1

THE CHEMICAL STRUCTURES AND PROPERTIES OF CONDENSED INORGANIC PHOSPHATES

For a proper understanding of the processes which take place in living organisms, a precise knowledge of the chemical structures of the compounds that participate in these processes is required. It is therefore deemed essential to present, even if only briefly, an account of present-day ideas of the chemical structures of condensed phosphates, hitherto often known by the long-obsolete terms 'metaphosphates' and 'hexametaphosphates'.

1.1 The Structures of Condensed Phosphates

The first mention of condensed inorganic phosphates dates back to 1816, when Berzelius showed that the vitreous product formed by the ignition of orthophosphoric acid was able to precipitate proteins (Van Wazer, 1958). Graham (1833) described a vitreous phosphate which he obtained by fusion of NaH_2PO_4. Believing that he had isolated a pure compound with the formula $NaPO_3$, Graham named this as a 'metaphosphate'. Shortly afterwards, however, Fleitmann and Hennenberg (1848), working in Liebig's laboratory, demonstrated that the 'metaphosphates' having the general formula MPO_3 (where M is hydrogen or a monovalent metal) were mixtures of closely related compounds which differed mainly in their degree of polymerization. The numerous investigations which were carried out over the next 100 years (for reviews, see: Ebel, 1951; Karbe and Jander, 1942; Teichert and Rinnmann, 1948; Topley, 1949; Van Wazer, 1958), although they provided a wealth of new

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data which shed much light on the structures and properties of this group of compounds, threw into perhaps even greater confusion both the chemical basis of the nomenclature of these compounds, and the names of the compounds themselves. This is perhaps hardly surprising, since these investigations were carried out with compounds of inadequate purity, using rather crude investigation methods. It was thanks to the work of Thilo (1950, 1955, 1956, 1959, 1962), Van Wazer (1950, 1958), Ebel (1951, 1952a–d, 1953a,b) and Boulle (1965) that the chemical structures and properties of this group of compounds were finally established, thus making it possible to bring order into their classification (Van Wazer and Griffith, 1955; Thilo and Sonntag, 1957).

According to the current classification, condensed phosphates are divided into cyclophosphates, polyphosphates and branched inorganic phosphates (or 'ultraphosphates').

1.1.1 Cyclophosphates

The true cyclophosphates (metaphosphates) have the composition which, since the time of Graham, has been incorrectly assigned to the whole group of condensed phosphates, i.e. MPO_3. These compounds are built up from cyclic anions. Only two representatives of this group have so far been investigated in detail – the cyclotriphosphate, $M_3P_3O_9$, and the cyclotetraphosphate, $M_4P_4O_{12}$, shown in Figure 1.1.

The existence of mono- and dimetaphosphates has not been demonstrated in practice, and is theoretically unlikely (Ebel, 1951; Thilo, 1959; Van Wazer, 1958). The possible presence of cyclopentaphosphates and cyclohexaphosphates in a mixture of condensed sodium phosphates was shown by Van Wazer and Karl-Kroupa (1956), followed by Thilo and Schülke (1965). In addition, more highly polymerized cyclic phosphates containing as many as 10 to 15 orthophosphoric acid residues have been observed in some samples of the condensed phosphates prepared by Van Wazer (1958). Furthermore, cyclooctaphosphate (Schülke, 1968; Palkina *et al.*, 1979) and cyclododecaphosphate (Murashova and Chudinova, 1999) have been obtained in the crystalline state.

It should be pointed out that the term 'hexametaphosphate', which is frequently encountered in the literature, refers in fact to the compound known as *Graham's salt*, which

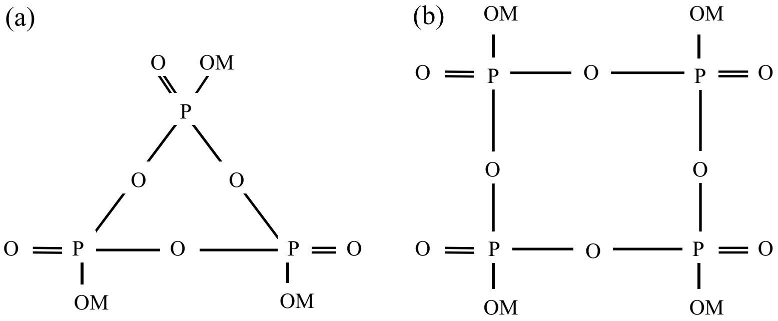
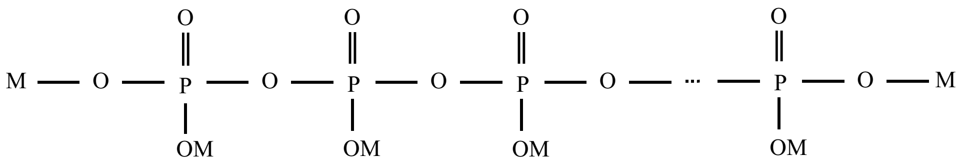


Figure 1.1 Structures of (a) cyclotriphosphate and (b) cyclotetraphosphate.



$$M-O-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle OM}{|}}{P}}-O-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle OM}{|}}{P}}-O-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle OM}{|}}{P}}-O-\cdots-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle OM}{|}}{P}}-O-M$$

Figure 1.2 Structure of a linear condensed phosphate (PolyP), where M is H^+ or a monovalent metal cation.

is a mixture of condensed sodium phosphates containing cyclic phosphates (including cyclohexaphosphate), but which is mainly composed of highly polymerized linear polyphosphates (Van Wazer and Griffith, 1955; Thilo and Sonntag, 1957).

1.1.2 Polyphosphates

Polyphosphates (PolyPs) have the general formula $M_{(n+2)}P_nO_{(3n+1)}$. Their anions are composed of chains in which each phosphorus atom is linked to its neighbours through two oxygen atoms, thus forming a linear, unbranched structure which may be represented schematically as shown in Figure 1.2. The degree of polymerization, n, can take values from 2 to 10^6, and as the value of n increases, the composition of the polyphosphates, i.e. the cation-to-phosphorus ratio, approximates to that of the cyclophosphates, which explains the belief which prevailed until recently that 'polyphosphate' and 'metaphosphate' were equivalent terms. Polyphosphates in which $n = 2$–5 can be obtained in the pure, crystalline state (Van Wazer, 1958), but members of this series in which n has higher values have been obtained in appreciable amounts only in admixtures with each other.

In contrast to the cyclophosphates, they are designated as 'tripolyphosphates', 'tetrapolyphosphates', etc., although the mono- and dimeric compounds are still called by their old names of 'orthophosphate' (P_i) and 'pyrophosphate' (PP_i), respectively. In addition, the highly polymeric, water-insoluble potassium polyphosphate ($n \sim 2 \times 10^4$), which has a fibrous structure of the asbestos type, is still called *Kurrol's salt*. We may mention in passing that the facile preparation of Kurrol's salt (by fusion of KH_2PO_4 at 260 °C), and the ease with which it is converted into the water-soluble sodium form by means of cation-exchange materials, has led to its frequent preparation and use in chemical and biochemical work as an inorganic polyphosphate.

Even better known is Graham's salt, the vitreous sodium polyphosphate ($n \sim 10^2$) obtained by fusion of NaH_2PO_4 at 700–800 °C for several hours, followed by rapid cooling. Graham's salt is a mixture of linear polyphosphates with different chain lengths. Fractional precipitation from aqueous solution by means of acetone (Van Wazer, 1958) affords less heterogeneous fractions with different molecular weights. For example, a sample of Graham's salt, in which the chains on average have 193 phosphorus atoms (i.e. $n \sim 193$), can be separated by this method, as shown in Figure 1.3.

As can be seen from this Figure, the sample contains molecules of different sizes. The fraction of highest molecular weight has $n \sim 500$, i.e. its molecular weight is of the order of

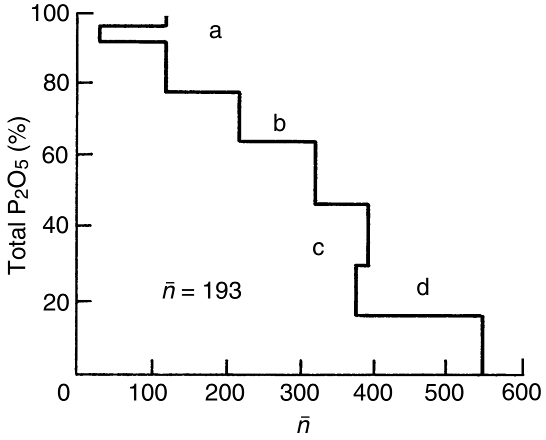


Figure 1.3 Distribution curve (by size) obtained for sodium polyphosphate molecules (Graham's salt, $n \sim 193$) after fractional precipitation, after Van Wazer (1958): (a) cyclic phosphates; (b), (c) and (d) linear polyphosphates.

40 000. It is interesting to note that the reason for the failure of Graham's salt to crystallize is that it consists of a mixture of homologous chains differing only in their lengths. Since all of the components of the homologous series of polyphosphates closely resemble each other, crystallization cannot take place with ease because molecules of different dimensions seek to displace each other on the growing crystal, thereby bringing its growth to a stop. When the chains are very long (such as is the case in Kurrol's salt), this does not occur, since the individual chains pass through many elementary cells of the crystal, and the chain length is not an important factor in determining the lattice parameters of the crystal (Van Wazer, 1958).

A second factor which determines the maximum chain lengths of the polyphosphates which are able to crystallize is the increase in polarity of the molecules which takes place as the degree of polymerization increases.

Two factors thus appear to be responsible for the failure so far to obtain linear polyphosphates containing 6–200 phosphorus atoms in a crystalline state: (1) the difficulty of crystallization from a mixture of similar compounds, and (2) the effect of polar groups on the molecules.

In addition to linear polyphosphates, Graham's salt usually contains very small amounts of cyclophosphates (see Figure 1.3). For example, a sample of Graham's salt with $n \sim$ 100–125 was shown by Van Wazer (1958) to contain 4 % of cyclotriphosphate, 2.5 % of cyclotetraphosphate, 0.8 % of cyclopentaphosphate, 0.5 % of cyclohexaphosphate, and fractional percentages of higher polymeric cyclophosphates. The compositions of two samples of Graham's salt obtained by Dirheimer (1964) are shown in Table 1.1.

The conformations of polyphosphate chains in the crystals depend on the nature of the metal cations. The period of the recurring unit changes depending on the charge, shape and electronic envelope structure of the metal cations. The structures of some crystalline

Table 1.1 Compositions of synthetic samples of Graham's salt (Dirheimer, 1964). The phosphorus contents of the poly- and cyclophosphates are expressed as a percentage of the total phosphorus contents of the compounds.

Polyphosphates and cyclophosphates	Sample 1	Sample 2
High-molecular-weight polyphosphate	68.1	75.1
Polyphosphates ($n \sim 5$–10)	17.3	13.6
Tetrapolyphosphate plus cyclotriphosphate	7.8	7.0
Tripolyphosphate	4.5	2.8
Pyrophosphate	2.3	1.5

polyphosphates with recurrence periods from 2 to 24 phosphate residues are shown in Figure 1.4.

1.1.3 Branched Inorganic Phosphates, or 'Ultraphosphates'

High-molecular-weight condensed phosphates which, unlike the linear polyphosphates, contain 'branching points', i.e. phosphorus atoms which are linked to three rather than two neighbouring phosphorus atoms, are known as branched phosphates (or 'ultraphosphates'). Such phosphates have a branched structure, a fragment of which is shown in Figure 1.5. In this type of structure, the individual polyphosphate chains are linked to form a 'network', which is the reason for the name given to this type of condensed phosphates. The existence of this group of phosphorus compounds was observed in some samples of both Kurrol's and Graham's salt, as identified by chemical methods (Van Wazer and Holst, 1950; Strauss and Smith, 1953; Strauss *et al.*, 1953; Strauss and Treitler, 1955a,b; Thilo, 1956, 1959; Van Wazer, 1958). In samples of Graham's salt with very long chains (of the order of several hundred phosphorus atoms), approximately one in every thousand phosphorus atoms is a branching point (Strauss and Smith, 1953; Strauss *et al.*, 1953; Strauss and Treitler, 1955a,b). The presence of branching in polyphosphate chains, or in other words, the presence of a *reticular structure*, can be detected by the decrease in the viscosity of aqueous solutions which occurs following dissolving the compounds in water (owing to the rapid hydrolysis of the lateral bonds, which are very unstable). Figure 1.6 shows how the proportion of lateral bonds in Graham's salt increases as the chain length is increased.

Although branched phosphates have not yet been found in living organisms (perhaps as a consequence of their unusually rapid hydrolysis in aqueous solution, irrespective of pH, even at room temperature), it is believed that their presence in biological materials cannot be excluded.

Information on the chemical compositions of the condensed inorganic phosphates, together with descriptions of their chemical and physico-chemical properties, can be found in several papers, reviews and monographs (Thilo, 1950, 1955, 1956, 1959; Van Wazer, 1950, 1958; Ebel, 1951; Griffith *et al.*, 1973; Ohashi, 1975; Corbridge, 1980). We shall

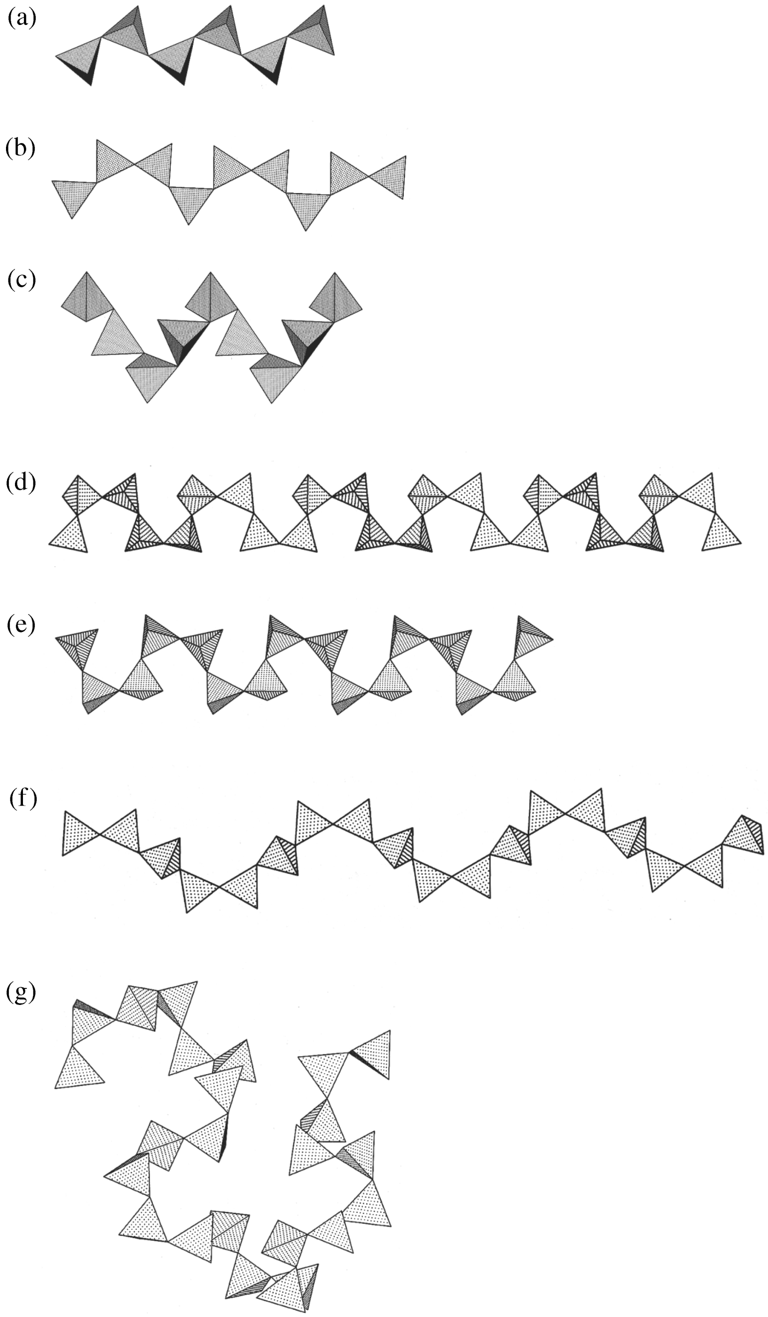


Figure 1.4 Structures of various crystalline polyphosphates: (a) $(Na_2HP_3O_9)_n$ (Jost, 1962); (b) $[Na_3H(PO_3)_4]_n$ (Jost,1968); (c) $(NaPO_3)_n$ (Immirzi and Porzio, 1982); (d) $(KPO_3)_n$ (Jost and Schulze, 1969); (e) $[Ca_2(PO_4)_3]_n$ (Schneider *et al.*, 1985); (f) $[(NH_4)Cu(PO_3)_3]_n$ (Tranqui *et al.*, 1969); (g) $[NaMn(PO_3)_3]_n$ (Murashova and Chudinova, 1997).

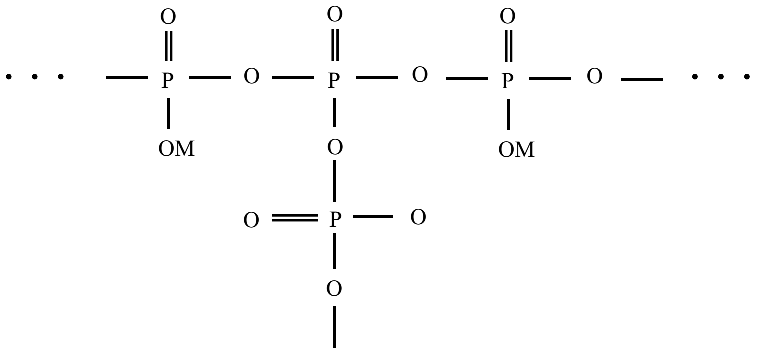


Figure 1.5 Structure of branched phosphate.

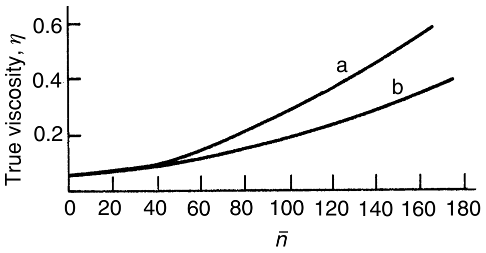


Figure 1.6 Changes in the viscosities of solutions of polyphosphates of different chain lengths on keeping for 12 h, where the abscissa represents the mean chain length as determined by end-group titration: (a) immediately after solution; (b) after keeping for 12 h (Strauss and Treitler, 1955b).

dwell here very briefly on those properties of condensed phosphates that are useful for their identification and chemical determination in living organisms.

1.2 Some Chemical Properties of Condensed Inorganic Polyphosphates

Polyphosphates are salts of acids that, in solution, contain two types of hydroxyl groups that differ in their tendency to dissociate. The terminal hydroxyl groups (two per molecule of polyphosphoric acid) are weakly acidic, whereas the intermediate hydroxyl groups, of which there are a number equal to the number of phosphorus atoms in the molecule, are strongly acidic (Van Wazer, 1958). Cyclophosphates do not contain terminal hydroxyl groups and, for this reason, the corresponding acids possess only strongly acidic groups which in solution are dissociated to approximately the same extent. Thus, titration of weakly and strongly acidic groups is a convenient means of determining whether a given condensed phosphate is a cyclo- or a polyphosphate. Moreover, this method provides a means of determining the average chain length of linear polyphosphates (Wan Wazer, 1950; Ebel, 1951; Samuelson, 1955; Langen and Liss, 1958a,b; Chernysheva *et al.*, 1971) It is interesting that this was the method used by Samuelson (1955) in showing for the first time that Graham's salt was not a cyclophosphate – as had been believed for almost 100 years – but a mixture of linear polyphosphates.

All alkali metal salts of condensed polyphosphoric acids are soluble in water. Potassium pyrophosphate is especially soluble, with, for example, 100 g of water dissolving 187.4 g of $K_4P_2O_7$ at 25 °C, 207 g at 50 °C, and 240 g at 75 °C. Exceptions to this rule are the water-insoluble Kurrol's salt (a macromolecular crystalline potassium polyphosphate), and the compounds known as Maddrell's salts (crystalline sodium polyphosphates of very high molecular weight). Kurrol's salt is readily soluble in dilute solutions of salts containing cations of univalent metals (but not K^+), for example, 0.2 M NaCl. It is worth mentioning that Graham's salt dissolves in water only when it is stirred rapidly. Without stirring, the compound forms a glue-like mass in water. Polyphosphates of divalent metals such as Ba^{2+}, Pb^{2+} and Mg^{2+} are either completely insoluble or dissolve to only a very limited extent in aqueous solutions. The polyphosphates of certain organic bases such as guanidine are also sparingly soluble in water (Singh, 1964). Other solvents (liquid ammonia, anhydrous formic acid, and organic solvents such as ethanol and acetone) dissolve only trace amounts of sodium and ammonium polyphosphates. Low-molecular-weight polyphosphates dissolve readily in very dilute aqueous alcoholic solutions, but addition of alcohol to these solutions rapidly reduces their solubility. Figure 1.7 shows that an ethanol–water mixture containing 40 % of ethanol is a very poor solvent for both potassium pyrophosphate and potassium tripolyphosphate (1.5 g per 100 g of solution).

Condensed phosphates, other than branched phosphates, are stable in neutral aqueous solution at room temperature. The hydrolysis of the P–O–P bond in linear polyphosphates such as Graham's salt liberates energy equivalent to approximately 10 kcal/mol (Yoshida, 1955a,b; Van Wazer, 1958), i.e. the same amount of energy as is liberated in the hydrolysis of the terminal phosphoric anhydride bonds in the adenosine 5′-triphosphate (ATP) molecule. Hydrolysis of the cyclotriphosphate also liberates this same amount of energy (Meyerhof *et al.*, 1953).

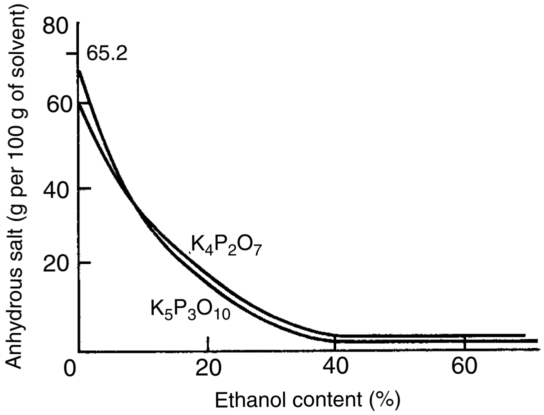


Figure 1.7 Solubility curves for potassium pyrophosphate and potassium tripolyphosphate in ethanol–water mixtures at 25 °C (Van Wazer, 1958).

The branching points in branched phosphates, in which one atom is bonded through oxygen to three other phosphorus atoms, are extremely labile. The rate of hydrolysis of the branching points in the reticular phosphates in aqueous solution at 25 °C, resulting in the formation of linear polyphosphates, is about 1000 times greater than that of the P–O–P bonds in the linear polyphosphates. Hydrolysis of the branching points liberates 28 kcal mol^{-1} (Van Wazer, 1958), which is much more than that liberated in the hydrolysis of the 'central' phosphoric anhydride bonds.

The linear polyphosphates and cyclophosphates are hydrolysed extremely slowly at neutral pH and room temperature in comparison with other polyacids such as polyarsenates and polyvanadates, and are unique in this respect. The 'half-hydrolysis time' for the P–O–P bonds in linear polyphosphates at pH 7 and 25 °C is several years (Van Wazer, 1958). The rate of hydrolysis of these bonds is increased by raising the temperature, reducing the pH, and by the presence in the solution of colloidal gels and complex cations. The hydrolysis of these bonds is dependent on the ionic strength of the solutions (Van Wazer, 1958).

When neutral solutions of polyphosphates are heated at 60–70 °C for 1 h, they are broken down quantitatively to cyclotriphosphate and orthophosphate. It has been shown that this hydrolysis does not occur randomly, but rather from the end of the polyphosphate chain (Thilo and Wieker, 1957). Thilo (1962) related the formation of cyclotriphosphates during the hydrolysis of linear polyphosphates in neutral solution (and even in non-aqueous solution) to the presence of a particular type of spiral secondary structure which makes it sterically possible for a rearrangement of the bonds to occur within the molecule with the formation of small closed chains (Figure 1.8).

In alkaline solutions, cyclophosphates undergo ring fission, even on gentle warming, to form linear polyphosphates with corresponding chain lengths (Ebel, 1951). Linear polyphosphates also undergo hydrolysis under alkaline conditions (Niemeyer and Richter, 1969, 1972), but more particularly under acidic conditions (pH, 3.5–4.0). Under these conditions, significant hydrolysis of the P–O–P bonds takes place even at room temperature, and here breakdown occurs along the length of the chains rather than from the ends of the chains,

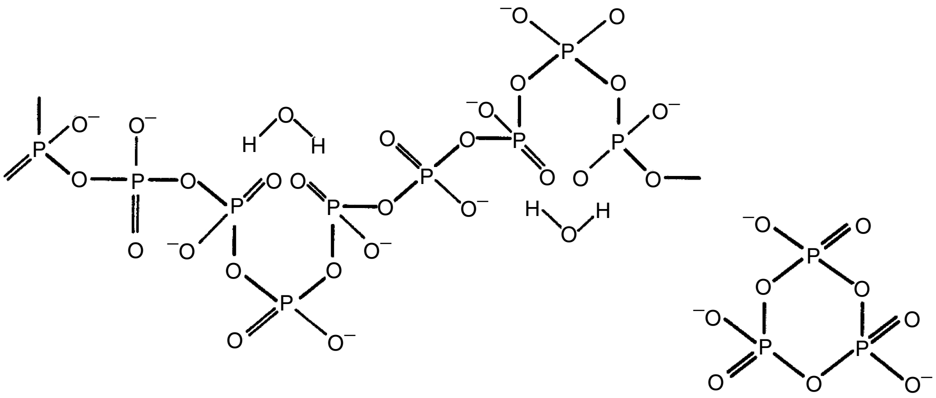


Figure 1.8 Illustration of the incomplete hydrolysis of linear high-molecular-weight polyphosphates to cyclotriphosphate and orthophosphate (Thilo, 1956, 1962).

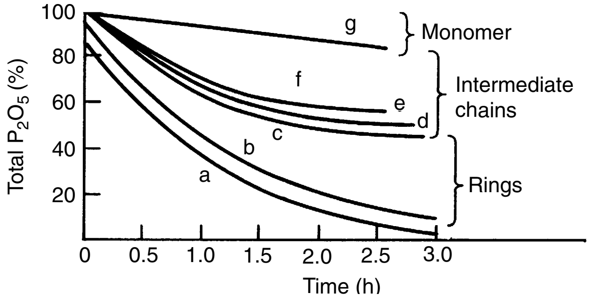


Figure 1.9 Results of a chromatographic examination of the hydrolysis products of Graham's salt at pH 4 and 90 °C: (a) high-molecular-weight polyphosphates; (b) cyclic phosphates containing four to six phosphorus atoms; (c) cyclotriphosphate; (d) pyrophosphate; (e) tripolyphosphate; (f) linear polyphosphates containing four to 15 phosphorus atoms; (g) orthophosphate (Van Wazer, 1958).

to form polymers with increasingly lower molecular weights, down to orthophosphate. The results of an investigation of the hydrolysis products of Graham's salt at pH 4.0 and 90 °C are shown in Figure 1.9. It can be seen from this figure that the proportions of the hydrolysis products (linear polyphosphates, cyclophosphates and orthophosphate) are very dependent on the duration of hydrolysis. When the reaction time is increased to 3 h, the higher polymeric polyphosphates disappear altogether, with the mixture consisting entirely of low-molecular-weight poly- and cyclophosphates and orthophosphate. When the pH of the solution is reduced to 1 and below, the extent of hydrolysis of polyphosphates to orthophosphate increases rapidly. Linear polyphosphates such as Graham's salt are completely hydrolysed after 7-15 min at 100 °C in 1 N HCI (Van Wazer, 1958).

1.3 Physico-Chemical Properties of Condensed Inorganic Polyphosphates

Apart from the low-molecular-weight polyphosphates and cyclophosphates, condensed inorganic phosphates are macromolecular compounds, and this affects their properties and behaviour in solution.

Aqueous solutions of polyphosphates of low ionic strength and pH values near neutral are very viscous, with the viscosity increasing with increasing mean chain length (Malmgren, 1948; Ingelman and Malmgren, 1950; Van Wazer, 1950). The presence of branched phosphates in any given sample of condensed phosphates results, as we have seen, in a very high initial viscosity which decreases rapidly following dissolution in water, even at room temperature (see Figure 1.6).

Polyphosphates in aqueous solutions of low ionic strength are capable of forming complexes with other polymers, especially proteins (Katchman and Van Wazer, 1954), *basic polypeptides* (Singh, 1964), *and nucleic acids* (Kulaev and Belozersky, 1958; Ebel *et al.*, 1958c). This ability increases as the chain length of the polyphosphate molecule increases. In acidic solution, these complexes separate as precipitates. The ability of condensed phosphates to precipitate proteins from acidic solutions has been known from a very early date (Perlmann, 1938; Ebel, 1951; Van Wazer, 1958; Wiame, 1958). It has been shown that this property of polyphosphates is due to a simple total charge, which is dependent on the pH. Katchman and Van Wazer (1954) showed that the higher the molecular weight of a water-soluble protein, then the less polyphosphate is required.

A similar polycation–polyanion interaction is found in the metachromatic reaction, in which high-molecular-weight polyphosphates cause a shift in the absorption maximum of cationic dyes, such as toluidine blue, towards shorter wavelengths (Ebel, 1951; Bergeron and Singer, 1958). This reaction essentially involves polymerization of the dye on the macromolecular anion (Wiame and Lefebvre, 1946; Wiame, 1947a,b). In the case of toluidine blue, addition of polyphosphate to the solution results in a change in colour from blue to violet–red, and a shift in the position of the absorption maximum from 630 nm (which is characteristic of solutions of the monomeric form of toluidine blue) to 530 nm (typical of the complex of polyphosphate and the polymerized dye) (Arloing and Richard, 1921; Damle and Krishnan, 1954; Tewari and Krishnan, 1959; Correll and Tolbert, 1964). However, only comparatively high-molecular-weight polyphosphates are capable of undergoing the metachromatic reaction, either in solution or on paper (Ebel and Muller, 1958; Tewari and Krishnan, 1959; Correll and Tolbert, 1964) Tripoly- and cyclotriphosphates, for example, do not react with toluidine blue (Kornberg, 1956).

Linear polyphosphates possess properties very similar to those of cross-linked, solid ion-exchange agents (Thilo, 1955). The behavior of polyphosphates as dissolved ion-exchange agents is yet further evidence of their ability to form complexes with counter-ions. Polyphosphates are known to be very good complexing agents for many metal ions (Van Wazer, 1958). This property is widely exploited in the fractionation of polyphosphates, and for other analytical purposes.

The information given above concerning the chemical and physico-chemical properties of the inorganic polyphosphates will assist in the better understanding and prediction of the behaviour of these compounds during their extraction from cells and their subsequent fractionation. Knowledge of these properties will facilitate the development and use of efficient and reliable biochemical procedures for the isolation, purification, identification and determination of polyphosphates.