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Interaction of metal ions with poly(1,3-propylene phosphate) – a synthetic analogue of natural teichoic acids

J. Ostrowska-Czubenko

Nicholas Copernicus University, Institute of Chemistry, Toruń, Poland

Abstract: The binding of metal ions to poly(1,3-propylene phosphate), (PPP), which can be considered as a synthetic analogue of natural teichoic acids, was examined. Infrared spectra of PPP in different ionic forms and of different degrees of polymerization were analyzed and some information on metal ion binding modes in M(I)-PPP and M(II)-PPP complexes, both in the condensed state as well as in aqueous solution, was obtained. It is concluded that the binding modes of sodium, potassium, cesium, calcium, and magnesium ions to phosphate groups of PPP are the same or similar and/or complexes of the same or similar structure are formed. In aqueous solution the analyzed metal ions are bound to poly(alkylene phosphate) chains mainly in the form of "outer sphere" complexes.

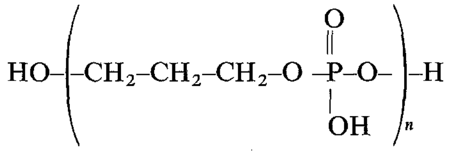
Key words: Polyphosphates – metal ion binding – IR studies

Introduction

Teichoic acids (TAs) are polymeric substances consisting of glycerol, ribitol or mannitol phosphate backbone upon which sugar residues and D-alanine may be substituted [1, 2]. They are important components of most Gram-positive bacteria and occur either in the cell wall or are associated with the cell membrane. TAs play various functions in the life processes of a cell. The most important one is binding and transport of metal ions, especially magnesium ions, from the environment to the cell interior [3, 4].

The information on the interaction of metal ions with natural teichoic acids is rather scarce [5–9]. Metal ion binding and metal ion transfer processes seem to be rather complex [3, 4, 8–10]. Both wall teichoic acids and membrane (or lipoteichoic) acids have different potential binding sites to metal ions. In addition, their cation's binding ability may change with cation type.

The complexity of the process of binding and transport of metal ions by natural teichoic acids prompted us to systematically study these phenomena for such model substances as poly(alkylene phosphate)s. Poly(1,3-propylene phosphate), (PPP), of chemical structure



$$HO\left(-CH_2-CH_2-CH_2-O-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle OH}{|}}{P}}-O-\right)_n-H$$

can be considered as a synthetic analogue of natural TAs belonging to a class of glycerol teichoic acids. PPP, in comparison with glycerol teichoic acids, is a structurally simpler polyelectrolyte. It contains functional phosphate groups in the chemical environment free of the complexities inherent in bacterial cells. Thus, the results of this paper should help us to better understand the role played by the phosphate groups of natural TAs in binding and transport of metal ions.

In our previous communications we have reported some information on the binding of metal ions obtained from the conductimetric [11, 12] and spectral measurements [13]. Now, we try to characterize polyion-counterion interactions in PPP-metal ion complexes by means of infrared spectroscopy.

Experimental

Materials

Poly(1,3-propylene phosphate)[1]), (PPP), was prepared by polymerizing trimethylene phosphonate in CH_2Cl_2 solution using triisobutylaluminium as the initiator, according to Penczek et al. [14]. The polymerization product was then converted into its acidic form (H-PPP) using N_2O_4 in CH_2Cl_2 solution as an oxydizing agent and fractionated by extraction with absolute ethanol and water. The details of synthesis, fractionation and determination of a degree of polymerization were described elsewhere [15].

In our experiments the samples of the average degree of polymerization $n = 3$, 8.5, 10.5 and $n > 20$ were used. The polymer of $n > 20$ in acidic form is insoluble in water, but dissolves in metal hydroxide solutions. According to Penczek et al. [14, 16], the molecular weight of this polymer can reach values up to $1 \cdot 10^5$, i.e., $n \simeq 800$.

Metal salts preparation

Sodium, potassium, cesium and barium salts of PPP were prepared by titration of H-PPP aqueous solution or H-PPP aqueous suspension (in the case of the polymer of $n > 20$) with the appropriate metal hydroxide solution, just to the equivalence point. Salt samples were then isolated from water solution by freeze drying and thoroughly dried in vacuum at 343 K. Magnesium and calcium salts were prepared by adding magnesium or calcium carbonate, followed by filtering off the unreacted carbonate and dried as the polysalts characterized above.

All chemicals used in this study were analytical grade and all bases were kept carbon dioxide free.

Infrared experiments

IR spectra of metal polyphosphates were obtained with Perkin-Elmer Model 75, Carl Zeiss Specord M-80 and UR 10 spectrophotometers. The frequencies were calibrated by the standard absorptions of polystyrene. Infrared measurements of solid samples were carried out on film or powder specimens. PPP samples of $n < 20$ were measured as films cast from anhydrous methanol solution (10% by weight) directly onto alkali metal halide plates or as Nujol mulls. PPP samples of $n > 20$ were measured as Nujol mulls and KBr pellets. Powders before mulling or milling with Nujol or anhydrous KBr (which were performed in a dry box) and films were thoroughly dried in vacuum at 343 K. KBr pellets were additionally dried before analysis. Spectra of water solutions were obtained with capillary films between KRS-5 plates.

Results and discussion

IR spectra of polyacid and polysalts

IR spectra of thoroughly dried poly(1,3-propylene phosphate) of different degree of polymerization and containing hydrogen and metal counterions, respectively, are shown in Figs. 1 and 2. As can be seen, remarkable changes are observed on neutralization of H-PPP. The bands which are greatly affected by neutralization are those near 2750, 2300, 1680, 900, and 505 cm^{-1}, respectively. These bands decrease in magnitude with an increasing degree of neutralization and disappear completely on salt formation. They were assigned earlier by us to acidic groups which were extensively hydrogen bonded [13].

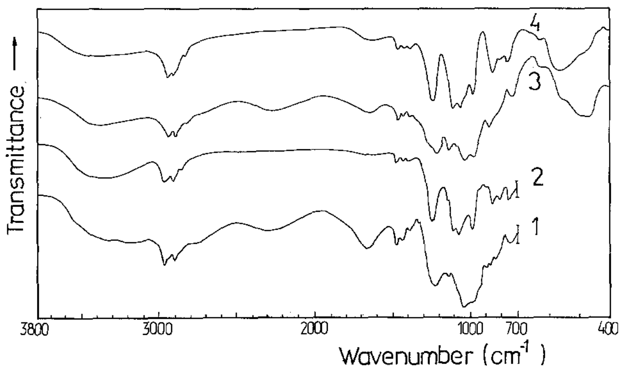


Fig 1. Infrared spectra of H-PPP and M-PPP of different degree of polymerization n: 1) H-PPP, $n = 3$; 2) Na-PPP, $n = 3$; 3) H-PPP, $n > 20$; 4) Na-PPP, $n > 20$

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[1] IUPAC nomenclature: α-hydro-ω-hydroxypoly[oxy(hydroxyphosphoryl)oxytrimethylene]

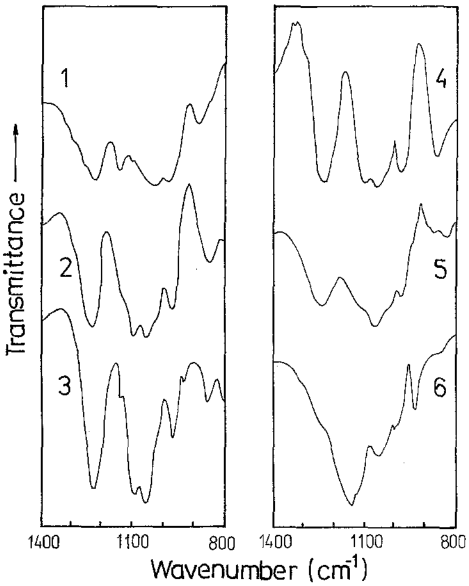


Fig. 2. Infrared spectra of H-PPP, M(I)-PPP, M(II)-PPP, and UO_2(II)-PPP in the phosphate stretching mode region: 1) H-PPP, $n > 20$; 2) Na-PPP, $n > 20$; 3) Na-PPP, $n = 3$; 4) *K-PPP*, $n = 10.5$; 5) *Mg-PPP*, $n = 3$; 6) *UO_2*-PPP, $n > 20$

Figure 2 displays the phosphate stretching mode region of the infrared spectra of M(I)-PPP and M(II)-PPP polymers. IR spectrum of UO_2(II)-PPP salt is also included for comparison. Several overlapping bands appear in the 1300–800 cm^{-1} frequency region, both in the case of polyacid as well as polysalts. Generally, in organophosphorus compounds the bands referring to stretching vibrations of P = O bonds, symmetric and asymmetric stretching vibrations of PO_2^- and PO_3^{2-} ions and stretching vibrations of C–O–(P), P–O–(C) and P–O–(H) groups appear in this frequency range [17, 18]. Although this spectral region is not fully understood, the bands observed near 1230 cm^{-1} in the infrared spectrum of water free H-PPP and near 1065 cm^{-1} and 1240 cm^{-1} in the spectra of M-PPP can be assigned unambiguously to the phosphoryl vibration in H-bonded POOH groups and to the P–O stretching modes of PO_2^- anion, respectively [13].

In Table 1 values of $\nu_{as}PO_2^-$ and $\nu_sPO_2^-$ frequencies for different M(I)-PPP and M(II)-PPP

Table 1. Absorption bands of PO_2^- ion for different M(I)-PPP and M(II)-PPP polysalts

			Condensed state			Aqueous solution	
Polymer sample	Degree of polymerization n	Ionic radius1) Å	$\nu_{as}PO_2^-$ cm^{-1}	$\nu_sPO_2^-$ cm^{-1}	$\Delta\nu PO_2^-$ cm^{-1}	$\nu_{as}PO_2^-$ cm^{-1}	$\nu_sPO_2^-$ cm^{-1}
Na-PPP	3	0.97	1240	1065	175		
	10.5		1240	1066	174	1208	1057
	> 20		1242	1069	173		
K-PPP	3	1.33	1234	1058	176		
	10.5		1235	1060	175	1208	1056
	> 20		1237	1061	174		
Cs-PPP	10.5	1.67	1230	1058	172	1206	1060
	> 20		1232	1059	173		
Mg-PPP	3	0.66	1240	1066	174		
	8.5		1242	1069	173		
	10.5		1244	1069	175	1209	1060
Ca-PPP	3	0.99	1230	1060	170		
	8.5		1235	1066	169		
	10.5		1240	1070	170	1206	1058
Ba-PPP	3	1.34	1225	1056	169		
	10.5		1228	1060	168		
UO_2-PPP	3		1173	1055	118		
	> 20		1180	1065	115		

[1] Source: Weast RC (ed), (1975–76) Handbook of Chemistry & Physics (56th ed). CRC Press, Cleveland

salts, both in condensed state as well as in aqueous solution, are summarized. These results indicate, that for solid, thoroughly dried polymer samples of the same degree of polymerization *n*, the symmetric and asymmetric stretching frequencies of the PO_2^- groups vary with the nature of the cation and show a general tendency to decrease with increasing counterion radius at constant counterion charge. Values of $\nu_{as}PO_2^-$ and $\nu_sPO_2^-$ for analyzed poly(1,3-propylene phosphate)s in aqueous solutions are independent on counterion type, both for mono- as well as divalent metal ions. These frequencies are practically the same for counterions of the same charge but of different ionic radius.

Metal ion binding modes in M-PPP complexes in the condensed state

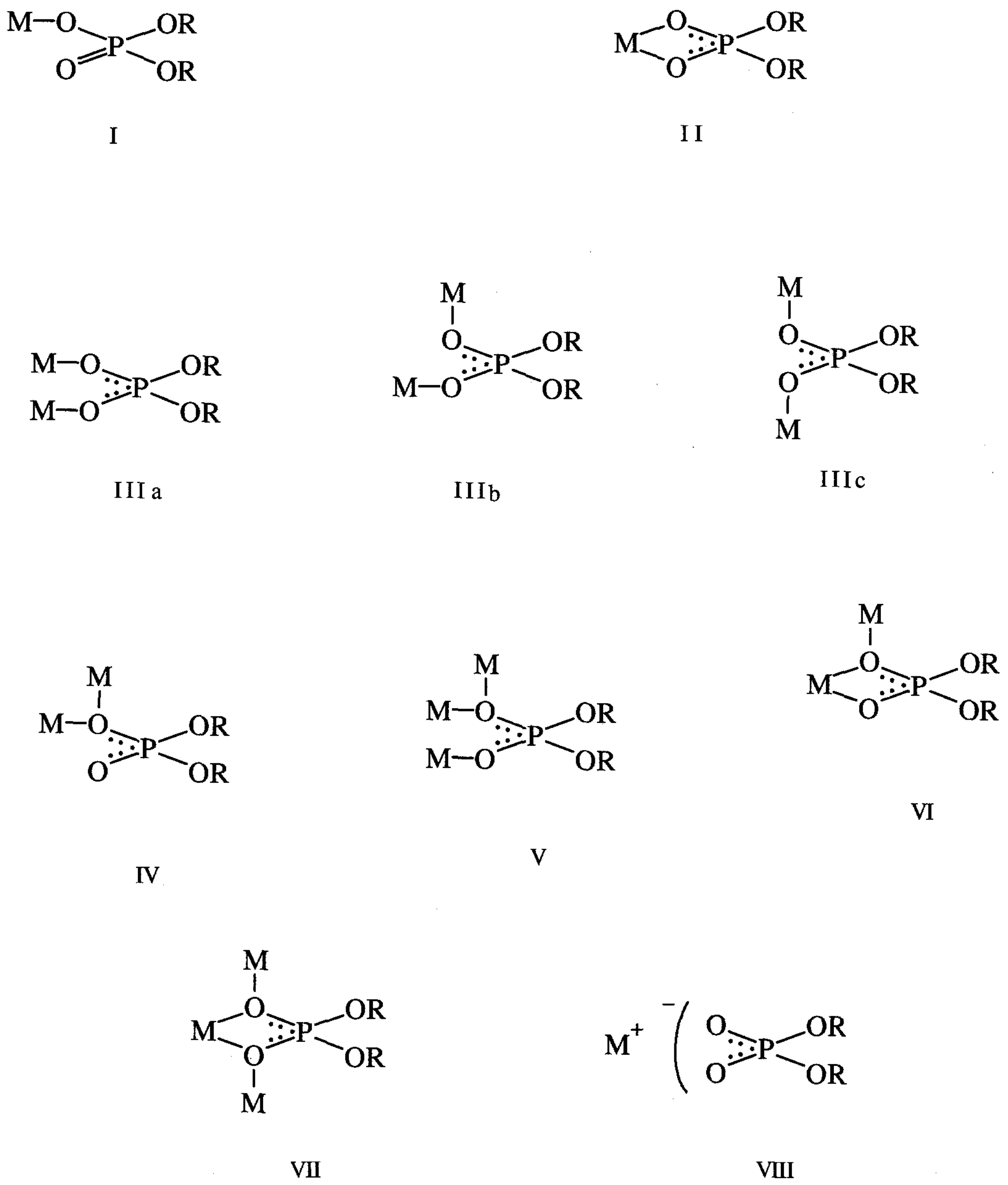
It can be supposed that $(RO_2)_2PO_2^-$ anion in the dialkyl phosphate esters in crystalline state can coordinate to metals in a number of ways, viz. as a unidentate ligand (structure I on Scheme 1), as a chelating bidentate ligand (II), as a bridging bidentate ligand in a different configuration (IIIa–IIIc), or as a monoatomic bridging ligand, either alone (IV), with additional bridging (V) or with chelation or bridging (VI, VII). In addition to coordination structures I–VII ionic structure VIII can also be formed. For non-crystalline solids the number of possible coordination structures can be, of course, reduced. We suppose that in this case structures I–III and VIII sufficiently describe a metal binding to the phosphate groups.

Generally, the nature of the functional group coordination can be readily evident form x-ray data. However, as we confirm by x-ray analysis, PPP samples do not show any tendency to crystallization. Data on crystal structure of low-molecular metal dialkyl phosphates are rather scarce. So, we have to try to obtain some information on the coordination modes of the PO_2^- group in poly(1,3-propylene phosphate)s from spectral data only, i.e., from the analysis of the PO_2^- stretching mode region. We assume that relationships between phosphorus-oxygen stretching frequencies and the type of phosphate group coordination for dialkyl phosphate complexes are similar to correlations between νCO_2 stretching frequencies and the nature of carboxylate group coordination generally used for metal carboxylates [19, 20]. Thus, unidentate, chelating and/or bridging coordination complexes can be expected to have the values of separations $\Delta\nu$ between νPO_2^- frequencies ($\Delta\nu PO_2^- = \nu_{as}PO_2^- - \nu_sPO_2^-$) different from ionic complexes. Values of $\Delta\nu PO_2^-$ substantially greater than ionic ones can be considered indicative of unidentate phosphate coordination (structure I). Separations significantly less than ionic values are characteristic for chelating (structure II). The $\Delta\nu PO_2^-$ values for bridging complexes III should be greater than those for chelating (bidentate) complexes, and close to the ionic values. A characteristic feature of the formation of the metal-oxygen bond in structures of type II or III consists of the occurrence in both cases of two equivalent P–O bonds, which are similarly affected by the metal ion. Therefore, one can expect that for phosphate complexes of type II and III, similarly to the carboxylate complexes, the replacement of one metal ion by another, will lead to the shift of $\nu_{as}PO_2^-$ and $\nu_sPO_2^-$ frequencies in the same direction.

For M(I)-PPP and M(II)-PPP polysalts $\nu_sPO_2^-$ and $\nu_{as}PO_2^-$ frequencies show general tendency to decrease with increasing counterion radius at constant counterion charge (Table 1). This means that in the dry condensed state the phosphate group vibrations are disturbed in a different way by different counterions. The observed insensitivity of $\Delta\nu PO_2^-$ to change of cation seems to indicate the formation of complexes with primarily ionic bonds between alkali metals and phosphate groups.

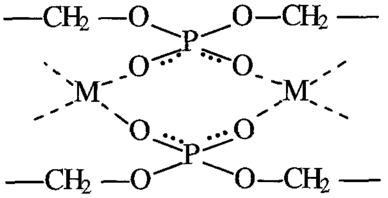
The results for PPP presented in Table 1 show also that the separation of the PO_2^- stretching frequencies for magnesium as well as for calcium complexes is similar to those found for sodium, potassium, and cesium salts. Thus, it is reasonable to assume that the binding modes of analyzed divalent and monovalent metal ions to phosphate groups are the same or similar and/or complexes of the same or similar structure are formed.

The similarities between the phosphate stretching mode region of the infrared spectra of M-PPP complexes (Fig. 2) to those of some simple dialkyl phosphate esters of known crystal structure [21, 22] lead to the conclusion that metal ions bind to the phosphate group of polymeric molecule to form similar structures, as observed for low-molecular model compounds. In the PPP

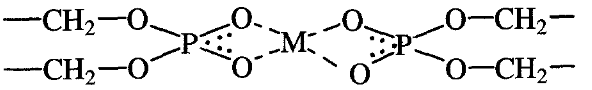


Scheme 1. Metal ion binding modes in M-PPP complexes

complexes with counterions specified above the dialkyl phosphate group probably has a symmetrical mode of coordination (structures II, III or VIII). For complexes of Ca(II) and Mg(II) structures IX and X are proposed in Scheme 2. Structure IX with dialkyl phosphate groups bridging neighboring metal atoms seems to be prevailing. Structure X with chelate rings is considered as less probable in our polymeric compounds. In the solid state both carboxylate groups [23] and phosphate groups [24] only rarely show the formation of strained four-membered chelate rings. Mostly, they act with their oxygen atoms as bridging groups between different metal ions. Hendry and Sorgenson in their recent report [25], based on work with small model systems, conclude that four-membered phosphate-metal ion chelates are unfavorable and irrelevant in

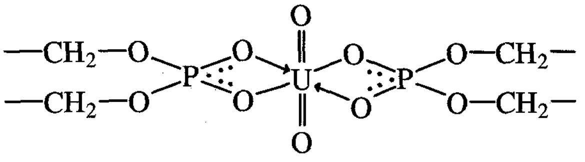


IX



X

Scheme 2. Metal ion binding modes in M(II)-PPP complexes; M = Mg(II), Ca(II)



XI

Scheme 3. Uranyl ion binding modes in UO_2(II)-PPP complexes

biological systems. Structure IX was also postulated earlier by Szemik et al. [21] for complexes of magnesium(II) and zinc(II) with diethyl phosphate esters on the base of x-ray and spectral studies. Stoyanov et al. [26] has proposed such a structure for magnesium(II), calcium(II), barium(II) and zinc(II) salts of di-(2-ethylhexyl)phosphoric acid.

The separation of the PO_2^- stretching frequencies for uranyl complex is distinctly lower than those found for M(I)-PPP as far as M(II)-PPP complexes. Moreover, the intensity of the $\nu_s PO_2^-$ band is higher than the intensity of the $\nu_{as} PO_2^-$ vibration. These findings are consistent with the formation of chelating complexes XI presented in Scheme 3.

Metal ion binding modes in M-PPP complexes in aqueous solution

In general, in aqueous solutions of metal polyphosphates counterions can either surround the polyphosphate chain in the form of a mobile ion cloud or they can be bound to the phosphate group [27]. In the first type of counterion binding, i.e., “ionic atmosphere binding”, the hydration shells of the counterions and of the charged sites on the polyions are not in contact and remain unaffected. In the second type of binding, i.e., “site” or “specific binding”, both metal ions and phosphate groups undergo changes in their state of hydration and “inner-sphere” complexes are formed. The modes of interaction of metal ions with PPP chains may be expected to be dependent on several factors: i) the type of cation (its electronic structure); ii) the environmental conditions, i.e., temperature, pressure, pH, state of sample (gel, crystal, dilute solution), concentration of binding ion, organic cosolvents, concentration of added salt, etc.; iii) degree of polymerization; iv) conformation of polymer chain, etc.

The spectral results obtained for M(I)-PPP and M(II)-PPP, presented in Table 1, i. e., the observed insensitivity of νPO_2^- frequencies to cation type, seem to indicate that in aqueous solution there is no direct contact between the polyphosphate groups and analyzed metal ions, and no site-binding occurs. Complexes formed between Mg^{2+}, Ca^{2+}, Na^+, K^+, Cs^+, and PPP are most probably “outer-sphere” type with indirect phosphate-metal ion binding via a hydrogen-bonded water molecule. The formation of outer-sphere complexes between alkali and alkaline earth metal ions and polymers with phosphate groups was observed earlier by many authors [28–31].

Binding of magnesium and calcium ions to PPP

As we have mentioned above, the main physiological function of teichoic acids in living organisms is to bind and selectively transport magnesium ions from the surrounding medium to the membrane region. In this respect it is very important to describe the manner in which magnesium binds to TAs.

The results of our spectral studies of PPP presented now, as well as the results of conductivity measurements published earlier [11, 12], seem to indicate that in aqueous solution at room temperature the binding of magnesium and calcium ions to poly(alkylene phosphate) chain is dominated by electrostatic interactions. These ions preferentially form outer-sphere complexes with PPP. It should be emphasized that Mg^{2+} and Ca^{2+} may also form inner-sphere complexes to some extent. But inner-sphere coordination or site-binding of these metal ions to the diester phosphate groups of PPP, if it even occurs, is negligible and therefore it is not observed in our IR experiments. Site-binding (tight localized binding) of alkali and alkaline earth cations can be expected to occur for poly(alkylene phosphate)s–polyelectrolytes with monovalent charged groups–only if the polymer chain is sufficiently flexible or the nearest-neighbor charge spacing is sufficiently close that two or more diester phosphate groups can cooperate in holding the cation. PPP chains, as we discuss below, do not satisfy these requirements.

The geometric form of poly(alkylene phosphate) anions in solution are considered to depend on the degree of polymerization. The studies of electrolytic properties of PPP performed in our laboratory by Wódzki et al. [15] suggest that the shorter chain polyphosphate anions are rigid, but longer ones exhibit a coiled structure. The high-molecular PPP ($n > 20$) undergoes a conformational transition with ionization from the so-called hypercoiled state (globular state, compact structure) to the extended coil state (loosely coiled structure) [32]. This transition occurs at the degree of neutralization α in the range from 0.3 to 0.4. A strong tendency of the PPP chain containing ionic phosphate groups to take the extended chain conformation (extended zig-zag form) was confirmed by Litowska et al. [33] by theoretical analysis using CNDO/2 method. A rigid rod or extended conformation is characteristic also for natural teichoic acids. Doyle et al. [34] reported this conformation for wall teichoic acid of *Bacillus subtilis* 168 in salt-free solution.

The distance between adjacent phosphate groups in the extended zig-zag form of PPP, about 7.7 Å, is large in comparison to the ionic radius of Mg^{2+} and/or Ca^{2+} ions. Thus, these ions cannot bind to the two adjacent phosphate residues simultaneously and form stable inner-sphere complexes. Mg^{2+} and Ca^{2+} ions could form an inner-sphere complex at one phosphate binding site only, but it would leave a local positive charge which seems to be unfavored. In conclusion, we can say that the absence of favorable binding sites in PPP, resulting from its tendency to take the extended chain conformation (extended zig-zag form), both in condensed state as well as in solution, seems to be the main reason for the formation of mainly outer-sphere complexes.

Our spectral results are in accordance with those obtained by Litowska from NMR analysis [35]. She has indicated the existence within the polymer gel complexes composed of six water molecules hydrating one magnesium ion and two phosphate groups, i.e. $(PO_2^- \cdot 3H_2O \cdot Mg^{2+} \cdot 3H_2O \cdot PO_2^-)$.

We can also state that in aqueous solution a specificity in polyion-counterion interaction is not observed. The binding modes of magnesium and calcium are the same or very similar and complexes of the same or similar structure are formed.

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Author's address:

Dr. Jadwiga Ostrowska-Czubenko
Nicholas Copernicus University
Institute of Chemistry
ul. Gagarina 7
87-100 Toruń, Poland