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Equilibria in interpolyelectrolyte reactions and the phenomenon of molecular "recognition" in solutions of interpolyelectrolyte complexes

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ABSTRACT. The results of studies on the equilibria in interpolyelectrolyte reactions occurring in homogeneous aqueous solutions of linear synthetic polyelectrolytes bearing unlike charges and accompanied by the transfer of polyions are described. The influence of the chemical nature of low-molecular-weight ions and also of the chemical nature, linear charge density, and the degree of polymerisation of polyelectrolytes on equilibria in interpolyelectrolyte exchange and substitution is analysed. The phenomenon of the molecular "recognition" of one set of polyions by others is described and the factors leading to the selective binding of polyelectrolytes are indicated.

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I. Introduction

The study of the reactions betweem chemically complementary linear macromolecules and also the structures and properties of the interpolymer complexes (IPC) formed constitute one of the most interesting and promising aspects of the development of the physical chemistry of macromolecular compounds. The knowledge of the main features of the occurrence of such interpolymer reactions (IPR) is essential for the creation and further development of a general theory of interpolymer reactions and interpolymer complexes. This refers fully also to interpolyelectrolyte reactions involving polyelectrolyte complexes (PEC). Cooperative interpolymer reactions are convenient models for the study of the processes in which biopolymers participate, for example, the self-assembly of complex structures such as viruses, for a deeper understanding of the mechanism of the self-regulation of enzyme systems, and for the elucidation of the principles of the action of artificial antigens and the creation of fundamentally new vaccines based on synthetic polyelectrolytes.

The study of the equilibria in interpolyelectrolyte reactions is also essential for the specific synthesis and the optimisation of the application of polyelectrolyte complexes in various branches of the national economy, for example as highly effective flocculants, stabilisers of colloidal dispersions, ion-exchange materials, membranes, binders, sorbents, structure-forming agents, physiologically active compounds, etc. This task is of particularly great current interest in connection with the prospects of using water-soluble polyelectrolyte complexes as carriers of biologically active substances, in particular enzymes, and their use in this state in biotechnology and medicine.

This review gives an account of the results of our systematic studies of the equilibria in interpolymer reactions accompanied by the transfer of polyions. The most important factors determining the direction of these reactions and the high selectivity of the interpolyelectrolyte interaction are specified and discussed.

II. Reactions involving interpolymer complexes

The reactions between chemically complementary polymers, i.e. polymers whose functional groups have an affinity for one another and whose geometrical structure does not hinder the formation of a fairly large number of intermolecular bonds per chain, have now been investigated in fair detail.[1–3] The reactive groups can be located both in the main chain and in the side groups and also on the surfaces of globular or other particles. The products of such reactions have come to be referred to as interpolymer complexes.

The nature of the forces by virtue of which interpolymer complexes exist can be various: hydrogen bonds [complexes of polycarboxylic acids with polyvinylpyrrolidinone, poly(vinyl alcohol), and poly(ethylene glycol);[4–9] complexes of biopolymers—for example, the native form of DNA [10]], electrostatic interactions (polyelectrolyte complexes[2,11,12]), van der Waals interactions [stereocomplexes of iso- and syndiotactic poly(methyl methacrylates)[13–15]], and donor–acceptor interactions (charge-transfer complexes[16]). Hydrophobic interactions may play a significant role in the stabilisation of interpolymer complexes in aqueous solutions.[17,18] The reason for the stability of such complexes is the cooperative nature of the interaction of the polymers with one another. The formation of interpolymer complexes is accompanied by an appreciable entropy gain compared with the analogous reactions involving low-molecular-weight reactants (for equal degrees of reaction) because after the formation of one bond between the units of the reacting macromolecules, the formation of all the subsequent bonds no longer entails the loss of additional translational degrees of freedom.

The physicochemical, mechanical, and other properties of interpolymer complexes differ significantly from those of their constituent components.[19] These polymeric compounds exist both in dilute solutions and in block and can be obtained either by mixing

the solutions of macromolecular reactants or as a result of matrix polymer reactions.[20–23]

One of the important properties of interpolymer complexes is their ability to enter into interpolymer exchange and substitution reactions in aqueous solutions. The essential feature of these reactions is the transfer of macromolecules from one interpolymer complex species to another. Interpolymer reactions involving interpolymer complexes stabilised by a system of hydrogen bonds were discovered and investigated systematically in the 1970s.[1,3,4–9,17–19,23–32]

The selectivity of the polymer–polymer interactions in systems containing more than two types of polymer molecules is also directly related to the interpolymer reactions. Such selectivity is a characteristic evolution-dependent property of the components of living organisms.[33,34] The selectivity of interactions is characteristic also of simpler polymer systems.[3,24,25]

III. Intermacromolecular reactions in solutions of polyelectrolytes bearing unlike charges

The formation of complexes between ionogenic polymers, manifested by a phase separation of the liquid–liquid type in their solutions, was discovered as early as the 1890s.[35] Such phase separation in solutions of polyelectrolytes, incorporating in their composition hydrophilic non-ionogenic groups for a low content of ionogenic groups, has been called complex coacervation.[36,37] According to modern ideas, coacervation is none other than the formation of polyelectrolyte complexes. The thermodynamic description of the phase separation process in the formation of a coacervate complex has been achieved, the composition and concentration of the complex have been predicted, and the influence of salts and various low-molecular-weight substances on its stability has been investigated.[38–41] The importance of the study of these questions arises from the fact that the coacervation phenomenon is used in various technological processes, for example in microencapsulation[42] or in the creation of medicinal preparations. Complex coacervation has been considered as a possible mechanism of the prebiological compartmentalisation, which constitutes an important stage in the prebiological evolution of organic compounds.[43] It therefore became necessary to investigate the interaction of such coacervate complexes with polyelectrolytes.[44–46]

The attempts to investigate complex formation in solutions of polyions bearing unlike charges with a high linear charge density were undertaken as early as the 1960s.[47–49] It was established that the precipitate of the polymeric complex formed as a result of the macromolecular reaction contains virtually equimolar amounts of polymeric components, while the precipitate itself is insoluble in any of the known individual solvents. Admittedly it is possible to dissolve it in ternary systems of the acetone–water–NaBr type, but this process takes place as a result of the decomposition of the complex — the polyelectrolytes in such systems hardly interact with one another. The combination of insolubility in familiar simple solvents with a high swellability in water, selectivity in the sorption of ions, and high biological compatibility have led to the application of such complexes as materials for medical-biological purposes.[50] However, the insolubility of stoichiometric complexes, formed by strong polyelectrolytes, as well as the insolubility of the coacervate complexes hinder the study of the structure of such species and also of the interpolymer reactions involving them.

The study of the cooperative interpolymer reactions in solutions of synthetic polyelectrolytes became possible after the discovery of non-stoichiometric polyelectrolyte complexes (NPEC) formed by polyions with a high charge density. Notable properties of non-stoichiometric polyelectrolyte complexes are their ability to dissolve in water and to participate in interpolyelectrolyte reactions occurring in homogeneous aqueous solutions. This factor gave rise to the possibility of using various physicochemical methods for the study of polymer solutions in order to investigate such reactions (light scattering, high-speed sedimentation in the analytical version, viscometry, etc.) and made it possible to avoid many complications which arise in the study of colloidal dispersions of insoluble stoichiometric polyelectrolyte complexes (SPEC).

IV. Water-soluble non-stoichiometric polyelectrolyte complexes

The procedure used to obtain water-soluble non-stoichiomdtric polyelectrolyte complexes was first described in detail by Tsuchida et al.[51] It consisted in mixing aqueous solutions of aromatic ionenes and sodium polystyrenesulphonate (NaPSS) in non-equivalent proportions. The complete dissolution of the polyelectrolyte complex is observed in the presence of a three-fold excess of the charged units of sodium polystyrenesulphonate relative to the charged units of ionene. After the introduction of excess amounts of ionene into a solution of sodium polystyrenesulphonate, an insoluble stoichiometric polyelectrolyte complex is obtained instead of the non-stoichiometric one, while the excess ionene remains in solution. These observations led the authors[51] to the conclusion that polymers of the ionene series have a special capacity for the formation of soluble non-stoichiometric polyelectrolyte complexes, which is in their view associated with the location of the charged groups in the ridge of the macromolecules. This conclusion was confirmed by the failure of the attempt to obtain water-soluble non-stoichiometric polyelectrolyte complexes from sodium polystyrenesulphonate and polytrimethylbenzylvinylammonium chloride, i.e. from a pair of electrolytes in which the ionogenic groups are located in the lateral substituents. It was soon shown for the reaction between sodium polyacrylate and 5,6-ionene bromide that aliphatic ionenes are also capable of forming water-soluble non-stoichiometric polyelectrolyte complexes.[52] This finding was also seemingly consistent with the hypothesis of the exceptional properties of ionenes, which, if one agreed with the conclusions of Gulyaeva et al.,[52] are due to the steric "accessibility" of the positively charged quaternary nitrogen atoms.

It was shown later that water-soluble non-stoichiometric polyelectrolyte complexes can be obtained, whilst maintaining certain conditions, from polyelectrolytes bearing unlike charges and having a wide variety of chemical structures.[53–55] It has been established[53] in relation to the sodium polymethacrylate (NaPMA)–poly-*N*-ethyl-4-vinylpyridinium bromide (PEVP) system that water-soluble non-stoichiometric polyelectrolyte complexes can be obtained for polyions each of which contains charged groups in their lateral substituents. In this case, i.e. when at least one weak polyelectrolyte (sodium polymethacrylate) is incorporated in the composition of the non-stoichiometric polyelectrolyte complex, there is a possibility of the formation of non-stoichiometric polyelectrolyte complexes under conditions where the interpolymer reaction is still far from completion. Such non-stoichiometric polyelectrolyte complexes, formed in acid media in the reaction of polymethacrylic acid

(PMAA) with poly-*N*-ethyl-4-vinylpyridinium bromide, are insoluble in water. The addition of alkali leads to the ionisation of the free carboxy-groups of the polymethacrylic acid, incorporated in the non-stoichiometric polyelectrolyte complex in excess, and to the dissolution of the non-stoichiometric polyelectrolyte complex.

In conformity with the terminology adopted nowadays,[2] the non-stoichiometric polyelectrolyte complex species contains a lyophilising polyelectrolyte (LPE) and the oppositely charged blocking polyelectrolyte (BPE). The units of the lyophilising polyelectrolyte are incorporated in the non-stoichiometric polyelectrolyte complex species in excess relative to the units of the blocking polyelectrolyte, i.e. the composition of the non-stoichiometric polyelectrolyte complex is φ = [BPE]/[LPE] and φ is less than unity. The fragments of the lyophilising polyelectrolyte chains and of the blocking polyelectrolyte chain, linked by interpolymer salt bonds, form relatively hydrophobic double-stranded sequences, which tend to segregate in aqueous solutions. The tails and loops of the lyophilising polyelectrolyte (see the left-hand side of Scheme 1) ensure the solubility of the non-stoichiometric polyelectrolyte species as a whole.

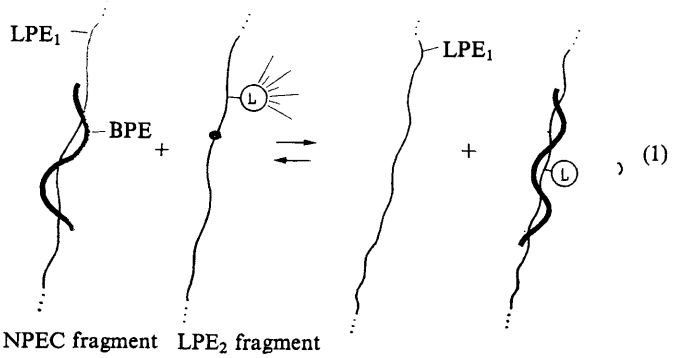
A simple and universal method of preparation of non-stoichiometric polyelectrolyte complexes consists in the direct mixing of aqueous solutions of polyelectrolytes bearing unlike charges taken in non-equivalent proportions in the pH range where they are both fully charged and in the presence of small amounts of a low-molecular-weight electrolyte.

Clearly in this synthetic method polymeric complexes of different composition, including complexes insoluble in water, should be formed as a consequence of inevitable local supersaturation. However, the final product—a water-soluble non-stoichiometric polyelectrolyte—is homogeneous as regards composition. The study of aqueous salt solutions of various non-stoichiometric polyelectrolytes by light scattering[55] showed that the macromolecular characteristics of the non-stoichiometric polyelectrolyte species are independent of the method of preparation, i.e. such complexes are equilibrium compounds. These findings show that interpolyelectrolyte reactions accompanied by the transfer of polyions do indeed take place in aqueous solutions.

Evidently, the observed[51] dissolution of the suspension of a stoichiometric polyelectrolyte complex incorporating sodium polystyrenesulphonate and ionene, which occurred after the addition of an excess of sodium polystyrenesulphonate, is also caused by the transfer of ionene chains from the stoichiometric polyelectrolyte complex species to the sodium polystyrenesulphonate chains introduced and by the appearance of new water-soluble non-stoichiometric polyelectrolyte species. However, the transfer of polyions, which occurs in this system also in salt-free media, is not due to structural-chemical characteristics[51] but is caused by the low linear charge density of ionene. The breakdown of the electrostatic complementarity of the interacting chains, as a result of both a change in the linear charge density and the introduction of low-molecular-weight ions capable of being bound specifically to the charged units of the macromolecules incorporated in the non-stoichiometric polyelectrolyte complex, accelerates the interpolyelectrolyte exchange.[56] In dilute salt-free solutions of electrostatically complementary polyelectrolytes, for example, in mixtures of sodium polymethacrylate and poly-*N*-ethyl-4-vinylpyridinium bromide,[57] the transfer of polyions hardly occurs, but, after the addition of low-molecular-weight electrolytes its rate increases sharply. The formation of soluble non-stoichiometric polyelectrolyte complexes is not observed in mixtures of salt-free solutions of electrostatically complementary polyions apparently for the same reason.[51]

V. Interpolyelectrolyte reactions

Cooperative interpolyelectrolyte reactions of various types, occurring in solutions of linear synthetic polyelectrolytes bearing unlike charges, are presented below in a generalised schematic form:



NPEC fragment LPE_2 fragment

where LPE_1 and LPE_2 are lyophilising polyelectrolytes competing for binding with the blocking polyelectrolyte. Predominantly the literature has reported interpolyelectrolyte reactions in solutions of non-stoichiometric polyelectrolyte complexes, where polyanions play the role of the lyophilising polyelectrolyte, while the blocking polyelectrolyte consists of polycations.

The transfer of the blocking polyelectrolyte to the lyophilising polyelectrolyte of the same chemical nature as that incorporated in the composition of the initial non-stoichiometric polyelectrolyte (in this case the initial and resulting non-stoichiometric polyelectrolytes are stabilised by interpolyelectrolyte salt bonds of the same kind) is referred to as the interpolyelectrolyte exchange reaction. Otherwise, if LPE_1 and LPE_2 have different chemical structures, then the transfer of the blocking polyelectrolyte to LPE_2 is called an interpolyelectrolyte substitution reaction. Apart from these two main types of interpolyelectrolyte reactions, the reaction involving the coupling of polyions bearing unlike charges has been investigated. The interpolyelectrolyte reactions described in this review take place in homogeneous aqueous solutions.

The occurrence of interpolyelectrolyte reactions involving a wide range of polyions has been established by various methods. The formation of products was detected on the sedimentograms for solutions of reaction mixtures in both the normal and scanning versions.[54,58–61] The molecular characteristics of the non-stoichiometric polyelectrolyte species—the products of the interpolyelectrolyte exchange—were measured by the light scattering method.[55,62–64] The interpolyelectrolyte substitution reaction was accompanied in individual instances by a change in viscosity or by the phase separation of the reaction mixtures, which made it possible to use viscometric and turbidimetric titration for their detection and investigation.[58,61] The rate of formation and decomposition of polymer–polymer complexes was recorded directly by the luminescence quenching method.[65] The advantages of this method include high sensitivity, which makes it possible to work with highly dilute polymer solutions, the possibility of studying opalescent solutions, the absence of a need for the introduction of a supporting low-molecular-weight electrolyte into the system, as happens, for example when light scattering and the high-speed sedimentation

method are used, and the possibility of continuous recording of rapid processes and hence the possibility of studying their kinetics.

The essential feature of the luminescence quenching method as applied to the study of interpolyelectrolyte reactions is as follows. A luminescent label L (see Scheme 1) is introduced into one of the polyelectrolytes, for example LPE_2. A blocking polyelectrolyte whose units are luminescence quenching agents, is selected. The formation (or decomposition) of the lyophilising polyelectrolyte LPE* carrying a luminescent label on reaction with the blocking polyelectrolyte is accompanied by a decrease (or increase) in the relative intensity of the luminescence of the solution I/I_0 (I_0 is the intensity of the luminescence of the LPE* solution). The degree of quenching is determined by the composition of the complex φ. By recording the change in I/I_0 in the reaction mixture containing all three polyelectrolytes, it is possible to follow the interpolyelectrolyte reaction.

The change in the quenching of the luminescence observed in the experiments constitutes direct evidence for the replacement of the LPE_1-BPE contacts by LPE_2-BPE contacts as a result of the interpolyelectrolyte reaction (1).

Salts of polycarboxylic acids — polymethacrylic and polyacrylic — bearing a luminescent label were used as the LPE*. The introduction of luminescent anthracene or pyrene groups into their chains was achieved by the interaction of fractions of the polyacids with anthryl- or pyrenyl-diazomethane by the method of Krakovyak et al.[66] In contrast to the copolymerisation method,[67] this procedure makes it possible to obtain specimens of labelled and unlabelled polyacids with the same molecular weight distribution (MWD). Polymeric amines played the role of blocking polycationic quenching agents. Polycations with pyridinium groups, which effectively quench luminescence, have been most widely used:[68] poly-*N*-ethyl-4-vinylpyridinium bromide together with its statistical copolymers and poly-4-vinylpyridine (PEVP-β).[69]

The question of the validity of the application of the luminescence quenching method in the study of interpolyelectrolyte reactions with selected polyelectrolytes is important. The kinetics of the quenching of luminescence in solutions of complexes of lyophilising polyelectrolytes bearing a luminescent label and polycationic quenching agents were investigated with the aid of a pulsed fluorimeter using single photon counting. The identity of the kinetics of the quenching of the luminescence of the NPEC* and the initial NaPMA* with the simultaneous decrease in luminescence intensity shows that the static quenching mechanism predominates in NPEC* solutions.[70]

This picture has been observed in solutions of various NPEC*, regardless of the composition of the latter, at different concentrations of the low-molecular-weight electrolytes introduced into the system and for different linear charge densities and degrees of polymerisation of the polyions. This means that in all the systems investigated the quenching mechanism is static, i.e. the lifetime of the contacts between the units of the polyions forming the NPEC* under the conditions investigated is significantly greater than the lifetime of the excited state of the label.[71]

VI. Equilibria in interpolyelectrolyte reactions

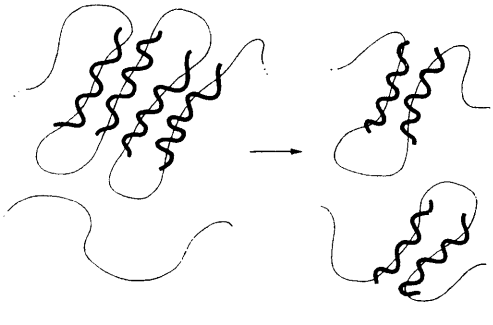
Before proceeding to the description of the results of the study of equilibria in interpolyelectrolyte exchange and substitution reactions, it is necessary to make the following comments. Low-molecular-weight electrolytes are known to exert an enormous influence on the behaviour and state of the polyelectrolyte complex species in solutions. At a high ionic strength of the solution, at which extensive decomposition of the cooperative system of interpolyelectrolyte salt bonds takes place, the uniform distribution of the blocking polyelectrolyte with respect to the lyophilising polyelectrolyte breaks down (the so called disproportionation is observed[63]), while in concentrated solutions of simple salts polyelectrolyte complexes are fully dissociated into the constituent polyions.[65] The results described below refer to interpolyelectrolyte reactions occurring under conditions far from such far-reaching action on polyelectrolyte complex species. In the dilute solutions of low-molecular-weight electrolytes investigated, the cooperative system of interpolyelectrolyte salt bonds in the initial polyelectrolyte complexes is still entirely stable as are those formed subsequently.

1. The equilibrium in an interpolyelectrolyte exchange reaction

(a) The uniform distribution of the blocking polyelectrolyte among the non-stoichiometric polyelectrolyte species

It follows from the results of the study of aqueous salt solutions of non-stoichiometric polyelectrolyte complexes by the light scattering method[55] that the molecular characteristic of the species of such complexes are independent of the method of synthesis, i.e. the non-stoichiometric polyelectrolyte complexes are equilibrium interpolymer compounds. The determination of the equilibrium distribution of the blocking polyelectrolyte among the polymeric complex species presupposes the transfer of blocking polyelectrolyte chains from one set of lyophilising polyelectrolyte chains to another, i.e. the occurrence of reaction (1) in the $LPE_1 = LPE_2$ version. We investigated the equilibrium in such an exchange reaction between a non-stoichiometric polyelectrolyte complex and a free lyophilising polyelectrolyte and also between non-stoichiometric polyelectrolyte complexes of different composition.

We shall consider initially the case where the degrees of polymerisation of the lyophilising and blocking polyelectrolytes $\overline{P}$ satisfy the condition $\overline{P}_{LPE} > \overline{P}_{BPE}$ and each non-stoichiometric polyelectrolyte complex species incorporates a considerable number of relatively short blocking polyelectrolyte chains. At the present time, this is the situation most frequently encountered in practice. The properties of such non-stoichiometric polyelectrolytes have been studied in detail and such complexes have in fact been used for the solution of many important problems, in particular for the creation of reversibly soluble immobilised enzymes.[72] The results of sedimentation studies[59] have shown that the transfer of blocking polyelectrolyte macromolecules from the non-stoichiometric polyelectrolyte complex to the free polyions of the lyophilising polyelectrolyte does indeed occur and results in the formation of a new non-stoichiometric polyelectrolyte whose composition is identical with that of the mixture of polyelectrolytes [reaction (2)]:



(2)

It follows from the comparison of the sedimentograms that in the solution of a mixture of a non-stoichiometric polyelectrolyte and a lyophilising polyelectrolyte a single component NPEC* is observed, its sedimentation coefficient not differing from that of a specially prepared non-stoichiometric polyelectrolyte complex having the same composition as the reaction mixture. The areas of the peaks on both sedimentograms also agree. The latter fact demonstrates convincingly that the interpolyelectrolyte exchange reaction (2) reaches completion during the experiment.

The results of the study of the same system by the luminescence quenching method using labelled NaPMA* (containing on average one anthrylmethyl methacrylate unit per 900 chain units) agree with the above conclusion. The relative luminescence intensity of aqueous salt solution of a mixture of NPEC* (NaPMA*–PEVP) having the composition $\varphi = 0.45$ and NaPMA with [NaPMA*] = [NaPMA] agrees with the value of I/I_0 for the solution of a non-stoichiometric polyelectrolyte (NaPMA*–PEVP) having the composition $\varphi = 0.23$ and incorporating half the amount of the polycationic quenching agent. The addition of an aqueous solution of the labelled NaPMA* to the solution of the non-stoichiometric polyelectrolyte complex (NaPMA–PEVP) leads to the same result.

Similar phenomena have been observed also on mixing solutions of non-stoichiometric polyelectrolyte complexes formed by a pair of the same polyelectrolytes but in different proportions.[59]

Thus interpolyelectrolyte exchange reactions lead to the appearance of non-stoichiometric polyelectrolyte complexes characterised by a uniform distribution of the blocking polyelectrolyte chains among the non-stoichiometric polyelectrolyte complex species, whose composition is identical to that of the mixture of polyelectrolytes formed ultimately. This has been explained[59] by the fact that, for a constant total number of interchain salt bonds and with retention of solubility, a higher entropy corresponds to a uniform distribution of the blocking polyelectrolyte among the non-stoichiometric polyelectrolyte complex species, i.e. the driving force of the transfer of polyions is the increase in the entropy of the systems. This constitutes a significant difference between the reactions investigated and those between linear polyelectrolytes and globular proteins[73–75] and also the reactions between polymethacrylic acid and poly(ethylene glycol) or poly-*N*-vinylpyrrolidinone[76] in which disproportionation is clearly manifested, i.e. there is a significantly non-uniform distribution of one of the macromolecular components among the species of the corresponding polymeric complexes.

The important question of the state and behaviour of the products of the interpolymer reactions—non-stoichiometric polyelectrolyte complexes whose composition is identical with that of the reaction mixture—remains obscure in the study of the exchange reaction. In particular, it is not clear whether the exchange of chains of the blocking polyelectrolyte between the non-stoichiometric polyelectrolyte complex species of the same composition, which are illustrated on the right-hand side of scheme (2), does occur. Evidently, this problem cannot be solved solely by studying the system examined above in the $\overline{P}_{LPE} \gg \overline{P}_{BPE}$ version. Even if such mutual exchange of the blocking polyelectrolyte chains between the non-stoichiometric polyelectrolyte complex species does occur, it is not accompanied by a change in the properties of the species themselves; in particular, it should not lead to an alteration of the luminescence intensity because it is not accompanied by a change in the number of label–quenching agent contacts.

However, it was found that such reactions are characteristic of non-stoichiometric polyelectrolyte complexes formed by pairs of polyelectrolytes with not unduly different degrees of polymerisation of the lyophilising and blocking polyelectrolytes. The ratio of the degrees of polymerisation of the lyophilising and blocking polyelectrolytes can be specified in such a way that it coincides with the ratio of the numbers of units [BPE]/[LPE] incorporated in the non-stoichiometric polyelectrolyte complex, i.e. $\overline{P}_{BPE}/\overline{P}_{LPE}$ = [BPE]/[LPE]. In a non-stoichiometric polyelectrolyte complex of this composition, there is on average only one blocking polyelectrolyte chain per lyophilising polyelectrolyte chain. If a free lyophilising polyelectrolyte is added to the solution of the given non-stoichiometric polyelectrolyte, then the "migration" of the blocking polyelectrolyte chains from the non-stoichiometric polyelectrolyte complex species to the chains introduced will naturally not be accompanied by a change in composition and such a process would correspond entirely to the conditions formulated above for the reaction involving the mutual exchange of blocking polyelectrolyte chains between the non-stoichiometric polyelectrolyte complex species.

We investigated the behaviour of the system obtained on mixing aqueous solutions of a free lyophilising polyelectrolyte and a non-stoichiometric polyelectrolyte complex formed by sodium polymethacrylate ($\overline{P}_{LPE} = 3000$) and poly-*N*-ethyl-4-vinylpyridinium bromide ($\overline{P}_{BPE} = 1300$) for which $\overline{P}_{BPE}/\overline{P}_{LPE} = 0.4$.[77] It follows from the results of high-speed sedimentation that free lyophilising polyelectrolyte polyions and non-stoichiometric polyelectrolyte complex species, whose composition is identical with that of the initial non-stoichiometric polyelectrolyte complex, do indeed coexist in the mixtures obtained.

The "migration" of the blocking polyelectrolyte chains derived from the species of the initial non-stoichiometric polyelectrolyte to the chain of the free lyophilising polyelectrolyte has been established also for the above system. The luminescent-labelled NaPMA* chain with the same degree of polymerisation as the sodium polymethyacrylate incorporated in the initial non-stoichiometric polyelectrolyte was used as the free lyophilising polyelectrolyte. The results of the study of this system by the high-speed sedimentation method indicate unambiguously the occurrence of reaction (2). Scanning at the wavelength corresponding to the absorption by the luminescent (anthryl) label make it possible to detect the LPE* in both fractions. The results presented demonstrate unambiguously that the interpolyelectrolyte exchange is completed before the end of the experiment, the latter requiring 3 h. It might be thought that similar exchange reactions occur also in solutions of a non-stoichiometric polyelectrolyte complex whose species contain more than one blocking polyelectrolyte chain; an example may be provided by the mutual exchange reactions between non-stoichiometric polyelectrolyte complex species illustrated on the right-hand side of scheme (2).

A similar picture has been observed also in mixtures of non-stoichiometric polyelectrolyte complexes formed by the same lyophilising high-molecular-weight sodium polymethacrylate and different blocking polycations—5,6-ionene bromide and poly-*N*-ethyl-4-vinylpyridinium bromide.[78] High speed sedimentation data show that a solution of a mixture of these two non-stoichiometric polyelectrolyte complexes contains one component which has a rate of sedimentation intermediate between the rates of sedimentation of the initial non-stoichiometric polyelectrolyte complexes. The occurrence of interpolyelectrolyte exchange is also confirmed by the behaviour of the mixture of non-stoichiometric polyelectrolyte complexes in solutions of low-molecular-weight electrolytes. The curve for the turbidimetric titration of a mixture of

non-stoichiometric polyelectrolytes does not coincide with the titration curves for either non-stoichiometric polyelectrolyte complex and occupies an intermediate position on the ionic strength scale for the solution. There is a single distinct jump on the turbidimetric titration curve. Mixtures of such polyelectrolyte complexes having other compositions behave similarly.

Thus the species of different non-stoichiometric polyelectrolyte complexes exchange in solution the blocking polyelectrolyte chains incorporated in their composition and the equilibrium in the exchange reaction shifts under the experimental conditions, i.e. at pH 7.5 and [NaCl] = 0.06 M, towards the formation of the final reaction product—a new soluble non-stoichiometric polyelectrolyte complex which incorporates both BPE_1 and BPE_2.

The variation of the degree of exchange can be followed by the luminescence quenching method because the introduction of ionene into the test solutions is accompanied by only a slight decrease in the luminescence intensity, whereas poly-*N*-ethyl-4-vinylpyridinium bromide effectively quenches the labels on the polyanion in the NPEC* species. The relative luminescence intensities I/I_0 of a solution of the mixture of non-stoichiometric polyelectrolyte complexes, which does not coincide with the values of I/I_0 for solutions of the individual non-stoichiometric polyelectrolyte complexes, occupies an intermediate position on the NaCl concentration scale, which agrees with the formation of mixed non-stoichiometric polyelectrolyte complexes in which some of the LPE* units are blocked by ionene, which is not a luminescence quenching agent.

The results show that the mutual exchange reactions investigated take place continuously, i.e. that the species of the non-stoichiometric polyelectrolyte complexes whose composition coincides with that of the reaction mixture exchange continuously the blocking polyelectrolyte chains with one another, the rate of exchange being controlled by the ionic strength of the solution. The reactions observed are none other than a form of thermal motion in polyelectrolyte complexes which are the products of reversible ionic reactions between polyelectrolytes.

(b) The influence of the degree of polymerisation of the lyophilising polyelectrolyte on the equilibrium in the interpolyelectrolyte exchange reaction

The influence of the length of the lyophilising polyelectrolyte chains on the equilibrium in the interpolyelectrolyte exchange reaction was investigated in solutions of non-stoichiometric polyelectrolyte complexes formed by sodium polymethacrylate and poly-*N*-ethyl-4-vinylpyridinium bromide fractions with different molecular weights.[79] We shall consider the results obtained when the three most characteristic conditions are fulfilled:

$$\overline{P}_{LPE} \gg \overline{P}_{BPE}, \quad \overline{P}_{LPE} \simeq P_{BPE}, \quad \text{and } \overline{P}_{LPE} \ll \overline{P}_{BPE}.$$

Sodium polymethacrylate (NaPMA*) specimens with $\overline{P}_W$ = 4100 and 500 and $\overline{P}_n$ = 50, which will henceforth be referred to as long, moderately long, and short polyanions respectively, and the poly-*N*-ethyl-4-vinylpyridinium bromide specimen with $\overline{P}_W$ = 500 satisfy these conditions. It is important to note that the labelled polymers NaPMA* were obtained by the reaction of chemically activated derivatives of the labels with sodium polymethacrylate fractions. This eliminates the question of the inconsistency between the $\overline{M}_W$ and the MWD for the labelled and unlabelled specimens.

The influence of the chain length of the lyophilising polyelectrolyte on the equilibrium in the exchange reaction

$$\text{NPEC(NaPMA}-\text{PEVP)} + \text{NaPMA}^* \Leftrightarrow \text{NPEC}^*\text{(NaPMA}^*-\text{PEVP)} + \text{NaPMA}, \quad (3)$$

is manifested particularly strikingly in systems containing lyophilising polyelectrolyte chains of different length. This follows from the results of the study by the luminescence quenching method of mixtures of polyions, prepared in different ways and containing long and short polyanions (Fig. 1). As can be seen from Figs. 2 and 3, reaction (3) does not occur in the absence of a low-molecular-weight electrolyte. The introduction of even insignificant amounts of a simple salt leads to the complete displacement of the short chains into the solution from their complexes with poly-*N*-ethyl-4-vinylpyridinium bromide (curves *2* and *3*). The reverse reaction, i.e. the insertion of short chains in the non-stoichiometric polyelectrolyte species with long lyophilising polyelectrolytes, does not occur under these conditions (curve *1*).

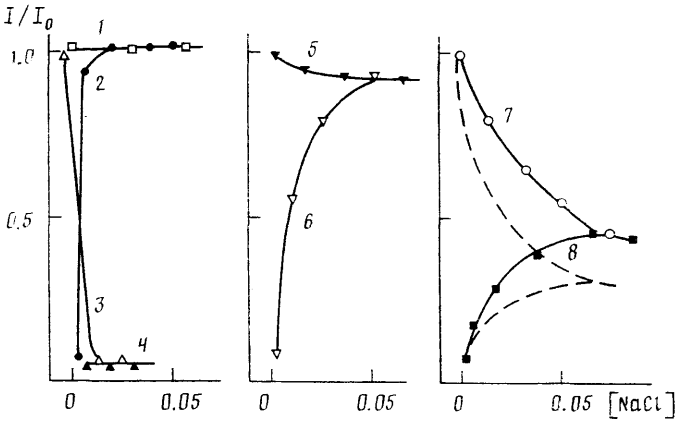


Figure 1. Dependence of I/I_0 on the NaCl concentration in mixtures of solutions of polyions containing polyanions polymerised to different extents: *1*, NPEC (P = 4100) and NaPMA* (P = 50); *2*, NPEC* (P = 50) and NaPMA (P = 4100); *3*, NPEC (P = 50) and NaPMA* (P = 4100); *4*, NPEC* (P = 4100); *5*, NPEC (P = 500) and NaPMA* (P = 50); *6*, NPEC* (P = 500) and NaPMA (P = 500); *7*, NPEC (P = 4100) and NaPMA* (P = 500); *8*, NPEC* (P = 500) and NaPMA (P = 4100).[79]

This result shows that in systems containing lyophilising polyelectrolytes of the same chemical nature, interpolyelectrolyte substitution of one set of chains by another can in essence occur. The displacement of short chains into the solution is accompanied by an entropy gain as a consequence of the liberation of a large number of short lyophilising polyelectrolyte chains previously immobilised in the NPEC or NPEC* species, which is apparently the reason for the virtual completion of reaction (3).

Very similar results were obtained in the study of mixtures containing moderately long and short polyanions (Fig. 1, curves *5* and *6*). In the absence of a salt, reaction (3) does not occur in this styrene either, while in aqueous salt solutions the displacement of short chains into the solution is observed. However, the differences

between the chain lengths and hence also the amounts of the chains in the non-stoichiometric polyelectrolyte species are insufficient to ensure such a high entropy gain when short lyophilising polyelectrolyte chains are displaced from the non-stoichiometric polyelectrolyte into the solution, so that a proportion of them, albeit extremely small, remains bound in the initial non-stoichiometric polyelectrolyte complex; $I/I_0 \neq 1$ at [NaCl] = 0.03 M.

This trend is manifested distinctly also in systems containing long and moderately long polyanions (Fig. 1, curves *7* and *8*). On attainment of equlibrium, which occurs at [NaCl] = 0.05 M, an appreciable proportion of the moderately long lyophilising polyelectrolyte chains remain in the non-stoichiometric polyelectrolyte complex. However, in this case too an appreciable amount of them pass into the solution. This is indicated by a higher value of I/I_0 corresponding to the state of equilibrium at [NaCl] = 0.05 M compared with I/I_0 for the NaPMA($\overline{P}_W$ = 500)–PEVP–NaPMA*($\overline{P}_W$ = 500) mixture at equilibrium (dashed curves).

Thus the ratio of the chain lengths of the competing lyophilising polyelectrolytes determines the direction of the interpolyelectrolyte exchange reaction in aqueous salt solutions.

(c) The influence of groups capable of stabilising additionally the polyelectrolyte complex on the exchange reaction equilibrium

It has been shown[80] that the presence in the polyanion of a small number of groups (one per hundreds of chain units) capable of interacting specifically with the polycation units in the non-stoichiometric polyelectrolyte complex formed can ensure the "recognition" by the polycation of such modified chains among unmodified polyanions.

Aqueous salt solutions of the non-stoichiometric polyelectrolyte complex formed by sodium polymethacrylate having $\overline{P}_W$ = 4100 and poly-*N*-ethyl-4-vinylpyridinium bromide having $\overline{P}_W$ = 600 were studied. Modified sodium polymethacrylate (NaPMA*) containing anthrylmethylmethacrylate (Ant-NA) groups or pyrenylmethyl methacrylate (Pyr-MA) units was used as the competing polyanion. The presence of the luminescent Ant-MA or Pyr-MA groups in the NaPMA* chains makes it possible to record the interpolyelectrolyte reaction by the luminescence quenching method:

$$\text{NPEC(NaPMA} - \text{PEVP}) + \text{NaPMA}^* \overset{K_e}{\Leftrightarrow}$$
$$\Leftrightarrow \text{NPEC}^*\text{(NaPMA}^* - \text{PEVP}) + \text{NaPMA}. \qquad (4)$$

The experiments were carried out as follows. A solution of the non-stoichiometric polyelectrolyte complex (NaPMA–PEVP), in which there is on average one polycation chain per polyanion chain, was prepared initially. The non-stoichiometric polyelectrolyte complex having the composition

$$\varphi = [\text{PEVP}]/[\text{NaPMA}] = \overline{P}_{\text{PEVP}}/\overline{P}_{\text{NaPMA}} = 0.147$$

corresponds to this condition. An aqueous salt solution of NaPMA was then added to the resulting solution so that the amounts of NaPMA and NaPMA* in the reaction mixture were identical and the time variation of *I* was recorded. The degree of completion of the reaction was judged from the value of I/I_0, where I_0 is the intensity of the luminescence of the aqueous solutions of NaPMA* at the same concentration as in the initial mixture. In the study of the reverse reaction, a solution of a labelled non-stoichiometric polyelectrolyte complex NPEC* (NaPMA*–PEVP), having the composition φ = 0.147, was prepared by a similar procedure and was treated with an aqueous salt solution of sodium polymethacrylate.

It was found that that presence in the NaPMA* chain on average of only three Pyr-MA units per 4100 methacrylate (MA) units already leads to the predominant binding of the polycation to the labelled polyanion, i.e. to an appreciable shift of the equilibrium in the exchange reaction (4) to the right. The equilibrium value of I/I_0, obtained in both the forward (Fig. 2, curve *1*) and reverse (curve *2*) reactions, is lower than expected on the assumption of a uniform distribution of poly-*N*-ethyl-4-vinylpyridinium bromide between the labelled and unlabelled polyanions (the dashed arrow in Fig. 2 indicates the value of I/I_0 for the NPEC* having the composition $\varphi = 0.5 \times 0.147 = 0.073$). With increase in the average number of Pyr-Ma units to 12 per 4100 MA units (Fig. 2, curves *3* and *4*), a virtually complete selectivity of the binding is attained: the vast majority of the sodium polymethacrylate polyanions in the non-stoichiometric polyelectrolyte complex are substituted by the NaPMA* polyanions and I/I_0 at equilibrium is identical with I/I_0 for the NPEC* solution having the composition φ = 0.147 (Fig. 2, continuous arrow).

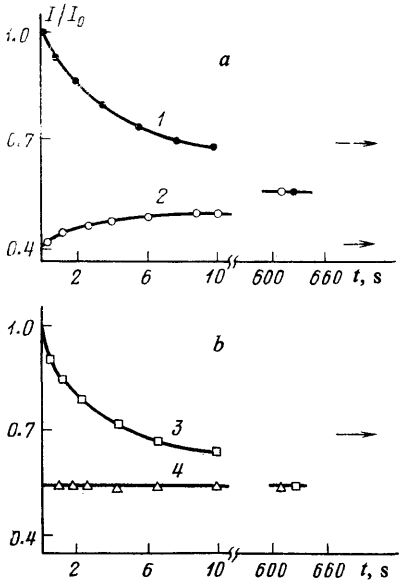


Figure 2. Kinetic curves for reaction (*4*); *1* and *3*, mixtures of the non-stoichiometric polyelectrolyte complexes NaPMA–PEVP and NaPMA*; *2* and *4*, mixtures of labelled non-stoichiometric polyelectrolyte complexes (NPEC*) NaPMA*–PEVP and NaPMA; [NaPMA*] = [NaPMA] = 1.3×10^{-4} M (curves *1* and *2*) and 3.2×10^{-5} M (curves *3* and *4*); [NaCl] = 0.08 M.[80]

The equilibrium constant of reaction (4) under the experimental conditions, involving the transfer of one polycation chain, i.e. [NaPMA] = [NaPMA*], is given by

$$K_e = X^2/(1-X)^2, \qquad (5)$$

where *X* is the fraction of the NPEC* (NaPMA*–PEVP) species formed as a result of reaction (4). If preferential binding of the polycation by the modified polyanion is observed (Fig. 2), *X* varies in the range $0.5 < X < 1$, which corresponds to the variation of the

equilibrium constant in the range $1 < K_e < \infty$. In the range $0.5 < X < 1$ in which we are interested, the relative luminescence intensity I/I_0 decreases linearly with increase in X. This makes it possible to determine X experimentally using the equation

$$X=0.5+0.5(I_l-I)/(I_l-I_\infty), \quad (6)$$

where I_l and I_∞ are the intensities of the luminescence of solutions of poly-*N*-ethyl-4-vinylpyridinium bromide and NaPMA* having the composition $\varphi = 0.073$ and $\varphi = 0.147$ respectively. Thus the determination of the equilibrium constant of reaction (4) reduces to the measurement of the quantities I, I_l, and I_∞, the calculation of X by Eqn. (6), and the calculation of K_e by Eqn. (5).

The equilibrium constants K_e of the interpolyelectrolyte reaction (4) involving NaPMA* with $\overline{P}_W = 4100$, differing in the number of Ant-MA and Pyr-MA units, are presented in Table 1.

Table 1. The quantities $\overline{M}_k$ and K_e for the exchange reaction (4) involving NaPMA* modified by different numbers of pyrenyl or anthryl groups {$\overline{P}_{NaPMA} = \overline{P}_{NaPMA^*} = 4100$, $\overline{P}_{PEVP} = 600$, [NaCl] = 0.08 M}.

No. of groups in NaPMA*	5	9	3	12
Chemical nature of groups	Ant-MA	Ant-MA	Pyr-MA	Pyr-MA
$\overline{M}_k$	2.5	3.5	1.5	5.5
K_e*	≃1	10	10	>200

* The accuracy of the determination of K_e is to within 10–15%.

The average number of labelled units $\overline{M}_k$ in contact with the units of the polycationic quenching agent in the NPEC* formed was calculated from the experimental values of I/I_0 for solutions of NPEC* having the composition $\varphi = 0.147$ by the formula

$$\overline{M}_k=\overline{M}(1-I/I_0), \quad (7)$$

where $\overline{M}$ is the average number of labelled units in the NaPMA* chain and the expression in brackets corresponds to the fraction of quenched labels.

Table 1 shows that the experimental values of $\overline{M}_k$ in the NPEC* with the composition $\varphi = 0.14$ are greater than the calculated values $\overline{M}_k = \overline{M}\varphi$, i.e. the average number of labels in the NaPMA* chains per contour length of the poly-*N*-ethyl-4-vinylpyridinium bromide chains bound electrostatically to them. It follows also from Table 1 that the equilibrium constant of reaction (4) is determined by the number of labels in the polyanion chain and their chemical nature. For example, the presence of five anthryl groups per 4100 MA units in the NaPMA* chains hardly disturbs the uniform distribution of poly-*N*-ethyl-4-vinylpyridinium bromide among all the polyanions present in solution ($K_e \simeq 1$) and does not therefore introduce perturbations into the occurrence of the corresponding interpolyelectrolyte reactions.[57,60,65,77] An increase in the number of anthryl labels to nine already displaces the equilibrium of reaction (4) appreciably to the right. Other conditions being equal, the pyrenyl labels influence K_e much more strongly than the anthryl labels. The "recognition" of the labelled polyanions by the polycations is probably determined by the decrease in free energy which is attained after the immersion of hydrophobic anthryl (pyrenyl) groups in the hydrophobic clusters formed by sections of the oppositely charged chains of the polymeric complex bound to one another. The free energy changes on transfer of anthracene and pyrene from octanol to water are respectively 24.9 and 29.0 kJ mol^{-1}.[82] This difference is apparently responsible for the better "recognition" of the pyrenyl groups compared with the anthryl groups. The stabilisation of the NPEC* (NaPMA*–PEVP) probably promotes also the formation of charge-transfer complexes (CTC) between the anthryl or pyrenyl groups (donors) and the pyridinium units (acceptors). Such charge-transfer complexes can be detected directly in the absorption spectra of the corresponding solutions.[81]

The higher experimental values of $\overline{M}_k$ compared with the calculated values can probably be accounted for by the same factors.

Thus a small number of molecular "anchors" with energies of the order of several kT is apparently sufficient to ensure a decided advantage for the labelled polyanion, whose length is greater by a factor of several hundreds than the length of the "anchor", in the competition with its unlabelled analogue for the binding of the polycation. In our view, this result is of fundamental importance for the understanding of the mechanisms of the selective biological action *in vivo* of functionalised polyelectrolytes (in particular, artificial immunogens—conjugates of high-molecular-weight linear polyions with a small number of antigens or antigen determinants[83]).

(d) The influence of the linear charge density of the blocking polyelectrolytes on the equilibrium in the interpolyelectrolyte exchange reaction

The influence of the linear charge density of the blocking polyelectrolyte on the equilibrium in the exchange reaction was investigated in solutions of non-stoichiometric polyelectrolyte complexes formed by sodium polymethacrylate and partly quaternised poly-4-vinylpyridines (PEVP-β).[84] The PEVP-β specimens, obtained by alkylating poly-4-vinylpyridine with ethyl bromide, were alkylated to different extents, the degree of alkylation being $\beta(\%) = 100n/(n + m)$, where n and m are respectively the mole fractions of the alkylated and non-alkylated units.

Under the experimental conditions, the non-alkylated units, distributed statistically along the polycation chain, are uncharged. The molar extinction coefficient at $\lambda = 257$ nm is $\varepsilon = 1800$ litre mol^{-1} cm^{-1} for the above pyridine units and $\varepsilon = 3600$ litre mol^{-1} cm^{-1} for the charged pyridinium units. Comparison of the UV spectra of solutions of the non-stoichiometric polyelectrolyte complex and PEVP-β at identical concentrations showed that the optical densities at $\lambda = 257$ nm are identical, i.e. under experimental conditions at pH 10 the charge on the PEVP-β chains is determined solely by the alkylated units, while the pyridine units of PEVP-β, incorporated in the non-stoichiometric polyelectrolyte complex species, disturb the continuity of the sequence of the charged quaternised unit. The study of the alkylation of poly-4-vinylpyridine by ethyl bromide showed that the reaction proceeds without the "neighbour effect", all three alkylation rate constants, calculated from the kinetic curve, being virtually identical.† This means that,

† The constants were calculated by L B Stroganov and A N Olonovskii—members of the Department of Macromolecular Compounds in the Faculty of Chemistry of Moscow State University.

up to the attainment of high degrees of quaternisation, the non-alkylated units are arranged along the chain in accordance with the statistical law.

The equilibrium constant of the exchange reaction

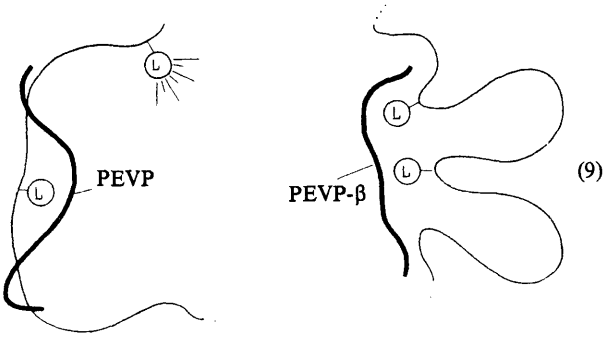
$$\text{NPEC(NaPMA} - \text{PEVP} - \beta) + \text{NaPMA}^* \overset{K_e}{\Leftrightarrow}$$
$$\Leftrightarrow \text{NPEC}^*(\text{NaPMA}^* - \text{PEVP} - \beta) + \text{NaPMA} \quad (8)$$

increases with decrease in the degree of quaternisation β. This follows from the results of the study of solutions of mixtures of polyelectrolyte by the luminescence quenching method[84] (Table 2). The quantities K_e were determined as described above for non-stoichiometric polyelectrolyte complexes incorporating the exhaustively alkylated poly-*N*-ethyl-4-vinylpyridinium bromide.

The sharp increase in K_e with decrease in the degree of alkylation of the polycation is accompanied by an increase in the number of labels covered by a single chain of the blocking polyelectrolyte in the NPEC*, which follows from the increase in $1 - I/I_0$ with decrease in β (Table 2).

An increase in the number of labels quenched by the blocking polyelectrolyte chain is accompanied by a decrease in the size of the non-stoichiometric polyelectrolyte complex species. This follows from the measurements by the quasi-elastic light scattering method of the effective sizes of the non-stoichiometric polyelectrolyte complex species incorporating PEVP-β alkylated to different extents.[84] It is important to note that the values of $\overline{M}_W$ measured by the light scattering method for all the test specimens proved to be constant and amounted to 445 000, which indicates the absence of associated species in the solutions.

Thus the increase in the number of defects in the structure of the non-stoichiometric polyelectrolyte complex with decrease in β, caused by the disturbance of the continuity of the sequence of quaternised blocking polyelectrolyte units, promotes the compaction of the non-stoichiometric polyelectrolyte complex and an increase in the number of labels covered by the polycationic quenching agent [Scheme (9)]. The appreciable decrease in the free energy of the system when an additional number of labels are immersed in the hydrophobic region of the NPEC* species is in fact the cause of the increase in K_e for reaction (8) with decrease in the degree of alkylation of the polycation.



(9)

Similar conformational transformations of the non-stoichiometric polyelectrolyte species have been observed on rupture of a proportion of the interpolymer salt linkages, stabilising the non-stoichiometric polyelectrolyte complex, under the influence of the low-molecular-weight electrolyte.[85] According to the authors,[85] the driving force of this process is the increase in the combinatorial entropy caused by the contribution of structures of the type illustrated on the right-hand side of Scheme (9). Evidently, the endeavour of the labels located on the LPE* to become immersed in the hydrophobic regions of the NPEC* species should promote still further such compaction. This is also indicated by the sharp decrease in the luminescence intensity of NPEC* solution when low-molecular-weight electrolytes are introduced into them.[71]

Table 2. The values of $1 - I/I_0$ and K_e for the exchange reaction (8) involving PEVP-β for different degrees of alkylation β:[84] $\overline{P}_{NaPMA} = \overline{P}_{NaPMA^*} = 4400$, $\overline{P}_{PEVP} = 500$, [NaCl] = 0.10 M; pH 10; in NaPMA* there is on average one Pyr-MA unit per 1400 methacrylate (MA) units.

β, %	40	55	80	90
$1-I/I_0$	0.74	0.78	0.62	0.59
K_e^*	>200	160	60	7

* The accuracy of the determination of K_e is to within 10–15%.

Thus non-stoichiometric polyelectrolyte complexes should be regarded as compounds which are extremely resistant to the disrupting effect of low-molecular-weight electrolytes and which are capable of exchanging their constituent polyions in aqueous salt media. Such exchange results in a uniform distribution of relatively short blocking polyelectrolyte chains among all the long lyophilising polyelectrolyte chains present in the solution. However, highly selective interaction of the polyions with the oppositely charged chains can be achieved, for example, by changing the degree of polymerisation or by slight chemical modification of the polyions. Thus in mixtures of relatively long and short sodium polymethacrylate chains the poly-*N*-ethyl-4-vinylpyridinium polycation is bound preferentially to the long sodium polymethacrylate chains. When the degrees of polymerisation of sodium polymethacrylate are significantly different, this competing interaction can lead to the complete displacement of short chains from the polyelectrolyte complex by long sodium polymethacrylate chains. The cation selects from the mixture of modified and unmodified polyanions macromolecules containing groups capable of additional stabilisation of the polyelectrolyte complex and binds them preferentially. This is manifested even in the presence of only one hydrophobic pyrene group per hundreds of sodium polymethacrylate chain units, whereupon such labelled chains are selected for incorporation in the polyelectrolyte complex almost without error.

2. Equilibrium in the interpolyelectrolyte substitution reaction

The previous section gave an account of the principal results of studies on equilibria in interpolyelectrolyte exchange reactions where the transfer of polyions is accompanied by the disruption and formation of interpolyelectrolyte contacts of the same type. This section will deal with the equilibria in interpolyelectrolyte substitution reactions where lyophilising polyelectrolytes of different chemical nature participate as competing polyanions. The effect involving the molecular "recognition" of polyions of a definite kind proves as a rule to be very pronounced in such systems. This effect has a bearing on the selective biological action of polyelectrolytes *in vivo* and can be used for the creation of systems for the specific transport of biologically active substances. Therefore, before

proceeding to the discussion of the experimental results, we shall consider briefly the available literature data on the selectivity of interpolymer substitution reactions.

(a) Molecular selectivity of interpolymer substitution reactions

The principal feature of interpolymer reactions is their cooperative character. The sharp increase in the stability constant of the polymeric complex as a function of the degree of polymerisation of the reacting macromolecules may serve as proof of this. It is described by the equation[29,30]

$$K_v = K_1^v = \exp(-v\Delta G_1^0/RT), \quad (10)$$

where K_v is the equilibrium constant for the binding of the oligomer incorporating v chains of the long chemically complementary chain-matrix and $K_1 = \exp(-\Delta G_1^0/RT)$ is the effective constant for the binding of the oligomer unit to the matrix unit. The term "oligomer" is conventional and indicates merely the fact that its chains are shorter than those of the matrix. Since the binding constant K_v increases rapidly with increase in the degree of polymerisation of the oligomer, it follows that, in a system containing oligomer chains of identical structure but of different lengths, one should expect the manifestation of selective binding of the longest chains. Such a case has been analysed theoretically.

Experimental confirmation of the model examined by Papisov and Litmanovich[29] has been obtained.[28] It was found that the fraction with the highest molecular weight participates in the reaction between polymethacrylic acid (PMAA) and a mixture of three fractions of poly(ethylene glycol (PEG) having different degrees of polymerisation $\overline{P}$:

$$\text{PEG}(\overline{P}=32\,000)+\text{PEG}(\overline{P}=180)+\text{PEG}(\overline{P}=30)+\text{PMAA} \Leftrightarrow$$
$$\Leftrightarrow \text{IPC[PMAA}-\text{PEG}(\overline{P}=32\,000)]+\text{PEG}(\overline{P}=30\,180). \quad (11)$$

The "selective complex formation" reaction (11) can be regarded in essence as an interpolymer substitution reaction. Indeed, at the instant of the mixing of the reactants, interpolymer complexes incorporating different oligomers are apparently formed and, as a result of the substitution of the relatively short oligomer chains by long ones, are converted into stable interpolymer complexes containing oligomer molecules. The selectivity of the binding of oligomers of the same chemical nature but with different degrees of polymerisation examined above, manifested strikingly in interpolymer reactions, is one of the fundamental features of polymer chemistry and has no analogue in the chemistry of low-molecular-weight compounds.

Apart from chain lengths, factors such as the chemical nature of the reactants, the structural complementarity of the interacting chains, the pH of the medium, etc. exert a significant influence on the occurrence and nature of the interpolymer reactions. We shall consider in what way each of these factors influences the equilibrium in reactions with participation of polymeric complexes.

The polymeric complex formation reactions in a system containing simultaneously high-molecular-weight chains and two kinds of complementary oligomers of different chemical structure were first investigated by Papisov et al.[26] and Bednar et al.[32] The objects of study were a high-molecular-weight polymethacrylic acid and the relatively short chains of poly(ethylene glycol), poly-*N*-vinylpyrrolidinone (PVPD), and poly(vinyl alcohol) (PVA). The interpolymer complexes formed in aqueous media in such systems are stabilised by the hydrogen bonds between the protonated carboxy-groups of the polymeric acid and the hydroxy-groups of poly(vinyl alcohol) or the corresponding nucleophilic groups of poly(ethylene glycol) and poly-*N*-vinylpyrrolidinone.[86] Hydrophobic interactions also play a significant role in the stabilisation of such interpolymer complexes.[18] In systems of this kind, the equilibria in the interpolymer substitution reactions (12) and (13) are displaced almost wholly to the right:

$$\text{PC(PMAA}-\text{PEG})+\text{PVPD} \rightleftarrows \text{PC(PMAA}-\text{PVPD})+\text{PEG}, \quad (12)$$

$$\text{PC(PMAA}-\text{PVA})+\text{PVPD} \rightleftarrows \text{PC(PMAA}-\text{PVPD})+\text{PVA}. \quad (13)$$

The highly selective binding of poly-*N*-vinylpyrrolidinone to polymethylacrylic acid in the multicomponent systems indicated is strikingly manifested in the processes involving the matrix polymerisation of methacrylic acid in a mixture of poly(vinyl alcohol) and poly-*N*-vinylpyrrolidinone [or a mixture of poly-(ethylene glycol) and poly-*N*-vinylpyrrolidinone]. In each of these cases, the rate of polymerisation of methacrylic acid is equal to its rate of polymerisation in the presence of poly-*N*-vinylpyrrolidinone alone and is independent of the presence of either poly(ethylene glycol) or poly(vinyl alcohol) in the polymerising system.[26] Under these conditions, the selectivity of the binding of polymethacrylic acid decreases in the sequence PVPD > PEG > PVA.

The substitution reactions can also be observed in systems containing two types of macromolecular matrices of different chemical nature and an oligomer capable of being bound to either of them. An example of such interpolymer reactions is provided by the substitution reactions in solutions of polymethacrylic acid of the interpolymer complex formed by polyacrylic acid (PAA) and poly(ethylene glycol):[27]

$$\text{IPC(PAA}-\text{PEG})+\text{PMA} \rightleftarrows \text{IPC(PMAA}-\text{PEG})+\text{PAA}. \quad (14)$$

The formation of a new interpolymer complex (PMAA–PEG) and the quantitative displacement into the solution of the polyacrylic acid previously incorporated in the initial interpolymer complex were observed in such a system by IR and NMR spectroscopic methods and also by the polarised luminescence method. Reaction (14) is endothermic and is accompanied by a gain in the entropy of the solvent (water). This can be explained by the fact that hydrophobic interactions play a decisive role in the stabilisation of the interpolymer complex (PMAA–PEG).[18] The important conclusion has also been reached that the matrix polymerisation of methacrylic acid in the presence of the interpolymer complex (PAA–PEG) is accompanied by a substitution reaction of type (14), whose rate is comparable to the rate of the polymethacrylic chain propagation in radical polymerisation, i.e. the substitution reaction takes place over a period of the order of 1 s.[27]

The influence of the chemical structure of the macromolecules on the selectivity of the interpolymer interaction in multicomponent systems has been examined.[28] Macromolecules capable of reacting with polymethacrylic acid with formation of the corresponding interpolymer complexes can be arranged in the following sequence in conformity with their ability to compete in substitution reactions:

$$\text{PEG}(\overline{P}=32\,000) > \text{polyacrylamide}\ (\overline{P}=80) > \text{PVPD}(\overline{P}=100) > \text{PEG}(\overline{P}=70) > \text{PVA}(\overline{P}=1000). \quad (15)$$

As can be seen, the influence of the molecular weight and of the chemical nature of the chain are manifested simultaneously in the intermacromolecular reactions. Thus, depending on the molecular weight, poly(ethylene glycol) can exhibit different reactivities in substitution reactions.

Systems in which the influences of both factors — the chemical nature and length of the chain — are manifested simultaneously lead to the possibility of the fine regulation of the matrix polymerisation processes. Cases of matrix polymerisation in the presence of two types of matrices differing in their chemical nature, namely "short" but "strong" and "long" but "weak", have been examined theoretically.[87] Model calculation showed that an interpolymer complex of the daughter chain with the shorter but "strong" matrix should be formed initially. Subsequently the daughter chain attains a length comparable to that of the matrix and an interpolymer reaction accompanied by the formation of a more stable interpolymer complex of the daughter chain with the long but "weak" matrix should take place.

The pH of the medium can play an important role in the selective interpolymer interaction. The influence of the pH on reactions of this type has been investigated[28] in relation to a three-component system containing a weak polymeric acid (polymethacrylic acid), a weak polymer base [poly-2-vinylpyridine (P2VP) or oligoethyleneimine (OEI)], and a non-ionogenic polymer [poly(ethylene glycol) or poly-*N*-vinylpyrrolidinone]. In an acid medium, the weak polymeric acid (polymethacrylic acid) exists predominantly in the unionised form, as a result of which an interpolymer complex between polymethacrylic acid and poly-*N*-vinylpyrrolidinone or poly(ethylene glycol), stabilised by a hydrogen bond, is mainly formed. In a neutral medium, both the polymeric acid and the polymeric bases are partly charged, so that a polyelectrolyte complex stabilised by salt bonds is produced. In an alkaline medium, where polymethacrylic acid is fully dissociated while the weak base is uncharged, the formation of both a polyelectrolyte complex and an interpolymer complex stabilised by hydrogen bonds is impossible, i.e. the interpolymer reaction does not occur at all. Thus the pH of the medium influences significantly both the ability of polymer chains to react with one another and the nature of the interactions stabilising the interpolymer complex.

Yet another important factor influencing the nature of the selective interpolymer interaction is the structural complementarity of the reacting chains. It has been shown[28] that the steric disposition of the functional groups is a decisive factor in the selective interaction in the following systems:

$$\text{P4VP} + \text{P2VP} + \text{PMAA} \rightleftarrows \text{IPC(PMAA} - \text{P4VP)} + \text{P2VP}, \quad (16)$$

$$\text{PGA} + \text{PEG} + \text{PMAA} \rightleftarrows \text{IPC(PMAA} - \text{PEG)} + \text{PGA}, \quad (17)$$

where P4VP is poly-4-vinylpyridine and PGA is polyglutamic acid. A characteristic feature of system (16) is the possibility of the formation of an interpolymer complex both as a result of electrostatic interpolymer interactions and as a consequence of the formation of interpolymer hydrogen bonds. Bearing this factor in mind, the formation of the interpolymer complex (PMAA–P4VP) in the presence of P2VP was explained precisely by the impossibility of the formation of strong hydrogen bonds between the polymethacrylic acid and poly-2-vinylpyridine units owing to steric hindrance. For the same reason the electrostatic interactions between the oppositely charged groups in the interpolymer complex PMAA–P2VP are much weaker than in the interpolymer complex PMAA–P4VP. The conformation of the polymeric reactants plays a decisive role in the reaction. In acid media, where reaction (17) was carried out, polyglutamic acid exists in the α-helical conformation, where the distance between the carboxy-groups of the acid does not correspond to the distance between the oxygen atoms in the neighbouring poly(ethylene glycol) units. The steric correspondence between the polymethacrylic acid and poly(ethylene glycol) chains is much better, as a result of which the insoluble interpolymer complex PMAA–PEG is formed predominantly in the PMAA–PEG–PGA ternary system. This is indicated by the results of the analysis of the supernatant, which contained almost the entire polyglutamic acid in the α-helical conformation introduced into the reaction.

(b) The influence of the degree of polymerisation of the polyions and of the chemical nature of the small ions on the equilibria in interpolyelectrolyte substitution reactions

We shall now proceed to the consideration of the equilibria in interpolyelectrolyte substitution reactions. An example may be provided by the reaction occurring in mixtures of solutions of the NPEC* formed by the high-molecular-weight labelled NaPMA* (P_W = 4100) and poly-*N*-ethyl-4-vinylpyridinium bromide (P_W = 170), and sodium polyphosphate (NaPP):[88–90]

$$\text{NPEC*(NaPMA*} - \text{PEVP)} + \text{NaPP} \rightleftarrows$$

$$\rightleftarrows \text{NPEC(NaPP} - \text{PEVP)} + \text{NaPMA*}. \quad (18)$$

The position of the chemical equilibrium in reactions of type (18) varies markedly, changing the direction of the process as a consequence of the change in the free energy of contact between a pair of units of the chemically complementary macromolecules. As already stated above, this is due to the cooperative nature of the interpolymer reaction.

Fig. 3 illustrates the dependence of the degree of conversion q on the NaCl concentration in reaction (18), which was carried out by mixing solutions of the NPEC* with sodium polyphosphate fractions with different $\overline{P}_n$. On addition of a salt-free solution of sodium polyphosphate to a salt-free solution of the NPEC*, the luminescence intensity does not change, i.e. in the absence of a low-molecular-weight electrolyte reaction (18) hardly occurs. The introduction of even insignificant amounts of NaCl into a system containing sodium polyphosphate with $\overline{P}_n$ = 230 leads to an appreciable increase in I (curve *1*). Hence it follows that the high-molecular-weight sodium polyphosphate substitutes NaPMA* in the initial NPEC*. However, with decrease in the length of the sodium polyphosphate chains, the rate of increase in I diminishes (curves *2*

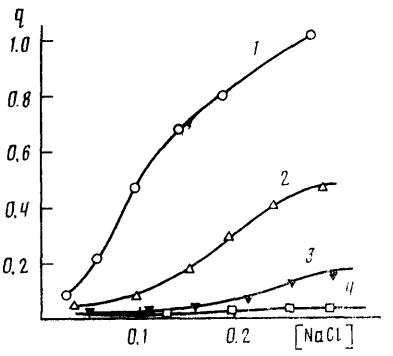


Figure 3. Dependence of the extent of reaction q in reaction (18) on the salt concentration in mixtures of solutions of the labelled non-stoichiometric polyelectrolyte complexes (NPEC*) NaPMA*–PEVP and sodium polyphosphate having different degrees of polymerisation: *1*, 230; *2*, 70; *3*, 50; *4*, 20; pH 10; [NaPMA*] = [NaPP]; φ = [PEVP]/[NaPMA*] = 0.33.[88]

and *3*) and finally does not change at all in solutions containing sodium polyphosphate with $\overline{P}_n$ = 20 (curve *4*). This means that in the last case reaction (18) hardly proceeds from left to right.

In order to elucidate the question whether kinetic or thermodynamic factors limit the occurrence of reaction (18), a study was made of the reverse reaction. It was found that, when [NaCl] > 0.05 M, the values of *I* for mixtures of the non-stoichiometric polyelectrolyte complex NaPP–PEVP and NaPMA* are identical with the values of *I* for mixtures having the same composition but obtained by adding sodium polyphosphate to NPEC*. This means that, when the ionic strength exceeds 0.05 M, reaction (18) is kinetically unhindered and equilibrium is established during the experiment. Consequently the observed dependence of *q* on the degree of polymerisation of sodium polyphosphate is determined by thermodynamic factors.

Fig. 4 shows that, by replacing one alkali metal cation, playing the role of the counterion in relation to PMA* and polyphosphate anions, by another alkali metal cation, it is possible to alter the direction of reaction (18) for fixed degrees of polymerisation of the polyelectrolytes. The addition of sodium polyphosphate ($\overline{P}_n$ = 70) to a solution of NPEC* in the presence of KCl does not alter *I* (curve *3*), i.e. the equilibrium in reaction (18) is fully displaced to the left. On the other hand, in LiCl solutions for [LiCl] > 0.05 M, equilibrium (18) is fully displaced to the right (curve *1*). An intermediate solution occurs in NaCl solutions (curve *2*).

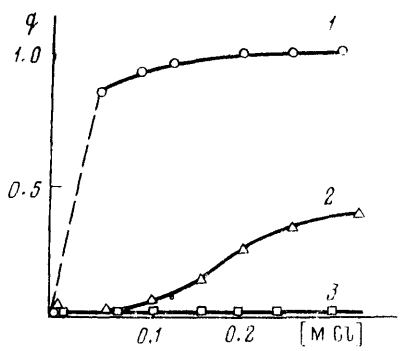


Figure 4. Dependence of the extent of reaction *q* in reaction (18) on the salt concentration [MCl] in mixtures of solutions of labelled non-stoichiometric polyelectrolyte complexes (NPEC*) NaPMA*–PEVP and sodium polyphosphate (P = 70) in the presence of various salts MCl: *1*, M = Li; *2*, M = Na; *3*, M = K; the conditions are the same as in Fig. 3.[88]

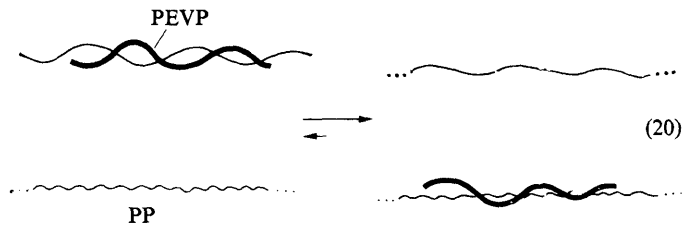
It must be emphasised that, over the entire range of variation of the concentrations of the salts and for any equilibrium position in reaction (18), almost all the units of the polycation are linked to the units of NaPMA* and sodium polyphosphate.

In order to account for the observed effects, we shall represent the free energy change in reaction (18) as the sum of two components:

$$\Delta G = \Delta G_r + \Delta G_s, \qquad (19)$$

where ΔG_r is the free energy change on replacement of the NaPMA*–PEVP contacts by the NaPP–PEVP contacts, while ΔG_s is the difference between the free energies of the reaction of the counterion with sections of the chains of the PMA* and polyphosphate polyanions.

If the degree of polymerisation of both polyanions is greater than that of the polycation, then the number of species in the system remains constant;



(20)

It follows from Fig. 3 that in NaCl solutions (curve 2) the equilibrium in such a reaction is displaced to the right, i.e. $\Delta G' = \Delta G'_r + \Delta G'_s < 0$. However, the decrease in the sodium polyphosphate chain length displaces the reaction equilibrium in the opposite direction, i.e. the overall free energy change in reaction (21) becomes positive: $\Delta G'' = \Delta G''_r + \Delta G''_s > 0$.



(21)

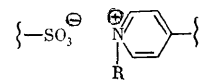
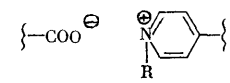
Since ΔG_s is independent of the chain length, i.e. $\Delta G'_s = \Delta G''_s$, the observed effect should be explained by the increase in $\Delta G''_r$, i.e. $\Delta G''_r > \Delta G'_r$. When the total number of salt bonds between the polyions is retained, the occurrence of reaction (21) is less favourable from the standpoint of entropy than that of reaction (20) owing to the decrease in the number of polymeric species in solution. This is correlated with data concerning the influence of the degree of polymerisation of sodium polyphosphate on the stability of the stoichiometric polyelectrolyte complexes NaPP–PEVP in salt media.[89]

For a fixed degree of polymerisation of the polyelectrolytes, ΔG_r remains unchanged on replacement of the alkali metal cation introduced into the system by another and it follows from the comparison of curves *1*, *2*, and *3* in Fig. 4 that the change of sign of ΔG is caused by the change in ΔG_s. The observed effect should be attributed to the difference between the free energies of the reaction of the PMA* and polyphosphate anions with different alkali metal cations. It follows from the turbidimetric titration curve[89] that the critical salt concentration at which the polyelectrolyte complex NaPMA*–PEVP dissociates decreases in the sequence KCl > NaCl > LiCl. In the case of the polyelectrolyte complex NaPP–PEVP, the series is reversed.

By replacing the counterion, it is possible to increase the reactivity of the short chains of the polyphosphate anion. In solution in LiCl, the polyphosphate anions with $\overline{P}_n$ = 20 are already capable of competing with the polymethacrylate anions in reaction (21). On the other hand, in KCl solution even long chains of polyphosphate anions with $\overline{P}_n$ = 230 are almost totally lacking in the ability to replace the polymethacrylate anions.

Thus the equilibrium in the interpolyelectrolyte reaction can be effectively regulated by the simple dissociation or growing of

polyion chains, i.e. by altering the degree of polymerisation. The replacement of one alkali metal cation by another can also induce a qualitative change in the state of multicomponent polyelectrolyte systems. The observed phenomena have no analogues in polymer systems. Presumably such cooperative effects constitute the basis of the regulatory processes in natural biopolymer systems.

The differences between the free energies of the reaction of polyanions of different chemical nature with the polycation as well as the differences mentioned above, observed in the interaction of the polyanions with different alkali metal cations, are evidently caused by the contribution of specific interactions together with the usual Coulombic forces. When such differences become considerable, they actually determine the direction of the interpolyelectrolyte reaction. Thus polyanions containing sulpho-groups, including heparin, sodium polystyrenesulphonate, sodium polyanetholesulphonate, potassium polyvinylsulphate, etc., almost wholly substitute polymeric carboxylate anions in their polyelectrolyte complexes with different polycations.[58,61,91] This is a consequence of the greater stability of the cooperative system of contacts of the type  compared with the contacts  in aqueous salt media (NaCl solutions) in which substitution reactions were investigated. Indeed, the dissociation of polyelectrolyte complexes formed by polyanions with sulpho-groups and by poly-*N*-ethyl-4-vinylpyridinium bromide requires a NaCl concentration higher by a factor of 4 than for the dissociation of the polyelectrolyte complex NaPMA–PEVP.[58]

(d) The influence of the length of the *N*-alkyl substituent in the poly-4-vinylpyridinium cation on the equilibrium in the substitution reaction

It has been observed[92] that the direction of the substitution reaction

$$\text{NPEC*(NaPMA*}-\text{PVPAlk)}+\text{NaPP} \rightleftarrows \text{NPEC(NaPP}-\text{PVPAlk)}+\text{NaPMA*} \qquad (22)$$

is determined, other conditions being equal, by the number of carbon atoms in the *N*-alkyl substituents of quaternised poly-4-vinylpyridines (PVPAlk). Virtually completely quaternised ($\beta > 90\%$) PVPAlk with $\overline{P}_W = 130$ as well as NaPMA* containing 11 pyrene labels and having $\overline{P}_W = 4100$ and sodium polyphosphate with $\overline{P}_W = 250$ were used in the experiments. Fig. 5 presents the dependence of the degree of conversion q in reaction (22) on the concentration of the added salt NaCl for PVPAlk with *N*-alkyl substituents for Alk = Me, Et, or Pr. The points on the curves correspond to equilibrium values. It follows from Fig. 5 that the number of carbon atoms in the *N*-alkyl substituent in PVPAlk determines the direction of reaction (22) at a fixed ionic strength of the solution. Thus the introduction of sodium polyphosphate into a solution of NPEC* incorporating PVPPr leads to a very slight alteration of q (curve *3*), i.e. equilibrium (22) is displaced to the left. On the other hand, in NPEC* solutions containing PVPMe the equilibrium is significantly displaced to the right (curve *1*), while an intermediate situation arises in solutions of a non-stoichiometric polyelectrolyte complex with PVPEt (curve *2*).

Such alteration of the direction of the interpolyelectrolyte substitution reaction (22) agrees with the difference between the stabilities of the corresponding initial polyelectrolyte complexes and the reaction products in aqueous salt solutions. In sodium chloride solutions, the stabilities of both the complexes NaPP–PVPAlk and the complexes NaPMA–PVPAlk diminish consistently in the sequence PVPMe > PVPEt > PVPPr.[93] Such decrease in the ability of the complexes to resist the disruptive effect of the salts in the homologous series of PVPAlk can apparently be accounted for by the steric shielding of the oppositely charged groups of polyions. However, it is significant that this effect is more marked in the case of the complexes NaPP–PVPAlk, where the replacement of one *N*-alkyl substituent in poly-4-vinylpyridine by another leads to more significant changes in stability. For the same reason, with increase in the length of the *N*-alkyl substituent in PVPAlk, the difference between the stabilities of the corresponding complexes decreases, which agrees with the decrease in the degree of conversion q in the substitution reaction (22) in the sequence PVPMe > PVPEt > PVPPr (Fig. 5).

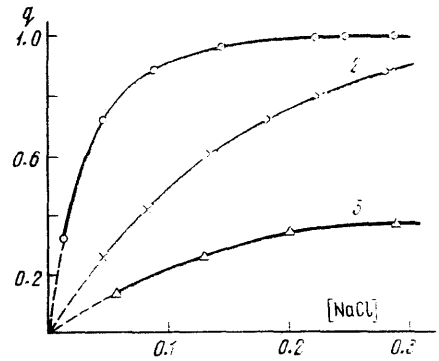


Figure 5. Dependence of the extent of reaction q in reaction (22) on the salt concentration in solutions containing different alkylated poly-4-vinylpyridines: *1*, PVPMe; *2*, PVPEt; *3*, PVPPr; φ = [PVPAlk]/[NaPMA*] = 0.2; [NaPP] = [NaPMA*]; pH 10.[92]

Thus, by virtue of the cooperative nature of the interpolyelectrolyte reactions, even very slight differences between the free energies of the interaction between pairs of units of chemically complementary chains is entirely sufficient to alter completely the direction of such processes. In many instances, a strikingly high selectivity of the interaction of the oppositely charged chains is manifested in systems of this kind. In a solution containing relatively long and relatively short polyanions with the same chemical structure, the polycation prefers to combine with long chains. By the method of trial and error, the cation recognises and selects that of the two cations which forms with it a more stable polyelectrolyte complex even if the difference between the energies of reaction per pair of oppositely charged units is very small. By adding different alkali metal salts to the reaction mixture (i.e. by varying the nature of the low-molecular-weight counterions), it is possible to alter the affinity of the competing polyanions for the polycation and to regulate thereby the direction and equilibrium position of the interpolyelectrolyte substitution reaction. The presence in the polyanion of a small number of groups (1 per hundreds of chain units), capable of interacting specifically with the units of the complementary chain in the non-stoichiometric polyelectrolyte

complex formed, can ensure the preferential binding, i.e. "recognition", by the polycation of a modified polyanion of this kind among the unmodified macromolecules.

This is responsible for the exceptionally high selectivity of the interpolypolymer interactions and the striking tendency of such systems to undergo self-organisation and also ensures the "self-assembly" of the non-stoichiometric polyelectrolyte complex species in aqueous salt solutions. The factors influencing the direction of the reactions investigated can be an effective means for regulating the interpolyelectrolyte exchange and substitution.

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