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Gel Chromatography of Condensed Phosphates

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The principles of the application of gel chromatography are discussed, together with its potentialities in the investigation of inorganic polymers of phosphorus with the aim of determining molecular dimensions, fractionating a mixture, obtaining individual phosphates, and also analysing their molecular-weight composition. The development and the achievements of the gel chromatography of condensed phosphates are surveyed. The phenomena occurring in a gel during the elution of inorganic compounds are described for several salts. A list of 91 references is included.

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I. INTRODUCTION

Molecular weight and degree of polydispersity are among the main characteristics of macromolecular compounds. Research has shown [1-6] that many properties of inorganic phosphate polymers depend directly on the size of the molecules. It is therefore very important to develop methods for investigating the molecular-weight composition of such polymers.

Almost all the methods known in the chemistry of macromolecular compounds—light scattering [1], ultracentrifuging [7,8], viscometry [1,9], chemical (end-group analysis) [10-12], and cryoscopic [13-15]—have been used to determine the mean molecular weight of condensed phosphates. Chromatographic methods of analysis (paper [16-18], ion-exchange [19-21], thin-layer [22-24], and electro-chromatography [25-27]) can be used to establish the molecular-weight distribution of polymers containing up to 10–14 phosphorus atoms in the chain. Precipitation [28-30] by means of various organic and inorganic reagents is, perhaps, the only method employed for the fractionation of macromolecular phosphates.

Promising new methods for isolating and separating condensed phosphates would contribute to further knowledge of their properties and structure. The production of porous gels varying in their permeability to molecules of different sizes was responsible for the rapid and widespread development of gel chromatography, a method of separation according to molecular weight†: since gel chromatography was first studied in 1959 on Sephadexes [31], more than 2000 publications have appeared on the application of the method.

Examples of hydrophilic gels used in gel chromatography are [32] dextrans—Sephadex (Sweden) and Molselekt (Hungary)—polyacrylamides—Bio-Gel P (USA) and Acrylex P (Hungary)—and agaroses—Sepharose (Sweden) and Bio-Gel A (USA). Organophilic examples include Sephadex derivatives and acrylic and polystyrene gels. Furthermore, dextran (ED, DED) and polyacrylamide (AM) hydrophilic gels and styrene—divinylbenzene (SDV) hydrophobic gels, similar in properties to the foreign products, have been manufactured in the USSR. [33] All the gels are produced as spherical granules of various sizes, and are divided into several types according to their porosity.

Gel chromatography was developed and used mainly for separating, isolating, purifying, and concentrating organic compounds, as well as for their molecular-weight analysis. A considerable number of reviews have been published on the theoretical basis of the method and its practical application in organic and biological chemistry [34-40].

The achievements and possibilities of gel chromatography have recently attracted the attention of inorganic chemists. The behaviour of halides, nitrates, sulphates, and perchlorates of alkali, alkaline-earth, and other uni- and bi-valent metals on Sephadexes and Bio-Gels has been investigated as a function of concentration, gel porosity, column height, eluent type, concentration, and pH, rate of elution, and temperature [41-52]. Gel chromatography has been used to study inorganic polymers such as phosphates [53-67], silicates [68], basic iron nitrates [69,70], and molybdates [71]. It has been employed to investigate complex formation with Trilon B [72,73] and other reagents [74,75]. Its application to the separation and investigation of various ions, complex compounds, and inorganic polymers has been reviewed [76,77].

These researches were decisive in establishing the processes occurring in a gel during the elution of electrolyte solutions. This knowledge served in turn as foundation for the practical application of gel chromatography to the determination of the sizes of ions, the separation and purification of inorganic substances, and the determination of the molecular-weight composition of inorganic polymers.

II. GEL CHROMATOGRAPHY OF INORGANIC COMPOUNDS

1. Processes occurring in the Gel during Elution

The solvent in a layer of a swollen gel can be regarded [34] as forming two phases—within and between the granules—so that the total volume of the layer can be expressed

(1)

where V_0 is the volume of solvent between the granules (external or free volume), V_i the volume of solvent inside the granules (internal volume), and V_m the volume of the

† The term *gel chromatography* is used in this Review as the most common in the chromatography of inorganic substances on gels.

gel matrix. Various phenomena may occur on the elution of dissolved molecules in such a system. Which phenomenon predominates is determined by the interaction of gel, solute, and solvent.

Numerous experimental results show that dissolved molecules are eluted from a gel chromatographic column in the order of their diminishing size or molecular weight. Such an inverse molecular-sieve effect is due to the fact that dissolved molecules of different sizes penetrate the swollen granules to different extents, which may be connected both with steric factors and with diffusion. The elution volume V_e of any solute is therefore intermediate between that of a substance which is completely excluded from the gel granules (equal to the free volume V_0) and the elution volume of a substance which is uniformly distributed between the two phases (equal to the total volume of solvent $V_0 + V_i$):

$$V_e = V_0 + K_d V_i, \qquad (2)$$

where K_d is the distribution ratio of the solute between the two solvent phases ($0 \leq K_d \leq 1$ in magnitude), which characterises the degree of penetration of solute inside the gel.

The above molecular-sieve mechanism is apparent in most cases of the gel chromatography of organic and inorganic substances; it is supported also by the consistency of experimental results with relations between elution parameters and molecular weights or sizes calculated on the basis of various models of gel structure [78-80]. However, several examples are known, especially in the elution of aromatic and heterocyclic compounds [81], their derivatives containing ionisable groups, and many inorganic salts [41], where the behaviour on gel chromatography cannot be satisfactorily explained solely by the molecular-sieve effect.

Secondary effects (secondary with respect to separation according to molecular weight, but in some cases decisive) are apparent in disturbances of the sequence of elution with diminishing molecular weight or size, in significantly overestimated ($V_e > V_0 + V_i$) or underestimated (in comparison with expectation from molecular size) elution volumes, in asymmetry of the chromatographic peaks, and in a dependence of the elution parameters on the concentration of the solute and on the nature and concentration of the eluent. These phenomena may result from specific interaction of the gel with certain substances and ions both in the separable mixture and in the eluent, as well as with their mutual complexes.

Abnormally high partition coefficients (or elution volumes) are a consequence of the retention of a substance in the column, owing to ion exchange or physical and chemical adsorption by the gel. The substance is then eluted by a volume of solvent exceeding $V_0 + V_i$, and the partition coefficient $K_d > 1$.

In several cases, on the other hand, substances are eluted from the column earlier than would be expected from their molecular size. This phenomenon, termed *ionic exclusion*, is attributed to the presence of a small quantity of negatively charged carboxylate groups in Sephadex, which repel solute anions, which are thus excluded from the gel granules and are eluted earlier.

Adsorption and ionic exclusion effects can be observed in the elution of sodium sulphate, chloride, nitrate, and perchlorate on Sephadex G-15 with 0.1 M sodium chloride solution [42]. The elution volumes of these salts increase in the sequence

$$Na_2SO_4 < NaCl < NaNO_3 < NaClO_4.$$

Sodium nitrate is eluted by a volume of solvent exceeding the total volume; the perchlorate is eluted still more slowly; and the partition coefficient of sodium sulphate (~0.1) is considerably smaller than the value to be expected from the radius of the hydrated sulphate ion [82]. Furthermore, experimental results on the elution of the chlorides of several metals with solutions of 0.1 M KCl + 0.01 M HCl, 0.1 M Na_2SO_4 + 0.005 M H_2SO_4, 0.1 M $NaNO_3$ + 0.01 M HNO_3, and 0.1 M $NaClO_4$ + 0.01 M $HClO_4$ on Sephadex G-25 showed that the partition coefficients of the metal ions in the nitrate and perchlorate systems were respectively 1.0–1.5 and 1.8–2.7, but those for the sulphate system were closely similar and small (up to 0.4). [43,44] These data indicate specific interaction between the gel and these ions, i.e. adsorption of nitrate and perchlorate ions and considerable exclusion of the sulphate ion.

Adsorption can be observed also in the elution of substances by eluents which by themselves are inert towards the gel. An example is the elution of zinc and cadmium chlorides on Sephadex G-10, which depends on the concentration of the eluting sodium chloride solution [45]: with increase in the eluent concentration chloride complexes are formed, whose adsorption on the gel produces values of $K_d > 1$.

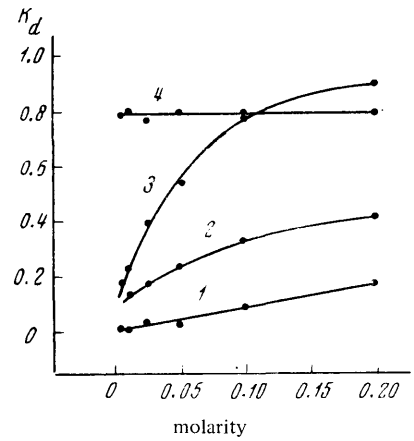


Figure 1. Effect of concentration of test material (in 50 ml of solution eluted with water on Sephadex G-10 at 35 ml h^{-1}) on its partition coefficient calculated from V_e, V_0, and V_i for [46]: 1) $Na_5P_3O_{10}$; 2) Na_2HPO_4; 3) NaCl; 4) glucose.

The ionic exclusion effect can be diminished by using a suitable eluent containing a supporting electrolyte to neutralise the action of the gel. Secondary effects are strengthened when water without a supporting electrolyte is used as eluent. In the elution of solutions of certain electrolytes with water, for example, their partition coefficients are relatively small and increase with the concentration of the solutions [46,47]. Use of an electrolyte as eluent increases the partition coefficients. The elution curves of non-electrolytes, e.g. glucose, do not depend on these factors (Fig. 1). To explain the processes occurring in a gel during the elution of electrolyte solutions the penetration of the supporting electrolyte into the interior of the gel phase was assumed to be incomplete owing to

steric and electrostatic factors[42,48]. Indications that part of the water involved in the swelling of a gel hydrates the polysaccharide chains of dextran[81] confirm the existence in the gel of a region which is inaccessible to dissolved molecules.

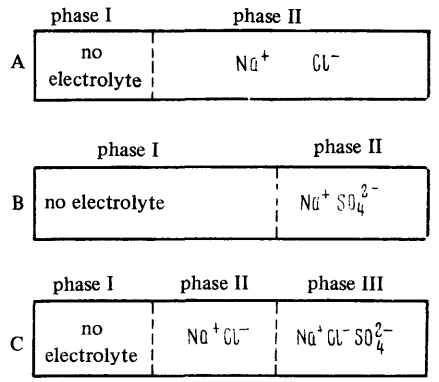


Figure 2. Diagram of distribution of ions in gel granules[42].

Thes properties of a gel are shown diagrammatically in Fig. 2. Their effect has been discussed in relation to the behaviour of magnesium ions in the $MgCl_2$–NaCl and $MgSO_4$–Na_2SO_4 systems[42]. If the supporting electrolyte, i.e. sodium chloride, penetrates inside the granules to only a limited extent, the interior of the gel is divided into two phases, as it were, distinguished by the presence or the absence of the given electrolyte (Fig. 2*A*). The degree of penetration by the electrolyte, i.e. the extent of phase (II), is determined mainly by the size of the larger ion. Since the effective ionic size increases from chloride through sodium to the sulphate ion, this phase will be smaller in the sulphate than in the chloride system (Fig. 2*B*). In a homogeneous sample–eluent system the efficiency of elution of magnesium ions will depend on the degree of penetration of the gegenion into phase (II), since the magnesium ion should be near its anion to preserve the electrical neutrality of the solution. In the chloride system the magnesium ions penetrate the gel to an extent determined by their size, since the chloride ion is smaller than the magnesium ion. In the sulphate system the penetration of magnesium ions is governed by the size of the sulphate ion, which is larger than magnesium. Thus the volume accessible to magnesium ions is smaller in the sulphate than in the chloride system. When a mixed solution of sodium chloride and sodium sulphate is used as eluent, the internal liquid phase is regarded as consisting of three regions (Fig. 2*C*), the additional phase (III) containing sodium, chloride, and sulphate ions. Since magnesium and sulphate ions interact to form complexes, penetration of the former into the gel will be determined by the relative sizes of phases (II) and (III). This may explain results obtained for the elution of magnesium chloride with solutions containing different ratios of sodium chloride and sulphate (Fig. 3).

It has already been stated that secondary effects are apparent also in the dependenee of the position and the shape of the elution curves on the concentration of the substance. The degree of asymmetry of the elution peak can be assessed by the ratio F/B,[46] where F and B are the distances from the ordinate for the peak to the ascending and descending portions of the curve measured at mid-height. For example, the effect of concentration on the elution volume and the symmetry of the peak has been investigated in the sample–eluent systems

$MgSO_4$–Na_2SO_4, $Mg(ClO_4)_2$–$NaClO_4$, $Mg(NO_3)_2$–$NaNO_3$[42], $MgCl_2$–NaCl, $SrCl_2$–NaCl, $BaCl_2$–NaCl[44].

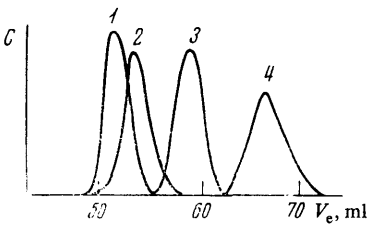


Figure 3. Variation of elution volume of Mg^{2+} ions from 0.01 M $MgCl_2$ (1 ml on Sephadez G-15 column 1.5 × 60 cm at 20–30 ml h^{-1}) with composition of eluent[42]: 1) 0.1 M Na_2SO_4; 2) 0.075 M Na_2SO_4 + 0.025 M NaCl; 3) 0.05 M Na_2SO_4 + 0.05 M NaCl; 4) 0.1 M NaCl; Here and on the other diagrams showing elution curves C denotes the relative concentration of the substance in the eluate.

Analysis of these results (Fig. 4) reveals three types of dependence of elution volume on concentration of the sample solution[44] according as $dV_e/d\lg[M]$ (i) vanishes (curves *1* and *2* at concentrations <0.1 M), (ii) <0 (curves *3*–*5* at <0.1 M and curve *6* at all concentrations investigated), and (iii) >0 (curves *1*–*5* at >0.1 M). In most cases the corresponding values of the ratio F/B were unity, <1, and >1.

These results reflect the processes occurring in the system. If a molecular-sieve mechanism alone operates, the elution curves are symmetrical, the partition coefficients are independent of concentration, and the relation $0 \leq Kd \leq 1$ is satisfied. With substances exhibiting symmetrical peaks at low concentrations and satisfying the above relation, the change in elution volume and in the shape of the peak with increase in concentration is attributed to diminished hydration of the ion and of the gel matrix with increase in the ionic strength of the solution, with a consequent increase in partition coefficient. Furthermore, the change in the degree of hydration may lead to the presence of hydrated ions of various sizes in the solution, whose elution curve will be non-Gaussian. A molecular-sieve mechanism then predominates. Asymmetry of the peaks and partition coefficients exceeding unity at low concentrations are a consequence of secondary processes.

2. Processing of Experimental Data and the Results of Gel Chromatography

Although the above secondary effects, representing an affinity for the gel phase, can sometimes be used for separation[34], the principal merit and the main use of gel chromatography consist in the relation between molecular

weight or size and the elution volume, which is possible when separation is due solely to a molecular-sieve mechanism. Various equations expressing the dependence of elution volume on molecular weight [34], "effective chain length" [83], Stokes radius [84], hydrodynamic volume [85], and molar volume [86] have been found for various substances. These equations contain parameters connected with the elution volume V_e/V_t and V_e/V_0, as well as partition coefficients, whose introduction permits comparison of results obtained with different columns and gels.

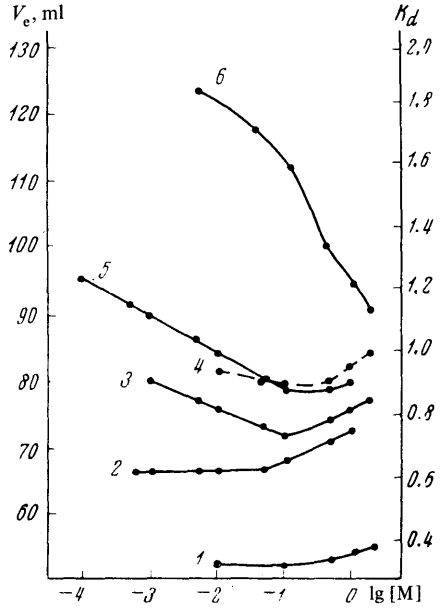


Figure 4. Dependence of elution volume and partition coefficient on sample concentration [M] (1 ml eluted at 20–30 ml h^{-1} on Sephadex G-15 column 1.5 × 60 cm with 0.1 M eluent) for the sample–eluent systems [42,44]: 1) $MgSO_4$–Na_2SO_4; 2) $MgCl_2$–NaCl; 3) $SrCl_2$–NaCl; 4) $Mg(NO_3)_2$–$NaNO_3$; 5) $BaCl_2$–NaCl; 6) $Mg(ClO_4)_2$–$NaClO_4$.

The partition coefficient characterises the behaviour of a substance in a gel, and a difference in its values for different substances may indicate differences in the mechanism of separation. From Eqn. (2) we have

$$K_d = \frac{V_e - V_0}{V_t} \qquad (3)$$

i.e. the free and internal volumes of the gel must be known in order to determine the partition coefficient. The former volume is determined by means of substances which are completely excluded from the gel and whose elution volume is $V_e = V_0$, while $K_d = 0$. A higher polysaccharide (dextran blue) and for inorganic systems a higher phosphate (e.g. Kurrol salt [53]) are most often used for this purpose.

The internal volume can be determined on the assumption that such compounds as hydrogen and sodium chlorides are uniformly distributed between the two phases, so that their elution volume $V_e = V_0 + V_i$ and $K_d = 1$. [46] The same hypothesis can be used to determine the internal volume for tritiated water THO. [53] However, comparison of the elution volumes of THO and $H_2^{18}O$ shows [87] that, owing to isotope exchange, the former has the rather larger elution volume. With the partition coefficient of the latter regarded as unity, those of tritiated water for Sephadexes G-10, G-15, and G-200 were determined as respectively 1.09, 1.06, and <1.02. [87]

In several cases the internal volume is determined by means of Eqn. (1), which gives

$$V_i = V_t - V_0 - V_m \ . \qquad (4)$$

The matrix volume of the gel is found from the specific volume of the latter (0.61). [88] The internal volume has been determined also [41] from the difference between the weights of the column containing the gel w, the empty column w_t, and the gel a transferred quantitatively from the column, washed with ethanol, and dried in a vacuum at 80°C for 6 h:

$$V_i = (w - w_t - a - \rho V_0)/\rho \qquad (5)$$

where ρ is the density of water.

In view of the presence in the gel of a region inaccessible to dissolved molecules, other workers [89] introduced the concept of an effective internal volume $(V_i)_{eff} = 0.8V_i$. It is advisable to use chloride and bromide ions—but not fluoride, iodide, sulphate, thiocyanate, copper(II), or iron(III)—for its determination.

Because of the uncertainty and difficulty of finding the internal volume, it is convenient to use the partition coefficient

$$K_{av} = \frac{V_e - V_0}{V_t - V_0} \qquad (6)$$

whose calculation depends on readily determinable quantities [80]. The relation between K_{av} and K_d is

$$K_{av} = K_d \frac{V_i}{V_i + V_m} \ . \qquad (7)$$

The difference in value between the two partition coefficients diminishes with increase in the porosity of the gel.

Partition coefficients are determined not only by the column method—i.e. under elution conditions—but also under static conditions [45,53,54]. The method is based on the postulate that the concentration of substances in the gel phase is the same as in the external liquid phase. Definite quantities of gel and solution of concentration C_1 of the substance in the eluent are stirred until equilibrium is established. After being allowed to settle, the concentration C_2 in the solution over the swollen gel is determined. We then have

$$K_d = 1 - \frac{V_0' + V_i'}{V_i'} \cdot \frac{C_2 - C_1}{C_2} \qquad (8)$$

where V_0' and V_i' are the free and internal volumes of the solvent. The sum $V_0' + V_i'$ is assumed to equal the volume of solution taken, and V_i' is determined by means of substances having $K_d = 0$.

Comparison of partition coefficients found by elution and under static conditions is used to estimate the contribution made by diffusion of the solutes to the separation [53,54]. Furthermore, the quantity $D = 100(C_1 - C_2)/C_1$ represents the contribution of adsorption, as a consequence of which $C_1 > C_2$ and $D > 0$. [45]

It was suggested[41] that the contributions of cation and anion to the partition coefficient of an ionic compound were additive:

$$K_d = \frac{s\Phi_c + t\Phi_a}{s+t} \qquad (9)$$

where t and s are the cationic and anionic charges, and Φ_c and Φ_a are the individual contributions of cation and anion to the partition coefficient. On the assumption that $\Phi_{Na} = 1$, the relative values of Φ_c and Φ_a were determined by a method similar to that used to find radii in a crystal. Good agreement was obtained with experimental partition coefficients for several salts on Bio-Gel P-2.

The use of gel chromatography to determine molecular weights, polydispersity, and molecular dimensions involves relating these characteristics with the elution parameters and employing the resulting mathematical and graphical formulae as calibration. In practice the elution volume is in most cases a linear function of the logarithm of the molecular weight. The best method for plotting a calibration straight line is to use monodisperse fractions of the test polymer, since the relation between molecular weight and molecular size may differ substantially for each type of compound. In several cases, however, calibration lines are obtained by means of standard specimens, e.g. narrow fractions of polystyrene or proteins[34].

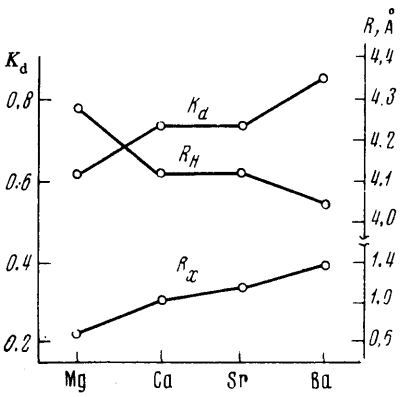


Figure 5. Comparison of partition coefficients and ionic dimensions of alkaline-earth metals[48] for 1 ml of 0.01 M solution eluted on Sephadex G-15 with 0.1 M NaCl + 0.01 M HCl at 20–30 ml h^{-1}: R_X = radius of ion in crystal; R_H = radius of hydrated ion.

The elution curve for the fractionation of a polydisperse polymer on an already calibrated column may be used to characterise its molecular-weight distribution. Analysis of the molecular weight and the quantity of each fraction obtained by preparative gel chromatography will also yield distribution curves.

Consideration of the various processes occurring during elution in a gel indicates that calibrations can be used to find ionic sizes or molecular weights when separation is due solely to a molecular-sieve mechanism. It is assumed that interactions among gel, substance, and eluent, apparent as adsorption, ionic exclusion, ion exchange, or complex formation, are absent.

The behaviour of inorganic salts in gel chromatography is in many cases consistent with a molecular-sieve mechanism. Comparison of the partition coefficients of alkaline-earth metal chlorides with the ionic radii, for example, shows (Fig. 5) that these salts are eluted in the sequence of diminishing size of the hydrated cations[48].

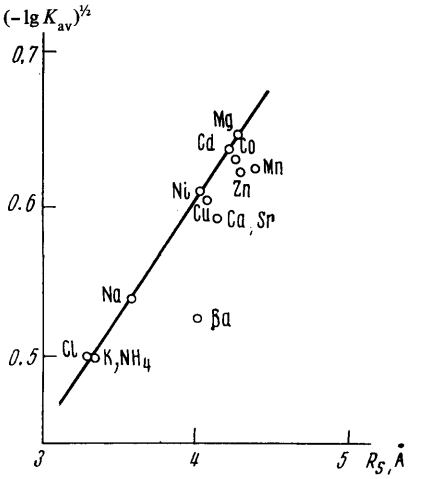


Figure 6. Relation between $(-\lg K_{av})^{1/2}$ and R_s for certain ions[43].

The model of a gel as a network of straight, rigid filaments, of infinite length and distributed at random in the gel[81], yields the relation

$$(-\lg K_{av})^{1/2} = A \cdot R_s + B \qquad (10)$$

between the partition coefficients and the radii R_s of dissolved molecules, where A and B are constants for a given gel. Fig. 6 illustrates the dependence of $(-\lg K_{av})^{1/2}$ on R_s for several ions eluted by 0.1 M potassium chloride solution on Sephadex G-15.[43] Results for ions which do not form chloride complexes and are not adsorbed on the gel lie on a straight line, which can be used as calibration for estimating the sizes of the ions.

The results given here illustrate the application of gel chromatography to the investigation of inorganic substances. It has been used to determine the sizes of various ions[43,48,72], as well as for the separation of ions[50–52] and the investigation of inorganic polymers. Information has been obtained on the properties of polysilicic acid[68], the existence of various types of polymeric molybdates[71], and the composition of $Fe(OH)_x(NO_3)_{3-x}$ polymers[69,70].

The rapid development of the application of gel chromatography to the investigation of inorganic polymers of phosphorus enables existing data to be generalised and further possibilities for the use of this method to be defined.

III. APPLICATION OF GEL CHROMATOGRAPHY TO THE INVESTIGATION OF PHOSPHATES

In order to establish rules for the gel-chromatographic behaviour of phosphates and to develop a technique, investigations were made on various individual phosphates [53–59], both linear and cyclic, and with different degrees of oxidation of the phosphorus, as well as specimens of polyphosphoric acids [58,60,61] and polyphosphates [62–67] having different molecular-weight distributions. Studies have been made of the effects of various factors on the behaviour of phosphates during chromatography—sample and eluent concentrations [55], pH, [54,55] rate of elution, temperature [57], gel porosity [55,57] and particle size [57], and the column parameters [57,58].

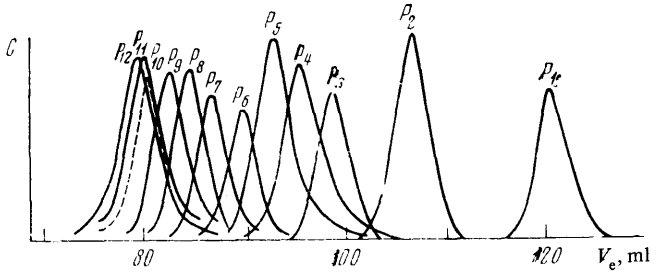


Figure 7. Elution curves for P_1-P_{12} linear polyphosphates [54] (1 ml containing 0.003–0.005 g-atom P litre^{-1} eluted on Sephadez G-25 column 1.5 × 90 cm with 0.1 M KCl of pH 7.0 at 20–30 ml h^{-1}).

Table 1. Partition coefficients of linear phosphates $Na_{n+2}P_nO_{3n+1}$ (0.003–0.005 g-atom P litre^{-1} eluted with 0.1 M KCl on Sephadex G-25).

P_n	K_d*	P_n	K_d	P_n	K_d	P_n	K_d
P_1	0.78	P_6	0.41	P_8	0.30	P_{12}	0.21
P_2	0.62	P_6	0.38	P_9	0.28	P_{13}	0.17
P_3	0.52	P_7	0.34	P_{10}	0.24		
P_4	0.46			P_{11}	0.23		

*Values of K_d for P_4-P_{13} phosphates calculated from those of K_{av}^{54}.

The concentration of phosphate in the eluate fractions was determined colorimetrically (Refs. 53–55 describe chemical analysis for phosphorus) and refractometrically with appropriate instruments, e.g. an interferometer [58] or a flow-type differential refractometer [57]. In the investigation of polyphosphoric acids, the pH of the eluate was measured by means of a pH-meter having a specially designed flow-type microcell [60,61]. The degree of polymerisation of phosphates in the eluate fractions was monitored by potentiometric titration [66,67] and by paper chromatography [58,60,65].

Many authors mention that phosphates are eluted from a gel column in the sequence of decreasing molecular weight. This relation has been found for linear phosphates containing 1–12 phosphorus atoms in the chain, eluted with 0.1 M potassium chloride solution on Sephadex G-25 [54] (Fig. 7), and for sodium ortho-, pyro-, and tripoly-phosphates on Sephadexes G-10, G-15, G-25, and G-50 [55] (Fig. 8). The partition coefficients of linear phosphates (Table 1) and of compounds containing up to four phosphorus atoms in different states of oxidation (Table 2) lie in the range $0 \leq K_d \leq 1$.

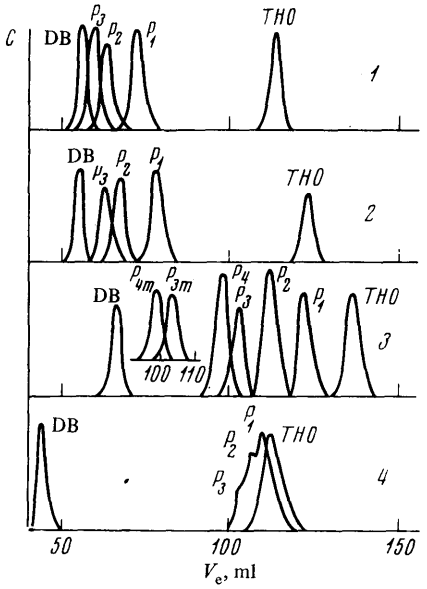


Figure 8. Elution curves of certain phosphates (1 ml of solution containing 0.002–0.005 g-atom P litre^{-1} eluted with 0.1 M KCl at 20–30 ml h^{-1}) on different Sephadexes [55]: 1) G-10 (column 1.5 × 90 cm); 2) G-15 (1.5 × 90); 3) G-25 (1.5 × 90); 4) G-50 (1.2 × 130). The peaks correspond to: P_1-P_4) polyphosphates containing a chain of 1–4 phosphorus atoms; P_{3m}) Na trimetaphosphate; P_{4m}) Na tetrametaphosphate; DB) dextran blue; THO) tritiated water.

Table 2. Effect of eluent concentration on partition coefficient K_d of phosphates [55]. (0.002–0.005 g-atom P litre^{-1} on Sephadex G-25).

Phosphate		Eluent			
formula	notation*	water	KCl solution		
			0.1 M	0.5 M	1.0 M
$NaPH_2O_3 \cdot H_2O$	P^1	—	0.80	0.83	0.83
$Na_2PHO_3 \cdot 5H_2O$	P^3	—	0.78	0.83	0.84
$NaH_2PO_4 \cdot 2H_2O$	P_1	0.36	0.78	0.83	0.84
$Na_3P_2HO_5 \cdot 12H_2O$	P^2-P^4	—	0.64	0.74	0.76
$Na_2H_2P_2O_6 \cdot 6H_2O$	P^4-P^4	—	0.67	0.77	0.79
$Na_3P_3H_2O_8$	P^3-O-P^3	—	0.59	0.66	0.69
$Na_3P_3HO_8 \cdot 4H_2O$	P^3-O-P^5	—	0.61	0.70	0.73
$Na_4P_2O_7 \cdot 10H_2O$	P_2	0.30	0.62	0.71	0.75
$Na_5P_3O_8 \cdot 14H_2O$	$P^4-P^3-P^4$	—	0.61	0.73	0.76
$Na_4P_3HO_8 \cdot H_2O$	$P^3-O-P^4-P^4$	—	0.50	0.62	0.70
$(NH_4)_5P_3O_9 \cdot xH_2O$	$P^5-O-P^4-P^4$	—	0.51	0.67	0.70
$Na_5P_3O_{10} \cdot 6H_2O$	P_3	0.21	0.52	0.62	0.69
$Na_3P_3O_9 \cdot 6H_2O$	P_{3m}	—	0.52	0.67	0.70
$Na_6P_4O_{11} \cdot xH_2O$	$P^4-P^4-O-P^4-P^4$	—	0.46	0.62	0.64
$Na_6P_4O_{10} \cdot 4H_2O$	$(-P^4-P^4-O-)_2$	—	0.48	0.62	0.65
$Na_4P_4O_{12} \cdot 4H_2O$	P_{4m}	—	0.46	0.60	0.65

*The superscript indicates the state of oxidation of the phosphorus, and the subscript the degree of polymerisation of the polyphosphate or metaphosphate (n_i).

The above results—the elution sequence, partition coefficients, and the symmetry of the peaks—are consistent with ideas on the sizes of polyphosphate anions and indicate that the mechanism of separation is based solely on a molecular-sieve effect.

Cyclic tri- and tetra-metaphosphates are eluted on Sephadex G-25 together with linear phosphates[55] (Fig. 8) according to their size.

Investigation of the dependence of the gel-chromatographic behaviour of polymeric phosphates on various factors has enabled important problems of technique to be solved and the most suitable parameters to be selected, whose use ensures the necessary conditions for a molecular-sieve mechanism to operate and for condensed phosphates to be analysed by means of calibration curves.

The availability of gels differing in porosity makes possible the analysis of polymers over a wide range of molecular weights. In the case of organic compounds limits have been found to the molecular weights, which are characteristic of each type of gel[34]. These limits may be different for inorganic substances, since other factors may then become more significant—ionic exclusion and hydration, and with phosphates the properties resulting from their polyelectrolyte nature.

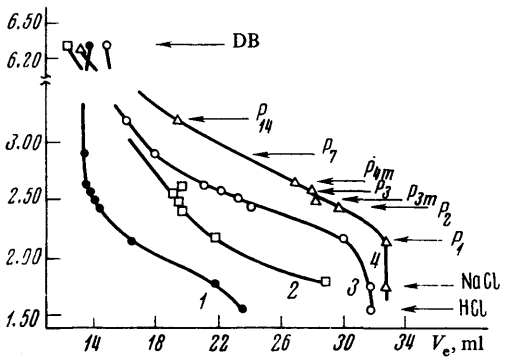


Figure 9. Dependence of relation between logarithm of molecular weight M [of phosphate] (eluted with 0.01 M NaCl) on gel porosity for columns 0.61–0.62 cm in diameter and 120–126 cm in height[57] of: 1) Sephadex G-10; 2) Bio-Gel P-2; 3) Sephadex G-25 (eluent 0.025 M Na_2HPO_4); 4) Sephadex G-50.

When an inhomogeneous sample of sodium polyphosphate of mean degree of polymerisation $\bar{n} = 14$ was eluted on Sephadexes G-25 and G-50, the partition coefficients of the component polyphosphates were closely similar and approached zero on Sephadex G-25, but distribution occurred throughout the accessible volume on G-50.[57] However, other factors—nature and concentration of eluent, quantity of sample solution—were not constant in this comparison. The shift in elution volumes and the difference in quality of separation characteristic of gels of different porosities are well illustrated by Figs. 8 and 9,[55,57] which show that the most satisfactory separation of oligophosphates is attained on Sephadex G-25.

The eluent may have a significant effect on the distribution of inorganic substances during elution. The influence of the supporting electrolyte on the separation of phosphates has also been studied. The partition coefficients of ortho-, pyro-, and tripoly-phosphates eluted with water on Sephadex G-25 are extremely low compared with the values obtained when potassium chloride solutions are used as eluent[55] (Table 2). The low values were explained by ionic exclusion, which is eliminated by the addition of potassium chloride to the eluent, for which purpose an electrolyte concentration <0.01 M was sufficient. The partition coefficients for anions with different states of oxidation of phosphorus increased slightly with the potassium chloride concentration (Table 2). Several explanations are possible for this effect: since polyphosphates possess the properties of polyelectrolytes, the size of their polyanions should diminish with increase in the ionic strength of the solution; another possible cause would be a decrease in the degree of hydration of the anions; furthermore, preliminary experiments showed[55] that the internal volume of Sephadex G-10 increased with the potassium chloride concentration of the eluent.

Examination of the behaviour of alkaline-earth metal chlorides in 0.1 M NaCl and 0.1 M NaCl + 0.01 M HCl showed[48] that the acid eluent gave the best separation. Such an eluent is undesirable with phosphates, owing to the increased probability of hydrolysis. The effect of the pH of the eluent on the partition coefficients was therefore examined. With linear phosphates[54] no appreciable changes in partition coefficients were observed with eluents having pH 2.0, 4.6, 7.0, and 9.2, obtained by adding respectively aqueous hydrochloric acid and phthalate, tris, and ammoniacal buffers to 0.1 M potassium chloride solution. Similar results were obtained in the separation of anions in which phosphorus was present in different states of oxidation[55]. Only on the addition of borate buffers of pH 7.0 and 9.2 were the partition coefficients lower, owing to electrostatic repulsion of phosphate ions and borate ions retained by the gel, which were able to interact with dextran depending on the pH.[90] The results confirmed yet again the need for careful choice of eluent if molecules are to be separated by a molecular-sieve effect.

Table 3. Dependence of partition coefficients K_d on concentration of sample[55] (on Sephadex G-25 eluted with 0.1 M KCl).

Phosphate*	Concentration, g-atom P litre^{-1}						
	0,001	0,005	0,01	0,05	0,1	0,5	1,0
P^3	0.78	0.78	0.78	—	0.79	—	0.80
P_1	0.78	0.79	0.79	—	0.80	—	0.80
P^4—P^4	0.66	0.66	0.66	0.69	0.71	—	—
P_2	0.62	0.63	0.63	—	0.69	—	—
P_3	0.52	0.50	0.51	0.55	0.57	0.66	0.70
P_{3m}	0.52	0.52	0.52	—	0.58	—	0.69
P^4—P^4—O—P^4—P^4	0.46	0.46	0.46	—	0.50	—	0.66
(—P^4—P^4—O—)$_2$	0.48	0.48	0.47	—	—	—	—
P_{4m}	0.46	0.46	0.45	—	0.50	—	0.63

* Notation as in Table 2.

In order to confirm the molecular-sieve mechanism it is necessary to determine the effect of the concentration of the test substances on their partition coefficients and the symmetry of the peaks. Table 3 lists values of K_d for certain phosphorus-containing compounds as a function of their concentration.

Fig. 10 illustrates the concentration dependence of the position and form of the elution peaks for sodium tripolyphosphate. With a sample concentration below 0.01 g-atom P litre^{-1} the elution curves are symmetrical, and the position of the peak remains unchanged. At higher concentrations the curves are displaced towards large volumes, and the peaks become unsymmetrical with $F/B > 1$. This was attributed to the presence of phosphate ions having different degrees of hydration. Furthermore, increase in the concentration of the polyphosphate is accompanied by a decrease in its dissociation[91], which with polyelectrolytes leads to a decrease in size of the polyanions. Similar results were obtained[57] for the effect of concentration on the elution parameters of sodium ortho-, pyro-, and tripoly-phosphates.

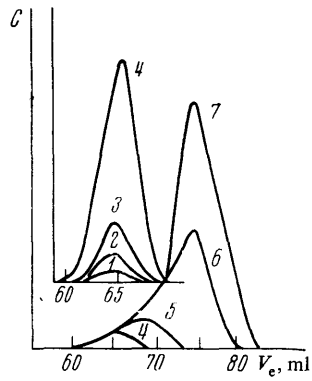


Figure 10. Dependence of elution curves for 1 ml of $Na_5P_3O_{10}$ (1.5 × 60 cm column of Sephadex G-25 eluted with 0.1 M KCl at 20–30 ml h^{-1}) on initial concentration[55] (g-atom P litre^{-1}): 1) 0.001; 2) 0.005; 3) 0.01; 4) 0.05; 5) 0.1; 6) 0.5; 7) 1.0.

Many authors state that the process becomes more efficient the longer the column, and this holds also for phosphates. The use of longer columns leads to a larger difference between the elution volumes of neighbouring polymer homologues and to better resolution of the peaks[58], but is accompanied by broadening of the curves, which has an adverse effect. Columns of height 60 and 500 cm give the same partition coefficients in the chromatography of a mixture of sodium ortho-, pyro-, and tripoly-phosphates on Sephadex G-25 with 0.01 M sodium chloride solution. The dependence of the elution of phosphates on the column parameters is obviously general in character.

In order to find the best conditions for the separation of substances a study was made[57] of the effect of various parameters on the resolution R of the peaks, defined by the equation

$$R = \frac{2(V_{e_2} - V_{e_1})}{w_{b_1} - w_{b_2}} \qquad (11)$$

in which the V_e are the elution volumes of adjacent peaks, and the w_b are their widths at the base. The resolution of sodium orthophosphate and tripolyphosphate is somewhat improved (Table 4) on decreasing the rate of elution, as a consequence of the diminished spreading of the bands. Raising the temperature has the opposite effect.

Completing our review of the different factors influencing the gel chromatography of phosphates and the techniques used, we can conclude that, within the limits investigated and allowing for actual applications, all the experimental conditions can be used for the gel chromatography of phosphates for analytical and preparative purposes.

Table 4. Effect of various factors on resolution R of $Na_5P_3O_{10}$ and Na_2HPO_4 peaks[57].

Experimental conditions		R
constant	variable	
Column dimensions, cm	Gel type	
diameter 0.61–0.62	Sephadex G-10	1.09
height 123.0–125.0	Sephadex G-25	1.47
	Sephadex G-50	1.35
	Bis-Gel P-2	0.87
Sephadex G-10 column, cm	Gel particle diameter, μm	
diameter 0.61	53–74	1.09
height 124.0–126.0	88–105	0.84
Sephadex G-25 (53–64 μm) (0.61 × 63.0 cm)	Rate of elution, ml min^{-1}	
temperature 22.0°C	0.16	1.69
	0.58	1.26
rate of elution 0.58 ml min^{-1}	22.0°C	1.26
	50.0°C	1.17

As mentioned earlier, various processes occurring in a gel as a result of interactions among the gel, test substance, and eluent affect the elution volumes (or partition coefficients), the elution sequence, the shape of the peak, and the dependence of these factors on the concentration of the substance. Investigations on phosphate polymers have shown that phosphates are eluted in the sequence of diminishing molecular weight, and that their partition coefficients in various gels lie in the range 0–1 and are constant with symmetrical curves over a certain concentration range. These results indicate the absence of interaction between phosphates and dextran. Furthermore, the identity of partition coefficients found under elution conditions and under static conditions indicates the absence of a diffusion contribution[53,54].

All the above facts indicate that, under certain conditions, the behaviour of phosphates on dextran gels is consistent with a molecular-sieve mechanism. It is therefore possible to use the method to separate and examine condensed phosphates, and important results in this field have already been obtained.

Firstly, it is now possible to obtain individual phosphates. When a solution of sodium polyphosphates having $\bar{n} = 3$, was fractionated on Sephadex G-15, components of a mixture with $n = 2–5$ were obtained in fairly pure form, isolated from the solution, and identified by paper chromatography and infrared spectroscopy.

Another important achievement of the application of gel chromatography to the investigation of phosphorus polymers is determination of the effective radii of many phosphate anions[54,55]. The radii were determined by means of a calibration line plotted from values of K_{av} and K_s for several ions, analogous to that shown in Fig. 6. The partition coefficients of the phosphorus-containing anions were calculated by means of Eqns. (6) and (7) with $V_i/(V_i + V_m) = 0.862$ for Sephadex G-25. The results show (Table 5) that these anions are considerably hydrated in aqueous solution, and that their size increases with the degree of polymerisation.

Finally, gel chromatography has been successfully used to determine the molecular-weight composition of a mixture of polyphosphates. A sample of sodium polyphosphates having $\bar{n} = 36$ was fractionated on Molselekt G-25,[66] the molecular weight of the polyphosphates in the eluate was determined by potentiometric end-group titration[11], and molecular-weight distribution curves were plotted from the fractionation results (Fig. 11). The reliability of the fractionation results is confirmed by the equality of the mean degree of polymerisation of the initial specimen and the sum of the products of the mole fraction x_i and the degree of polymerisation n_i of the ith fraction:

$$\bar{n} = \Sigma x_i n_i \quad . \qquad (12)$$

Table 5. Anionic radii R_S of phosphates[54,55] obtained from $(-\lg K_{av})^{1/3} = A \cdot R_s + B$

Phosphate*	R_S, Å	Phosphate	R_S, Å	Phosphate	R_S, Å
P^1	4.3	$P^4—P^3—P^4$	5.7	P_5	7.2
P^3	4.4	$P^3—O—P^4—P^4$	6.6	P_6	7.6
P_1	4.4	$P^5—O—P^4—P^4$	6.4	P_7	8.0
$P^2—P^4$	5.5	P_3	6.4	P_8	8.3
$P^4—P^4$	5.5	P_{3m}	6.4	P_9	8.6
$P^3—O—P^3$	5.8	$P^4—P^4—O—P^4—P^4$	6.8	P_{10}	9.0
$P^5—O—P^3$	5.7	$(—P^4—P^4—O—)_2$	6.8	P_{11}	9.2
P_2	5.7	P_4	6.9	P_{12}	9.3
		P_{4m}	6.9	P_{13}	9.8

*For notation see Tables 1 and 2.

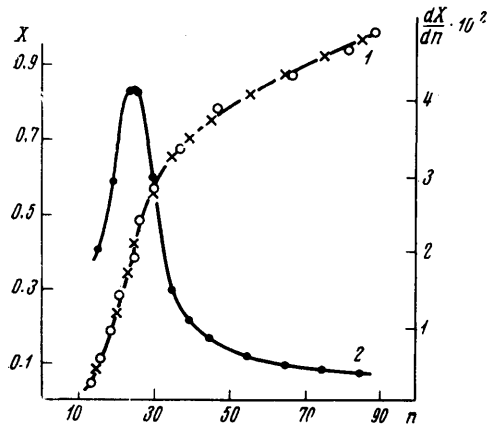


Figure 11. Numerical distribution curves for sodium polyphosphate with $\bar{n} = 36$ (0.5 g in 5 ml eluted on Molselekt G-25 column 1.3 × 80 cm with water at 26 ml h^{-1}):[66] 1) integral; 2) differential; ×) points chosen for graphical differentiation.

For the same purpose gel chromatography has been used to analyse a mixture of polyphosphates on Sephadex G-100.[67] Such investigations require a considerable quantity of the specimen, exceeding that at which the partition coefficient is still independent of concentration. Although a linear relation $V_e = k \lg M$ was obtained in these investigations, it can hardly be regarded as a calibration.

The calibration line (Fig. 12) for phosphates containing up to thirteen phosphorus atoms in the chain, determined by the use of individual phosphates obtained by ion-exchange chromatography[21], merits close attention in connection with the application of calibrated columns. Its possibility for calibration purposes was verified[54] by first determining by potentiometric titration the mean degree of polymerisation of a few polyphosphate specimens, and then chromatographing them on Sephadex G-25 with 0.1 M potassium chloride solution of pH 7.0. The concentration of the polyphosphates in each fraction was determined and also, from the calibration line, the degree of polymerisation, from which $\bar{n}$ in the initial specimen was calculated by a slightly modified form of Eqn. (12)

$$\bar{n} = \frac{\Sigma w_i}{\Sigma (w_i/n_i)} \qquad (13)$$

where w_i is the number of phosphorus atoms in the ith fraction. The good agreement between results obtained by the two methods showed the possibility of using this calibration (Table 6).

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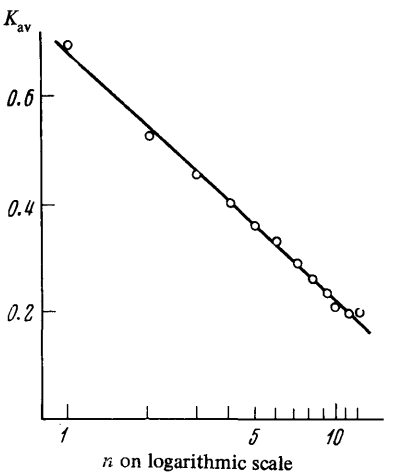


Figure 12. Relation between K_{av} and $\lg n$ for elution on Sephadex G-25 with 0.1 M KCl (pH 7.0) at 20–30 ml h^{-1}.[54]

Table 6. Comparison of mean degrees of polymerisation $\bar{n}$ of sodium polyphosphates obtained by gel chromatography and by potentiometric titration[54].

Method	$\bar{n}$					
Gel chromatography	3.2	8.4	9.3	9.5	12.0	14.0
Potentiometric titration	3.0	8.2	9.1	10.1	11.0	13.1

Gel chromatography has opened up new possibilities for the study of condensed phosphates. The achievements of the method, which was developed mainly for organic

chemistry, biochemistry, and clinical medicine, have proved useful for investigating inorganic phosphate polymers. The gel chromatography of phosphates has in turn confirmed several aspects of the method and has helped to expand its field of application.

REFERENCES

1. U.P.Strauss, E.H.Smith, and P.L.Wineman, J.Amer.Chem.Soc., 75, 3935 (1953).
2. T.Tsubaki, M.Hattori, and M.Tanaka, Kolloid Z., 225, 112 (1968).
3. L.N.Reutovich, E.A.Fomina, V.Ya.Rotanina, L.A.Leont'eva, and Yu.I.Gerzhberg, Trudy LenNIIGiprokhima, No.4, 72 (1971).
4. P.di Bello and A.M.Pradel, Interceram., 17, 232 (1968).
5. O.A.Pirogov, L.A.Babkina, M.I.Kuz'menkov, V.V.Pechkovskii, and G.Kh.Cherches, Ogneupory, No.5, 55 (1973).
6. R.Pfanstiel and R.K.Iler, J.Amer.Chem.Soc., 74, 6059 (1952).
7. O.Lamm and H.Malmgren, Z.anorg.Chem., 245, 103 (1940).
8. O.Lamm and H.Malmgren, Z.anorg.Chem., 252, 256 (1944).
9. J.R.Van Wazer, J.Amer.Chem.Soc., 72, 906 (1950).
10. J.R.Van Wazer and K.A.Holst, J.Amer.Chem.Soc., 72, 639 (1950).
11. J.R.Van Wazer, E.J.Griffith, and J.E.McCulogh, Analyt.Chem., 26, 1755 (1954).
12. L.V.Kubasova, G.G.Aleksandrova, and T.D.Pozharskaya, "Khimiya i Tekhnologiya Kondensirovannykh Fosfatov" (Chemistry and Technology of Condensed Phosphates), Nauka, Alma-Ata, 1970, p.37.
13. P.Nylen, Z.anorg.Chem., 229, 30 (1936).
14. P.Bonneman, Compt.rend., 204, 865 (1937).
15. A.Boulle and R.Jary, Compt.rend., 235, 1029 (1952).
16. J.Ebel, Bull.Soc.chim.France, 20, 991 (1953).
17. A.E.R.Westman and J.Growther, J.Amer.Ceram. Soc., 37, 420 (1954).
18. E.Karl-Kroupa, Analyt.Chem., 28, 1091 (1956).
19. R.F.Jameson, J.Chem.Soc., 752 (1959).
20. L.M.Ukrainskaya and L.I.Kuznetsov-Fetisov, Izv. Vys.Ucheb.Zaved., Khim.i Khim.Tekhnol., 13, 1694 (1970).
21. S.Ohashi, N.Tsuji, Y.Ueno, M.Takeshita, and M.Muto, J.Chromatog., 50, 349 (1970).
22. T.Rössel, Z.analyt.Chem., 197, 333 (1963).
23. T.Rössel and H.Kiesslich, Z.analyt.Chem., 229, 96 (1967).
24. T.Iida and T.Yamabe, J.Chromatog., 41, 163 (1969).
25. B.Sansoni and R.Klement, Angew.Chem., 65, 422 (1953).
26. B.Sansoni and L.Baumgartner, Z.analyt.Chem., 158, 241 (1957).
27. T.R.Sato, Analyt.Chem., 31, 841 (1959).
28. L.T.Jones, Ind.Eng.Chem., Analyt., 14, 536 (1947).
29. J.R.Van Wazer, J.Amer.Chem.Soc., 72, 647 (1950).
30. W.Dewald and H.Schmidt, Z.anorg.Chem., 272, 253 (1953).
31. J.Porath and P.Flodin, Nature, 183, 1657 (1959).
32. A.A.Lur'e, "Sorbenty i Khromatograficheskie Nositeli" (Sorbents and Chromatographic Supports), Khimiya, Moscow, 1972.
33. S.B.Makarova and E.V.Egorov, Khim.Prom., 344 (1972).
34. H.Determann, "Gel-Chromatographie" (Translated into Russian), Mir, Moscow, 1970.
35. K.H.Oltgelt and D.Moore, "Fractionation of Polymers" (Translated into Russian), Mir, Moscow, 1971, p.110.
36. B.G.Belen'kii, L.Z.Vilenchik, and D.D.Novikov, in "Novoe v Metodakh Issledovaniya Polimerov" (Advances in Methods for the Investigation of Polymers), Mir, Moscow, 1968, p.81.
37. K.H.Oltgelt, Adv.Chromatog., 7, 3 (1968).
38. H.Determann, Adv.Chromatog., 8, 3 (1969).
39. J.F.Johnson and R.S.Porter, Progr.Polymer Sci., 2, 201 (1970).
40. D.M.W.Anderson, I.C.M.Dea, and A.Hendrie, Talanta, 18, 365 (1971).
41. D.Saunders and R.Pecsok, Analyt.Chem., 40, 44 (1968).
42. T.Ogata, N.Yoza, and S.Ohashi, J.Chromatog., 58, 267 (1971).
43. Y.Ueno, N.Yoza, and S.Ohashi, J.Chromatog., 52, 321 (1970).
44. N.Yoza, T.Ogata, and S.Ohashi, J.Chromatog., 52, 329 (1970).
45. T.Tarutani and M.Watanabe, J.Chromatog., 75, 169 (1973).
46. P.A.Neddermeyer and L.B.Rogers, Analyt.Chem., 40, 755 (1968).
47. D.Eaker and J.Porath, Separation Sci., 2, 507 (1967).
48. N.Yoza and S.Ohashi, J.Chromatog., 41, 429 (1969).
49. G.Wormser, Compt.rend.C, 263, 805 (1966).
50. B.Z.Egan, J.Chromatog., 34, 382 (1968).
51. H.Ortner and H.Spitzy, Z.analyt.Chem., 238, 167 (1968).
52. H.Ortner and H.Spitzy, Z.analyt.Chem., 238, 251 (1968).
53. S.Ohashi, N.Yoza, and Y.Ueno, J.Chromatog., 24, 300 (1966).
54. Y.Ueno, N.Yoza, and S.Ohashi, J.Chromatog., 52, 481 (1970).
55. Y.Ueno, N.Yoza, and S.Ohashi, J.Chromatog., 52, 469 (1970).
56. G.Kura and S.Ohasi, J.Chromatog., 56, 111 (1971).
57. P.A.Neddermeyer and L.B.Rogers, Analyt.Chem., 41, 94 (1969).
58. V.V.Pechkovskii, A.S.Shul'man, M.I.Kuz'menkov, and G.Kh.Cherches, Neorg.Materialy, 6, 1984 (1970).
59. K.Ujimoto, T.Nakamura, H.Asada, N.Yoza, Y.Takashima, and S.Ohashi, J.Inorg.Nuclear Chem., 32, 3177 (1970).
60. V.V.Pechkovskii, A.S.Shul'man, M.I.Kuz'menkov, G.Kh.Cherches, and S.V.Plyshevskii, Izv.Vys. Ucheb.Zaved., Khim.i Khim.Tekhnol., 14, 538 (1971).
61. V.V.Pechkovskii, A.S.Shul'man, M.I.Kuz'menkov, S.V.Plyshevskii, and G.Kh.Cherches, Tekh. Ekonom.Inform., Ser."Metody Analiza Kontrolya Proizvodstva v Khim.Prom." (NIITEKhIM), No.6, 3 (1970).
62. K.J.Bombaugh, W.A.Dark, and K.N.King, J.Polymer Sci.C, 21, 131 (1968).
63. V.V.Pechkovskii, A.S.Shul'man, A.D.Alekseev, and L.N.Shchegrov, Neorg.Materialy, 5, 2168 (1969).

64. V.V. Pechkovskii, A.S. Shul'man, and L.N. Shchegrov, Dokl. Akad. Nauk Beloruss. SSR, **14**, 144 (1970).
65. V.V. Pechkovskii, M.I. Kuz'menkov, A.S. Shul'man, S.S. Gusev, G.Kh. Cherches, T.I. Barannikova, and S.P. Martynchik, "Issledovaniya v Oblasti Neorganicheskoi Tekhnologii. Soli, Okisly, Kisloty" (Researches on Inorganic Technology—Salts, Oxides, Acids), Nauka, Leningrad, 1972, p. 87.
66. V.V. Pechkovskii, M.I. Kuz'menkov, and G.Kh. Cherches, Neorg. Materialy, **9**, 105 (1973).
67. S. Felter, G. Dirheimer, and J.P. Ebel, J. Chromatog., **35**, 207 (1968).
68. T. Tarutani, J. Chromatog., **50**, 523 (1970).
69. T.G. Spiro, S.E. Allerton, J. Renner, A. Terzis, R. Bels, and P. Saltman, J. Amer. Chem. Soc., **88**, 2721 (1966).
70. R.A. Henry and L.B. Rogers, Separation Sci., **3**, 11 (1968).
71. C.A. Streuli and L.B. Rogers, Analyt. Chem., **40**, 653 (1968).
72. N. Yoza, T. Ogata, Y. Ueno, and S. Ohashi, Bull. Chem. Soc. Japan, **41**, 2550 (1968).
73. T. Deguchi, Nippon Kagaku Zasshi, **92**, A26, 458 (1971); Chem. Abs., **75**, 83780b (1971).
74. Y. Matsumoto, M. Shirai, and H. Saito, Bull. Chem. Soc. Japan, **41**, 2542 (1968).
75. N. Yoza, H. Matsumoto, and S. Ohashi, Analyt. Chim. Acta, **54**, 538 (1971).
76. N. Yoza, Kagaku-no Reiki, **25**, 1041 (1971); Ref. Zhur. Khim., 7G11 (1972).
77. N. Yoza, Bunseki Kagaku, **20**, 505 (1971); Chem. Abs., **75**, 70892 (1971).
78. J. Porath, Pure Appl. Chem., **6**, 233 (1963).
79. P.G. Squire, Arch. Biochem. Biophys., **107**, 471 (1964).
80. T.G. Laurent and J. Killander, J. Chromatog., **14**, 317 (1964).
81. B. Gelotte, J. Chromatog., **3**, 330 (1960).
82. E.R. Nightingale, J. Phys. Chem., **63**, 1381 (1959).
83. J.G. Hendrickson and J.C. Moore, J. Polymer Sci. A-1, **4**, 167 (1966).
84. J.K. Ackers, Biochemistry, **3**, 723 (1964).
85. Z. Grubisec, P. Rempp, and H. Benoit, J. Polymer Sci. B, **5**, 753 (1967).
86. W.B. Smith and A. Kollmansberger, J. Phys. Chem., **69**, 4157 (1965).
87. N.V.B. Marsden, J. Chromatog., **58**, 304 (1971).
88. K.A. Granath, J. Colloid Sci., **13**, 308 (1958).
89. H.J. Zeitler and E. Stadler, J. Chromatog., 74, 59 (1972).
90. B. Lindqvist, Acta Chem. Scand., **16**, 1794 (1962).
91. C.F. Callis, J.R. Van Wazer, and P.G. Arvan, Chem. Rev., **54**, 777 (1954).

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