3



Losada *et al.*[62] briefly described the radical polymerization of cationic monomers containing two quaternized amino groups. However, this research was not further developed because the polymerization products are unstable and hydrolyze even in neutral media. Interesting results were obtained in the study of polycations with two cationic sites per monomeric unit. Their synthesis was reported.[63, 64] However, the drawback of this approach is that it involves four steps.

These difficulties were overcome by Izumrudov *et al.*[65] Supercharged polycations were prepared in one step by the alkylation (under relatively mild conditions) of the same starting compound, poly(4-vinylpyridine) (**1**), with different bromoalkyltrimethylammonium bromides, similar to the synthesis of modified chitosan described in the previous

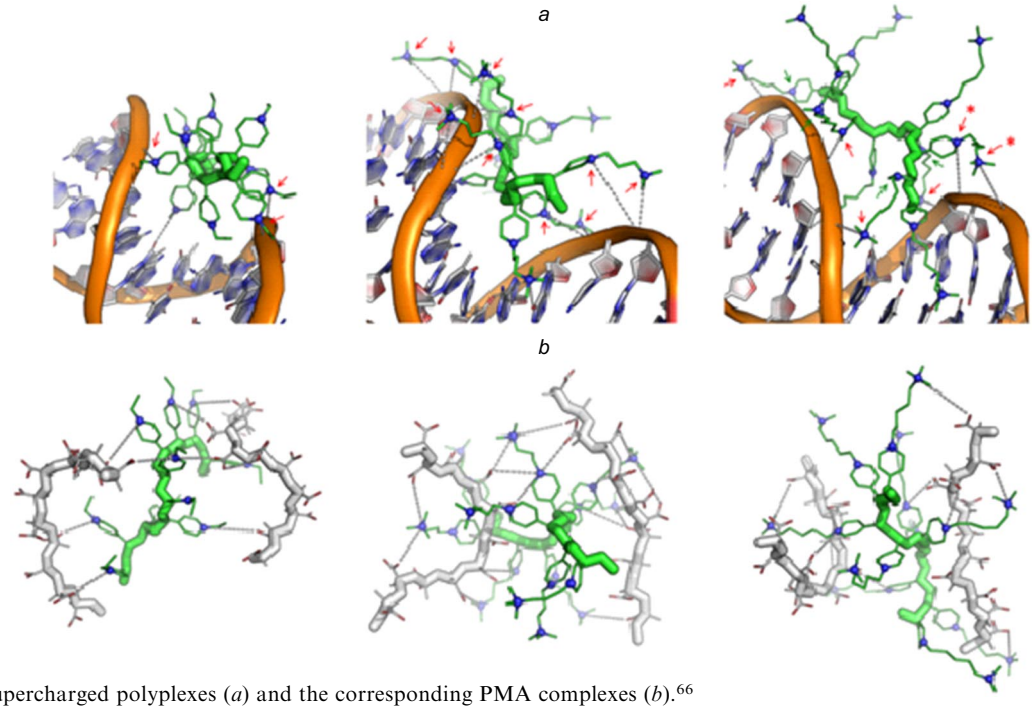


Figure 4. Supercharged polyplexes (*a*) and the corresponding PMA complexes (*b*).[66]
The nitrogen atoms bound (red arrows) and not bonded (green arrows) to DNA phosphate bases are indicated.

Section. The supercharged polycations thus prepared have three (compound **2**) or five (compound **3**) methylene groups in the spacer between the charges. In these polycations, each modified unit contains two pH-insensitive fully charged groups that are accessible to polyanions. The ion pairing, which was monitored by fluorescence quenching using pyrenyl-labelled polymethacrylate, resulted in the formation of soluble complexes. The most stable complexes were those containing a polycation with a short spacer. In the case of partially alkylated copolymers, the binding in slightly alkaline solutions becomes weaker with decreasing degree of alkylation of the polycation, but it becomes significantly stronger at pH $\leqslant$ 7.0 due to stabilization of the system by hydrogen bonds and the appearance of additional ion pairs between protonated pyridine groups.

This effect allows the fine control and tuning of the system in neutral media, thereby holding a potential for practical applications in biotechnology.

In the further study of polycations and their complexes,[66] native DNA was used instead of the PMA anion. This replacement made it possible to explain abnormally high stability of the polyplexes formed by supercharged polycations in aqueous salt media, which has been observed earlier but remained unclear.[67–70] The main conclusions drawn from the experiments,[66] which provided evidence of an important role of the steric factor in these interactions (in particular, the spontaneous formation of tetrahedral structures), were confirmed by model investigations that revealed the factors responsible for the structure, stability and properties of supercharged polycations (Fig. 4).

6. Microcapsules and nanogels with controllable stability

The formation of polyelectrolyte multilayers on charged surfaces was analyzed.[3] Multilayers are nanosized systems highly prone to self-assembly and self-adjustment, which is of particular interest for materials science, biomedicine and pharmaceutics. The breakthrough in these areas was due to the development and improvement of new technological approaches. The so-called layer-by-layer (LbL) technique for the fabrication of polyelectrolyte nanofilms, nanogels and microcapsules with controllable stability has an important place. The advantage of the LbL technique is its simplicity. The multilayers are fabricated by the alternate treatment of the substrate of any shape by dilute solutions of oppositely charged polyelectrolytes. Nevertheless, the proper and efficient application of this method for the fabrication of nanofilms requires that a number of conditions are met. These conditions can be found in model studies. The scope and prospects of application of polyelectrolyte multilayers in different fields were discussed.[3]

The prospects of practical use of the LbL technique were demonstrated by Mussabayeva *et al.*,[71] who synthesized and characterized polyelectrolyte microcapsules as new targeted delivery systems of two antituberculosis drugs. The simultaneous encapsulation of oral drugs, pyrazinamide and moxifloxacin, was accomplished for the first time using multipolyelectrolyte layers. Gellan gum and sodium alginate were employed as biopolymer matrices. The efficiency of the simultaneous encapsulation was monitored by UV-Vis spectroscopy. Microcapsules were treated with the cationic polysaccharide (chitosan) and the polyanion Eudragit S100, and the charges of all deposited nanolayers were determined by ζ-potential measurements for three, five and ten polyelectrolyte bilayers. The structures of microcapsules were studied by scanning submicroscopy. The *in vitro* drug release was evaluated at different pH values mimicking different regions of the gastrointestinal tract. The study demonstrated that the drugs had a prolonged action, which was enhanced with increasing number of bilayers. Thus, five layers were sufficient for the prolongation of drug release to 24 h.

The encapsulation of pyrazinamide, moxifloxacin and isoniazid by polyelectrolyte multilayers composed of cationic chitosan and the anionic polyelectrolyte dextran sulfate was performed on biopolymer matrices (gellan gum, pectin and sodium alginate). The capsules were studied by scanning electron microscopy. The efficiency of encapsulation and drug release are indicative of the prolonged action of microcapsules.[72]

Protein protease inhibitors having different specificity to trypsin, chymotrypsin and elastase were encapsulated together with the target protein into polyelectrolyte microparticles by the LbL technique.[73] The formation of an insoluble protein – polyanion complex is the step in which encapsulation was the most efficient. The simultaneous immobilization of the inhibitor and the protein has no significant effect on physicochemical properties of the microcapsules, in particular on their pH sensitivity, under conditions mimicking the transit through different regions of the gastrointestinal tract after oral administration. The most pronounced protection against proteolytic enzymes was achieved in the case of simultaneous release of the protein and the inhibitor from microparticles. Therefore, this is a promising approach to improve bioavailability of the protein after oral administration.

The experimental data on the fabrication of protein microparticles with controllable stability using the LbL technique and investigations of their properties are analyzed in a review.[74] Different applications of proteins as adsorbed polyions, methods of incorporation of proteins into matrices (aggregates and microspheres) for subsequent deposition of biopolyelectrolytes and the encapsulation of proteins into multilayer charged particles with different permeability were considered. Special attention was paid to biocompatible and biodegradable microparticles, which act as depot systems, *i.e.*, they are able to efficiently protect biologically active substances against harsh medium in the body and quantitatively release protein preparations (hormones, enzymes and peptides) once a certain acidity of the solution is achieved. This ability is of particular interest for the development of targeted oral drug delivery systems.

Polyelectrolyte complexes of chitosan with gellan gum were successfully applied to deliver oral[75] and nasal[76] drugs. The encapsulation of drugs into particles composed of two oppositely charged acrylate copolymers, Eudragit® EPO (EPO) and Eudragit® S100 (S100), as well as Eudragit® RL PO (RL) and Eudragit® L 100-55 (L55), enables their controlled release; the prolonged drug action was more than 7 h.[77–80]

Prado and Matulewicz[81] gave a systematic analysis of the synthesis methods, properties and fields of application of polyelectrolyte complexes of cationic polysaccharides, such as starch, dextran, cellulose and its derivatives, hemicellulose, pectin, chitosan and seaweed polysaccharides. The results of toxicological studies of the polysaccharides were summarized. The structures of many complexes of cationic

polysaccharides are similar to those of hydrogels. This allows the expansion of the scope of application of these complexes. In particular, they can be used instead of synthetic materials for biomedical and environmental applications.

The properties and applications of gellan gum, a natural anionic polysaccharide, are discussed in the review.[82] This polysaccharide is commercially available in two forms, as high acyl and low acyl gellans. It is widely used in the food industry for gelling and stabilizing. Gellan gum forms complexes with cationic polyelectrolytes, in particular with polysaccharides and proteins, that are suitable for targeted drug delivery. These complexes hold promise in food biotechnology, the repair of damaged tissues and the fabrication of superconducting hydrogels for microelectronics.

Hydrogel nanoparticles with a hydrodynamic radius of 40 – 50 nm in reverse micelles were prepared.[83] All amino groups of the nanoparticles were protonated in acidic media, but almost one third of them were inaccessible to the relatively flexible PSS anion, while two thirds were accessible to the rigid DNA double helix. Complexes of nanogels with plasmid DNA hold promise for transfection. The products of interaction of nanogels with small interfering RNAs suppressed the expression of the marker enzyme. The replacement of the linear polyamine in both systems with nanogel leads to a significant increase in the delivery efficiency. Transfection experiments revealed the dependence of the activity of nanogel on the degree of cross-linking. The maximum activity was achieved using the cross-linking agent at a concentration of 5 mol.%. These data provide evidence that the proposed approach is suitable for the synthesis of cationic nanogels as carriers of genetic material to living cells.

A comparative study of interactions of linear poly[2-(dimethylamino)ethyl methacrylate] and its cationic nanogels having different cross-linking densities with DNA and sodium polystyrene sulfonate was reported.[84] All amino groups of nanogels proved to be accessible for protonation; however, their ability to form ion pairs with polyanions depends on the degree of cross-linking; the higher the degree of cross-linking, the lower the ability. The investigation of nanogel complexes with cell cultures using a calcein pH-sensitive probe revealed a gradual increase in cytoplasmic fluorescence with increasing degree of cross-linking due to calcein leakage from acidic compartments to the cytosol. This fact indicates that amino groups, which are predominantly buried inside the gel, are protected against ion pairing with polyanions of plasma membranes and, as a consequence, they can exhibit buffer properties, being entrapped in acidic endosomes, *i.e.*, have lyso(endo)somolytic capacity. These data are indicative of an important contribution of network structures to the proton sponge behaviour of weak polycations.

Of particular note are approaches based on modification of nanoparticles by interpolyelectrolyte reactions, which impart required functionality to the particles. Thus, due to the magnetostatic field, the nanoparticles are demanded in many areas, from medicine to catalysis. A facile method was developed for the preparation of thin polyelectrolyte shells on the surface of magnetic nanoparticles.[85] This method can be applied to fabricate shells stable in a wide pH range and bearing both positive and negative charges.

Brito *et al.*[86] synthesized magnetite nanoparticles stabilized by a polymer matrix composed of the chitosan complex and the PSS anion. Both positively and negatively charged matrices were prepared by varying the polymer concentrations in interpolyelectrolyte reactions, the magnetic properties of magnetite nanoparticles being preserved and independent of the charge of the matrix.

7. Suppression of protein aggregation and prevention of protein denaturation

Interpolyelectrolyte reactions can be used to construct nanosized complexes as targeted delivery systems of different physiologically active substances, in particular proteins. In many cases, the bound protein should be utilized after the targeted delivery. The study of the proteolysis of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) in the complex with polyelectrolyte[87] demonstrated that polyanions can enhance the proteolytic degradation of GAPDH by proteinase K and thermolysin due probably to destabilization of the protein structure. This process is reversible because the release of the enzyme from the complex with polyelectrolyte (even if the polyelectrolyte is tightly bound to the protein, as in the case of the sulfated polyanion or a supercharged polycation) is accompanied by partial or complete reactivation of GAPDH depending on the nature of the polymer or environmental conditions. The complexation with the sulfated polymer was found to enhance the proteolytic degradation of prion fibrils by proteinase K. This may be used to treat pathologies associated with amyloid aggregation.

Polyelectrolytes are promising tools for protecting proteins against aggregation. The results of comparative study of the ability of the synthetic PSS anion and natural chaperones of different types to prevent protein aggregation were reported.[88] The PSS anion was found to have chaperone-like activity, which was retained at high temperatures (even at 60 °C), whereas this effect disappeared at 50 – 55 °C in the case of chaperones. These results demonstrate that

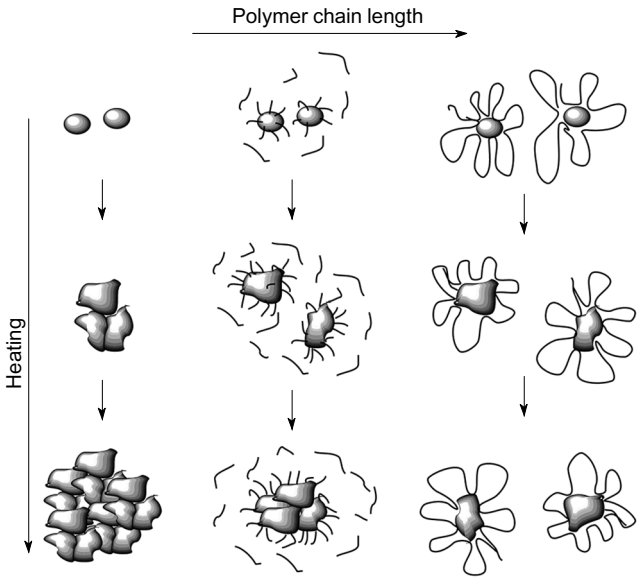


Figure 5. Schematic representation of the influence of the polymer chain length on protein thermal aggregation.[66]
Short-chain polymers (oligomers) are low active.

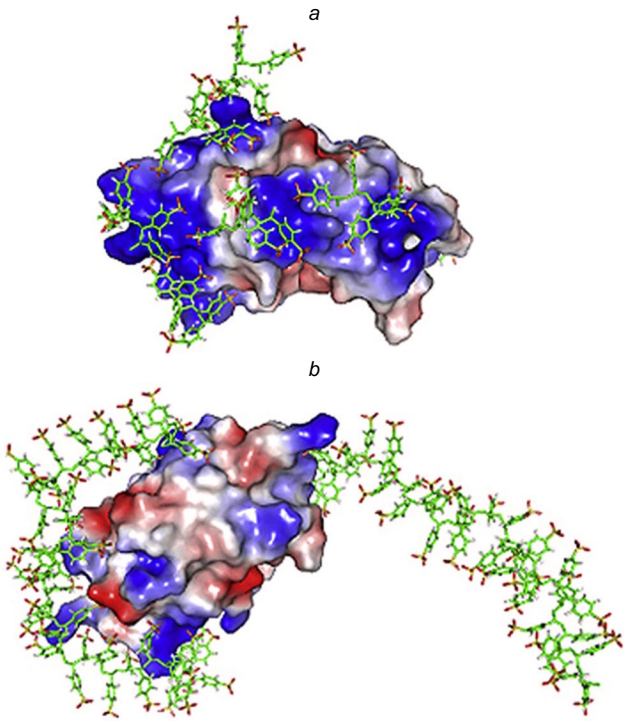


Figure 6. Insoluble (*a*) and soluble (*b*) protein – polyelectrolyte complexes.[90]

synthetic polyelectrolytes can be employed to construct artificial chaperones.

The suppression of undesirable protein aggregation is a challenging problem. The protein thermal aggregation was reduced or completely prevented using synthetic polyelectrolytes, the efficiency of which increased with increasing chain length (Fig. 5).

Sulfate and sulfonate polyanions were found to efficiently solubilize protein aggregates of different nature (Fig. 6).[89,90]

A new approach was developed based on solubilization of protein aggregates by polysulfoanions. For the first time it was shown that polysulfoanions can solubilize amorphous and amyloid protein inclusion bodies and thermal aggregates. The treatment of prion protein inclusion bodies with sulfonate polymers led to a significant decrease in the fraction of amyloid structures; the application of the enzyme GAPDH led to even partial recovery of enzymatic activity after the thermal treatment of the protein with polymers. This approach may be useful for the extraction of recombinant proteins from inclusion bodies, as well as for the improvement and development of therapy for diseases associated with protein metabolism disorders.

8. Investigation of interpolyelectrolyte complexes

8.1. Model issue

In order to optimize targeted drug delivery systems using polycations, it is necessary to know the influence of molecular characteristics of cationic polymers on physicochemical parameters of polyplexes and their effect on the transfection efficiency. To tackle this issue, San Juan *et al.*[91] synthesized a series of model polymers based on the quaternized polycation with the same degree of polymerization but differing by the number of methylene groups (n) in N-alkyl moieties, which varied from 1 to 6. Besides, the degree of quaternization of pyridine groups was varied from 25% to 95%. In the case of alkylated polycations, the efficiency of transfection of a plasmid vector became detectable at $n = 4$, significant at $n = 5$ and again undetectable at $n = 6$. A decrease in the degree of alkylation of the polymer from 95% to 65% resulted in an up to a ninefold increase in the transfection efficiency. This effect was attributed to the proton sponge mechanism, namely, to the influence of protonation of nonalkylated pyridine moieties of polycations in slightly acidic media. The experimental data supported by the fluorescence quenching assay demonstrate the advantage of the use of partially alkylated tertiary amino groups of polyamines with medium-length alkyl side chains for the preparation of efficient nonviral transfection agents.

Zhiryakova and Izumrudov[92] presented a comprehensive overview of investigations of polyplexes using the fluorescence method based on the competitive binding of intercalating dyes to the DNA double helix. The interaction of a cationic (bio)polymer with DNA can be accompanied by the competitive displacement of the intercalating cationic dye, *e.g.*, ethidium bromide, from the double helix into the solution, resulting in fluorescence quenching of the probe. This effect served as the basis for the investigation of cooperative electrostatic binding of polymer components giving polyplexes. An example are studies of polyplexes based on a wide range of model polycations. These data can be used to evaluate the cooperativity of the binding and design systems with controllable stability. The latter is particularly promising for the construction of polyelectrolyte systems capable of efficiently delivering genetic material to cells.

The fluorescence quenching assay of interactions of the labelled PMA* anion with aliphatic and aromatic surfactants with different structures in aqueous and aqueous salt media demonstrated that the binding of surfactants to a polyanion molecule can be efficiently controlled by varying pH of solutions, the surfactant concentration, the number of methylene groups in alkyl moieties and positions of amino groups.[93] A significant and successive increase in stability of the complexes upon the replacement of quaternary amino groups of surfactants by the tertiary, secondary and primary amino groups is indicative of an important role of the steric factor in ion pairs. This effect may be particularly useful for the construction of polymer colloidal systems with controllable stability in aqueous salt media.

Interactions of the poly(*N*-oxyethyl-4-vinylpyridinium) cation containing OH groups in repeating units with the PMA anion and DNA were studied.[94] Stability of the resulting polyelectrolyte complexes against the destructive effect of the salt is influenced by the presence of OH groups in a different manner. In the case of DNA, the stabilization of the complexes is almost unchanged in the pH range of 5.5 – 9.0, whereas the complexes with the PMA anion are significantly stabilized due to a hydrogen bonding system between OH groups of the polycation and CO_2H groups of polycarboxylic acid. Despite much higher stability of the complexes of the low-charged PMA anion in slightly acidic media, polycations predominantly bind to the highly charged DNA double helix, which is indicative of the major role of electrostatic interactions in complexation. The results of the study [94] may be useful for the construction

of pH-responsive biopolyelectrolyte systems with a feedback and controlled stability.

The state of the art and advances in the field of experimental research on solutions of polyelectrolyte complexes based on biopolymers, in particular oligomeric fragments, chitosan or nucleic acids, are analyzed in a review.[95] Soluble biopolyelectrolyte complexes were shown to be promising for solving challenging practical problems. For example, methods were developed for suppressing thermal aggregation of globular proteins without loss of their functionality or for making chitosan (or its complexes) soluble at physiological pH and ionic strength of the solution without loss of catalytic activity and structural changes.

Compared to polycarboxylic acids, polysulfate and polysulfonate anions much more efficiently suppress protein thermal aggregation but they generally cause protein denaturation. Different polymers, in particular polyphosphate anions, were used to study the effect of charged groups on stability and thermal aggregation of the model enzyme GAPDH.[96] The interaction of the protein molecule with the polyanion and the effect of the bound enzyme on the protein structure were evaluated by isothermal calorimetry and differential scanning calorimetry. Highly polymerized polyphosphate anions were shown to be able to quantitatively suppress the aggregation but at substantially higher concentrations of the polymer compared to the PSS anion or dextran sulfate taken at the same concentration. The influence of polyphosphates on the enzyme structure and its catalytic activity is much weaker compared to sulfo-containing polyanions with the same degree of polymerization, particularly in the case of short-chain PSSs. These observations can help to elucidate the mechanism of control over the protein functionality based on the introduction of charged groups of different nature by post-translational modification of proteins.

Polyelectrolytes are increasingly used for delivery of drugs and genetic material to cells, protein immobilization and suppression of protein aggregation. Polysulfoanions occupy a special place among polyelectrolytes. They exhibit the most pronounced suppression effect but have an undesirable impact on the structure and catalytic activity of the bound enzyme. The factors controlling the denaturation of the model cationic protein GAPDH in the presence of polysulfoanions and polyphosphate anions with different degrees of polymerization were revealed in the study.[97] The titration of the enzyme with a polyanion solution was performed in two steps. The protein structure remained virtually unchanged till the end of the first step; the further titration led to protein denaturation. The polyanion : protein molar ratio corresponding to the onset of the second step increased with increasing polymer chain length and was diminished for highly polymerized polyanions, which did not cause protein denaturation. The knowledge of the factors responsible for protein denaturation is necessary for the adequate modelling of interactions between proteins and natural polyelectrolytes.

8.2. Interpolyelectrolyte complexes in soil ecology

The ability of polyelectrolytes and polyelectrolyte complexes to efficiently interact with various charged dispersed colloidal particles, which are highly sensitive to changes in the environment (temperature, pH, ionic strength of the solution, *etc.*), was employed in the development of soil amendments. The treatment with solutions of these compounds can prevent water and wind erosion and, correspondingly, the spread of various contaminations (chemical, radioactive, salt, *etc.*).[98] This area of research is of utmost urgency, in particular in relation to global desertification and terrible consequences of accidents at nuclear power plants.

There is a wealth of theoretical and experimental data on the technology of the preparation of polymers and their deposition on the surface of dispersed particles. Qualitative and quantitative compositions of mixtures of oppositely charged synthetic and natural polymers suitable for the preparation of polymer binders were found.[99] Special attention was paid to the development of techniques for coating of the surface soil layer with various polymer materials, including the preparation of insoluble complexes composed of a polycation and a polyanion followed by the conversion of the complex into a soluble state by adding a low-molecular-mass electrolyte, as well as by the successive impregnation with polycation and polyanion solutions. In this case, a polyelectrolyte complex is synthesized directly in the surface soil layer. Protective soil – polymer crusts against air (in an aerodynamic tube) and water (in a hydrodynamic apparatus) flows were studied in laboratory tests. The mechanical strength and temporary stability of polymer crusts were evaluated in field tests at different polymer levels under conditions of atmospheric precipitations of variable intensity and at a variable wind speed. Besides, the environmental compatibility of the developed polymer binders in vegetation experiments was assessed and the migration of toxicants (radionuclides, salts, heavy metals) was studied by their fixation and accumulation in a soil – polymer film.

Polyelectrolyte complexes have a broadly variable hydrophilic – lyophilic balance, which is responsible for their efficient adsorption on the surface of dispersed particles of different nature.

For artificial irrigation,[100 – 102] a solution of a complex is prepared by mixing aqueous solutions of anionic and cationic polyelectrolytes in the presence of one (or several) water-soluble salt, such as alkali metal, ammonium, calcium or magnesium salts. After drying on the soil or ground surface, a soil – polymer crust is formed. The thickness of the crust is determined by the component ratio in the mixture, their consumption and, correspondingly, the depth of penetration. It was experimentally found that the order of the addition of the components is not so important. It should be noted that the mixing of dry components or their suspensions in organic media does not give rise to complexes. The molecular mass of the polymers can be varied in a wide range, the optimal percentage of charged units of one polyelectrolyte being in the range from 5% to 50% of another polyelectrolyte, and the sign of the charge of the polyelectrolyte, taken in excess, is of little importance.

In the so-called two-solution method, aqueous solutions of polyelectrolytes are prepared separately in individual vessels with the total equimolar charge-to-charge ratio being kept at charge neutrality.[103] The soil is exhaustively saturated with a solution of the first polyelectrolyte followed by the treatment with the second polyelectrolyte. In this case, an insoluble complex is formed directly on the surface soil layer after the deposition of a solution of the second polyelectrolyte (Fig. 7).

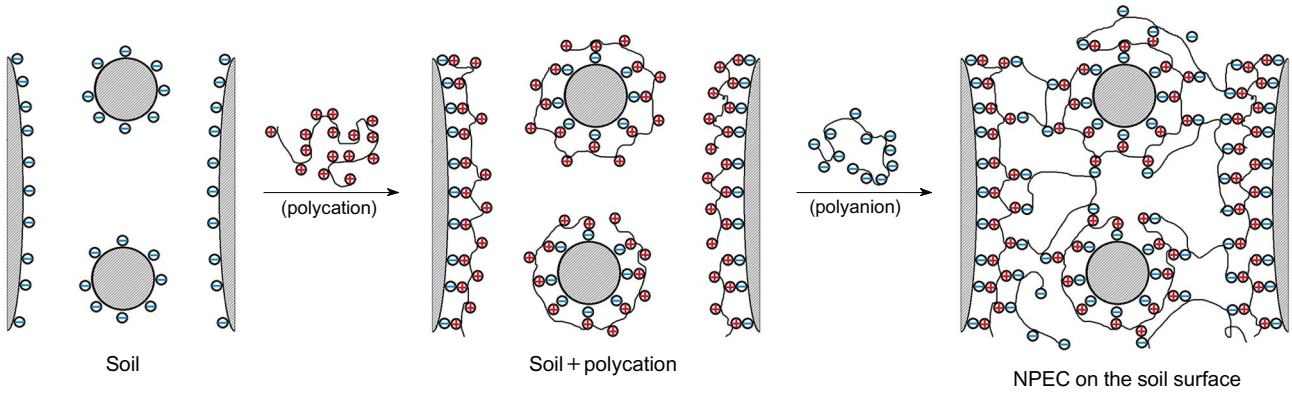


Figure 7. Schematic representation of soil saturation with solutions of complexes.

Both procedures have the following environmental advantages:

— the use of nontoxic aqueous solutions of polyelectrolytes and salts;

— long-term high efficiency of soil stabilization (for more than 3 – 5 years);

— the absence of undesirable soil salinization;

— permeability of crusts to air and water;

— the large-scale application in the case of environmental disasters and industrial accidents.

The above-mentioned first method is characterized by the formation of homogeneous crusts. In the last method, the successive introduction of polyelectrolytes does not lead to the formation of a homogeneous crust but results in a nonhomogeneous continuous or local cohesion of dispersed particles.

New polymer formulations based on NPECs with an excess of an anionic polymer were developed by Panova *et al.*[104, 105] A negatively charged complex can interact with cationic colloidal particles of the soil bonded to heavy metal cations. The formulations are suitable for the suppression and prevention of erosion of soils and grounds contaminated by highly toxic metals.

The use of secondary raw materials (industrial waste) as polyelectrolyte amendments is a challenging and attractive problem.[106, 107] Biopolymers and their complexes are environmentally friendly. Hataf *et al.*[108] used chitosan for soil aggregation and found that it has a short-term effect exclusively in wet soils. Due to their structural features, polymer complexes are more suitable materials for soil sabilization than individual polymers. Shulga *et al.*[109] developed a new environmentally friendly lignosulfonate-based glue, which is a lignin-containing polyelectrolyte complex, for stabilization of sandy soils. It was shown that the rheological properties of the complex, which are associated with its stabilizing ability, and, consequently, the fractional composition and properties of the soil aggregates can be changed by varying the composition of the complex.

The results of field and laboratory tests of soil – polymer systems in an aerodynamic tube and the modelling of stability at a wind speed of 25 m s^{-1} are indicative of a significant (more than fivefold) decrease in the entrainment of treated soils.[110]

It is important that soil amendments based on polyelectrolyte complexes are safe for soils, plants and animals. The treatment with such compounds results in the formation of a soil – polymer crust facilitating plant growth.[111] Thus, experiments with cotton crops[112] showed that this treatment leads to the improvement of germination and assists in quick growth and strengthening of root system. This is attributed to the greenhouse effect created by the soil – polymer crust, which slows the evaporation of soil moisture from the surface of the field.

The assessment of environmental compatibility of polyelectrolyte complexes based on carboxymethylcellulose and urea formaldehyde resin performed for many years in cotton farming[113] clearly demonstrated that these binders are beneficial to the vegetation period. They facilitate the creation of required agrophysical conditions for uniform germination and finally lead to an increase in the cotton yield by 4 – 5 centners per hectare.

It is worth noting that insoluble polyelectrolyte complexes were used starting from the 1970s as coagulants for the treatment of toxic and radioactive mining waste slag and slurry, metallurgical waste and waste from concentration plants and tailing ponds in order to prevent dustiness of air by small particles (not larger than 0.25 – 0.1 mm). The developed approaches rely on stabilization of soils, grounds and other disperse systems *via* interactions of polyelectrolyte complexes based on synthetic polyelectrolytes [hydrolyzed polyacrylonitrile, PAA, poly(*N*,*N*-dimethyldiallylammonium chloride), urea formaldehyde oligomers, *etc.*] and natural polymers (carboxymethylcellulose, lignosulfonate, carboxylated lignin) with the charged surface of dispersed particles and their cohesion. This leads to the formation of a water-insoluble protective soil – polymer structured layer of a thickness of 3 – 5 mm.

The most impressive results were obtained when using polyelectrolyte complexes for the elimination of consequences of the Chernobyl disaster. Starting in 1986 and over 12 years, amendments for soils and grounds with unique efficiency were synthesized and examined in order to localize dust-like contaminations.[99] The rational use of drifting sands in agriculture was considered by Allamuratov *et al.*[106] The studies on the synthesis of water-soluble polymer composites based on lignosulfonates with phosphorus-containing industrial waste coming from chemical plants were performed in laboratory and field conditions in order to stabilize drifting saline sands of the dried Aral Sea bed.

The soil treated with amendments has a stable cloddy structure for at least five years. This ensures plowing without mechanical forces and additional costs and provides a

favourable water air regime and soil salinity in accordance with biological parameters of the growth and development of perennial crops and climatic conditions. Radioactively polluted soils of the Semipalatinsk Nuclear Test Site were studied for their remediation.[114] The ability of carboxyethyl 3-aminocrotonate/acrylic acid and its derivatives involved in interpolymer complexes to protect against wind erosion (anti-deflation effect) and capture radioactive ^{90}Sr was evaluated. Apparently, the fact that the polyampholyte is more efficient as a stabilizing and ^{90}Sr-capturing agent compared to the corresponding complexes of homopolymers is attributed to the nature of the polyampholyte, its hydophobicity, the surface charge of complex particles and the ability to form ionic, hydrogen and coordination bonds.

The optimal compositions of ameliorants, agents that reduce irrigation erosion and increase water-absorbing ability, porosity, stabilizing properties and soil fertility, and conditions of their use are disclosed in a patent document.[115] Economically affordable and agriculturally valuable ameliorant composites that reduce nutrient loss *via* surface runoff during irrigation, were found.

A nonstoichiometric complex of chitosan with sodium carboxymethylcellulose (Na-CMC) of the composition [CHT] : [Na-CMC] = 30 : 70 was synthesized.[116] The treatment with a solution of this complex led to an increase in the mechanical strength of structured soil samples and improvement of water permeability of the soil, which is indicative of aggregation of its particles and an increase in stability against water and wind erosion. The duration of the film formation on a polymer substrate and formation of soil ameliorants at room temperature was 3 days.

Anti-erosion measures using NPECs require very low consumption of polymers, on the average, from 20 to 100 kg per hectare.[99] In model experiments on dark and light chestnut soils of the dry-steppe zone of Kazakhstan,[116] 2.5 kg of chitosan and 14 kg of CMC per hectare of the soil were consumed for the preparation of a mechanically stable and water-permeable soil – polymer film of an average thickness of 0.5 mm using a two-solution method for the introduction of the dry polymers.

Evidently, the harmful impact of biopolymers is not so acute compared to synthetic polymers. Nevertheless, attempts were made to study degradation of biopolymers. For instance, the biodegradation of cellulose-based biopolymer films was investigated in organic compost at a humidity of 85% at 20 and 60 °C.[117] Under these conditions, the biopolymer degrades to form oligomers that are taken up and metabolized by microorganisms. The decomposition of the composite was found to occur more rapidly than the decomposition of the individual polymer. Almost 100% biodegradation giving carbon dioxide and water is achieved within 10 – 12 weeks at moderate temperature, thereby closing the natural cycle of substances.

The contamination of soil with so-called engineered nanomaterials (ENM) is an environmental concern. It is necessary to prevent transport of engineered nanomaterials through soil. The transport properties of carbon nanotubes, fullerenes and gold nanoparticles through a quartz sand-packed column were studied.[118] Quartz sand served as a simple soil model. The treatment of ENM-contaminated sand with a mixture of oppositely charged polyelectrolytes, in particular PAAC and polyacrylic acid, in the presence of sodium chloride leads to flocculation of ENMs and their adsorption on the sand surface. This approach proved to be universal for different types of nanomaterials (carbon, metal and oxide nanoparticles), provided efficient accumulation of ENMs (up to 90%) and showed high performance.

Therefore, the results of experimental and theoretical studies demonstrate that polyelectrolyte complexes are promising soil amendments, which will make it possible to convert low-potential lands into agricultural use.

9. Conclusion

The analysis of a wide range of polyelectrolyte systems that differ in the nature and structure of their polymer components confirms the validity of conclusions made previously in studies of water-soluble nonstoichiometric polyelectrolyte complexes. A unique combination of stability of the complexes and their ability to be involved in competitive interpolyelectrolyte reactions, some of which are characterized by high selectivity, underlies the self-assembly of NPEC particles that occurs upon mixing of solutions of polymer components. Complexes remain stable over a wide range of external conditions (pH, ionic strength, temperature) and then show a rapid, reversible and highly sensitive response to environmental changes by changing the phase state.

The main general features revealed in studies of complexation, the phase behaviour and competitive interpolyelectrolyte reactions demonstrate that NPECs can be considered as new polymer compounds that are formed in equlibrium reactions and are characterized by a continuous exchange between polymer components in aqueous salt media. The ability of NPECs to be involved in competitive reactions, which can be highly selective, provides self-assembly and self-tuning of complex particles. The distinguishing feature of some polyelectrolyte complexes is their solubility in aqueous media giving so-called polyelectrolyte systems with a feedback, which undergo controlled phase separation caused by changes in external conditions. These features and properties of NPECs are fundamental and can be extended to polyelectrolyte complexes of different chemical nature, in particular, to complexes of globular proteins, enzymes, antibodies and nucleic acids.

Investigations of the phase separation of NPECs are required for the practical application of NPECs in biotechnology, materials science, the targeted delivery of nucleic acids to the cells and the development of new processes and materials for regenerative medicine and tissue engineering. These principles underlie the development of efficient methods for the separation and purification of proteins and nucleic acids, the improvement and upgrade of immunoenzymatic assays, the suppression of enzyme thermal aggregation, the design of reversibly soluble biocatalysts, multilayer ultrathin films and capsules, artificial chaperones and systems for delivery of genetic material to cells. Virtually all systems considered in the present review deserve special investigation.

A number of issues in this area of research remain to be addressed to achieve further progress. It seems necessary to employ rigid aromatic polyphenylene pyridine dendrimers as models in investigations of dendriplexes of nucleic acids. Available flexible aliphatic polyamidoamine dendrimers used for this purpose have significantly different structures, the difference being particularly pronounced between low and higher generation dendrimers, while rigid aromatic dendrimers retain their shape under these conditions and, therefore, are more convenient for model theoretical studies.

Besides, the factors responsible for abnormally high stability of NPECs based on poly(sulfo acids) (polysulfates and polysulfonates) in aqueous salt media are unknown. This distinguishing feature provides stable formation of polyelectrolyte multilayers widely used for the fabrication of nanomaterials but it interferes with the preparation of water-soluble NPECs, which are synthesized by mixing solutions of polyelectrolyte components.

Finally, another issue closely related to the use of poly(sulfo acids) is the development of protein – polyelectrolyte systems, in which the complexation with the charged chains suppresses and prevents aggregation of protein globules without noticeable loss of their catalytic activity. According to the available data, this problem would be expected to be solved, which will be particularly useful for the proper folding of protein chains during their synthesis.

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