Single-column ion chromatography of linear polyphosphates[1]

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Abstract

The retention characteristics of condensed linear phosphates, P_2 to P_{13}, on a strongly basic quaternary amine anion exchanger were studied. A single-column chromatographic system with indirect UV detection was tested, dependencies of the polyphosphates' retention on the concentrations of pyromellitic acid and EDTA in the mobile phase and on the pH of the eluent were investigated. A linear relationship between the logarithm of the capacity ratios and the number of phosphorus atoms in a phosphate molecule was found, which can be used for identification of long-chain linear polyphosphates, as standards are not available. Mixtures of glassy sodium–potassium condensed phosphates were separated under optimum isocratic conditions.

Keywords: Polyphosphates; Pyromellitic acid

1. Introduction

Chromatographic techniques are widely used for the analysis of condensed phosphates. Paper and thin-layer chromatography were applied in the past [1,2], but high-performance liquid column chromatography is preferred at present. This method is useful for the separation, identification and quantification of components of polyphosphate mixtures containing both cyclic and linear condensed phosphates. It is useful for the characterization of these products to estimate the average chain length ($\bar{n}$) or to get a fingerprint for a qualitative description of the sample. Various separation and detection modes have been applied for linear phosphate analyses in ion

chromatography (IC). Glassy phosphate mixtures were fractionated by gel permeation chromatography (GPC) on Sephadex G columns [3,4] and GPC was used for the characterization of Graham's and Kurrol's salts [5]. It is possible to separate ortho- to triphosphate and cyclotriphosphate on a strongly acidic cation exchanger, in the H^+ form, on the basis of the ion-exclusion principle [6]. Ion-pair chromatography (IPC) systems for polyphosphate identification and determination have been described. Octadecylsilica and styrene–divinylbenzene copolymer columns have been used for the separation of neutralized polyphosphoric acids; both isocratic and gradient elution modes are suitable for these purposes [7]. Short-chain condensed phosphates in detergents have been determined by IPC [8]. Ion-exchange chromatography (IEC) is the most recommended method for the analysis of polyphosphates. Various separation and detection systems have been tested. The stationary phase consists of a

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polystyrene or ethylvinylbenzene resin, copoly-merized with divinylbenzene and modified with ion-exchange groups, the aqueous mobile phases contain salts of mono-, di- or tribasic inorganic or organic acids and modifiers and the pH of the eluent is adjusted to the optimum value. Dionex systems with conductivity detection make use of sodium hydrox-ide solutions as mobile phases. Several anion-ex-change columns are recommended for polyphosphate separations: Hamilton PRP-1 [7,8] and PRP-X 100 [9], HPIC-AS 7 [10–12], HPIC-AS 5 [13], HPIC-AS 4A [14], PAX 100 [15] and Ion Pac AS 11 [16] (all from Dionex), TSK-gel IEX 220 SA [17,18] and TSK-gel SAX [19,20]. Three detection modes are most frequently used for the IC of polyvalent ions including condensed phosphates, namely, direct photometric detection combined with post-column derivatization, indirect photometric detection (IPD) and conductivity detection. In the case of photo-metric detection and post-column derivatization, polyphosphates, after passing through the separation column, are hydrolysed to orthophosphates, forming coloured complexes with an appropriate reagent. Iron(III) complex is detectable in the wavelength range 310–330 nm [9–11], molybdovanadate reagent forms a yellow complex absorbing at 410 nm [12,19,21,22], molybdate and orthophosphate ions react to yield the molybdenum blue complex, which is detected at 830 nm [17,18,20,23–26]. Indirect photometric detection is universally applicable for inorganic anions including polyphosphates. Prefer-ably, sodium naphthalenetrisulfonate [9] and trime-sate [27] are used as eluents. The conductivity detection in suppressed [13,15,16] or non-suppressed mode [9] and other detection modes, such as flame photometric [8], refractometric [28] and inductively coupled plasma atomic emission spectroscopic de-tection [7], were used for the IC of polyphosphates.

This paper describes the retention behaviour of linear polyphosphate homologues (P_2 to P_{13}) on a macroporous hydroxyethyl methacrylate-based low capacity anion exchanger in pyromellitic acid (PA) solutions (as the mobile phases). Using a PA eluent, these multivalent anions can be separated in about 30 min by isocratic elution mode and then detected by IPD. Until now, this detection mode was used only for P_2–P_4 oligomers, with trimesate [27] and naph-thalenetrisulfonate [9] as eluents.

2. Experimental

2.1. Apparatus

The equipment used included a HPP 5001 linear pump, a LCI 30 injector with 0.5 and 20 μl loops, a LCD 2040 UV–Vis variable-wavelength detector operated at $\lambda = 270$–330 nm, a CI-105 integrator and a TZ 4620 two-channel linear recorder, all from Laboratory Instruments (Prague, Czech Republic). Separation and guard glass columns (150×3 mm I.D. and 30×3 mm I.D., respectively; Separon HEMA 1000-S Q-L from Tessek, Prague, Czech Republic) were used, with a nominal particle size of 10 μm, an ion-exchange capacity of 0.1 mmol/g and an exclu-sion limit of M_r $800 \cdot 10^3$–$2 \cdot 10^6$. This rigid, macro-porous sorbent, with strongly basic quaternary am-monium functional groups (a hydrophilic matrix is synthesized by suspension copolymerization of 2-hydroxyethyl methacrylate and ethylene dimethacry-late), is chemically stable in the pH range 2–12 [29]. The void volume of the analytical column ($V_0 = 0.88$ ml) was determined as the elution volume of ^2H_2O measured with the aid of a differential refractometer (RIDK 101, Laboratory Instruments). Polyphosphate samples were characterized by ^{31}P-NMR using a Brooker AMX-360 spectrometer; infrared (IR) spec-tra were measured with a Perkin-Elmer DE-684 model and the number-average chain length of polyphosphate mixtures ($\bar{n}$) was determined using a titration procedure [31].

2.2. Chemicals

The mobile phases were prepared by dissolving the calculated amount of pyromellitic acid (Riedel-de Haën, Seelze, Germany) and the disodium salt of ethylenediaminetetraacetic acid (EDTA; Lachema, Brno, Czech Republic) in demineralized water, purified by filtration in a Milli-Q+ system (Milli-pore, Bedford, MA, USA) and adjusting the pH to required value by the addition of a few drops of sodium hydroxide solution.

The samples of pyrophosphate and triphosphate were obtained from Fosfa (Břeclav, Czech Republic) and long-chain linear polyphosphates of P_4–P_{13} were prepared in our laboratory [30] and analysed by

^{31}P-NMR and IR spectroscopy. Linear polyphosphates were determined to be the main components of the products. They may contain only very small concentrations of cyclic metaphosphates, as no corresponding elution peaks of cyclics were identified on chromatograms of prepared phosphate glasses. Aqueous solutions of phosphates were injected onto the separation column.

2.3. Procedure

The retention volumes, V_R, of polyphosphates in mobile phases with various component concentrations and pH values were measured at a flow-rate of 0.4 ml/min and the capacity ratios, $k' = (V_R - V_0)/V_0$, were calculated.

3. Results and discussion

3.1. Effect of the concentration of pyromellitic acid on the retention of polyphosphates

The retention of linear polyphosphates decreases with increasing concentration of PA in the mobile phase at constant pH and EDTA content in the eluents. The logarithmic dependencies of the capacity ratios, k', on the PA concentration in the eluent, c_{PA}, are linear in the range studied with correlation coefficients of $r < 0.999$, the values of straight line slopes differ, however, from theoretical ones, corresponding to the relationship

$$\log k' = -x/y \log c_{PA} + \text{const.} \qquad (1)$$

where x and y are the charges of the polyphosphate and PA anion, respectively (Table 1). A possible explanation of the deviations of the observed slope values from the experimental ones consists of non-stoichiometric interaction between the polyphosphate or eluent anions and the ion-exchange groups of the sorbent. A similar conclusion was arrived at by Shamsi and Danielson [9] for naphthalenetrisulfonate eluent and P_2-P_4 homologues.

3.2. Effect of EDTA concentration on polyphosphate retention

The use of PA solutions containing EDTA anions as the eluent resulted in excellent separations of linear condensed phosphates, whereas ill-resolved and irreproducible chromatograms were obtained when PA solutions without EDTA anions were used as the mobile phase. EDTA in the eluent significantly improved the symmetry of elution curves and reduced peak-tailing. Similar behaviour was found previously for ion-exchange chromatography of polyphosphates in potassium [12,18] or sodium [23–25,32,33] chloride eluents with or without EDTA sodium salts. Trimesate mobile phases with EDTA were also recommended [34]. The positive effect of the EDTA in the mobile phase consists of masking heavy-metal ions, especially Fe^{3+}, present at trace concentrations in the eluents and in preventing complexation and hydrolysis of polyphosphates. The

Table 1
Theoretical and experimental straight line slopes of log k' vs. log c_{PA} dependencies

n_P	pH=4.5, $y = -3$				pH=9.0, $y = -4$			
	x	T	E	E/T	x	T	E	E/T
2	-2	-0.67	-0.82	1.22	-4	-1.00	-0.71	0.71
3	-3	-1.00	-1.17	1.17	-5	-1.25	-1.17	0.94
4	-4	-1.33	-1.38	1.03	-6	-1.50	-1.57	1.05
5	-5	-1.67	-1.75	1.04	-7	-1.75	-2.15	1.23
6	-6	-2.00	-2.30	1.15	-8	-2.00	-2.52	1.26
7	-7	-2.33	-2.89	1.24	-9	-2.25	-2.83	1.26
8	-8	-2.67	-3.35	1.25	-10	-2.50	-3.25	1.30

PA = pyromellitic acid.
n_P = the number of phosphorus atoms in a phosphate molecule.
x, y = charges of phosphate and PA anions, respectively.
T, E = theoretical (see Eq. (1)) and experimental straight line slopes, respectively, of log k' vs. log c_{PA} dependencies.

effects of metal ions on the hydrolysis of linear phosphates have been reported by previous investigators [35,36] and experimental observations [37] show that the presence of ions of multivalent metals promotes the hydrolysis of P–O–P linkages at pH values above 4 and that hydrolytic degradation of polyphosphates during anion-exchange chromatography could be prevented by adding EDTA to the eluent [32]. Retention times of linear polyphosphates are constant in the range of $c_{EDTA} = 2 \cdot 10^{-6}$–$2 \cdot 10^{-3}$ M, at a constant PA concentration in the mobile phase, $c_{PA} = 10^{-2}$ M, and a constant eluent pH. At higher EDTA concentrations, the retention of phosphates decreases (Fig. 1). Symmetric elution curves and good peak resolution were achieved at $c_{EDTA} = 8.10^{-5}$ M.

3.3. Effect of eluent pH on polyphosphate retention

The dependencies of log k' of polyphosphates on the eluent pH show a minimum at pH~6–8 at constant PA and EDTA concentrations in the mobile phases (Fig. 2). PA is almost completely dissociated under these conditions ($pK_{a,1} = 1.92$, $pK_{a,2} = 2.87$, $pK_{a,3} = 4.49$, $pK_{a,4} = 5.63$, [38]) and further increases

Fig. 2. pH Dependencies of the retention of polyphosphates. Pyromellitic acid and EDTA concentrations in the mobile phase were $c_{PA} = 1 \cdot 10^{-2}$ M and $c_{EDTA} = 8 \cdot 10^{-5}$ M, respectively. Curves: P_2, P_3, P_4, P_5 = pyro- to pentaphosphate.

in the pH of the eluent do not influence the elution strength of the mobile phase. On the other hand, deprotonization of phosphate anions occurs in solutions at pH>8 and their retention on an anion-exchange column increases up to a pH value of ~10, when the dissociation is complete. At pH<6, the elution strength of the mobile phase decreases, polyphosphate anions, however, are highly deprotonized until pH~4–5, and the retention of phosphate anions is strong in this pH range. There are two system peaks on chromatograms in eluents of pH<7 that can make the identification and quantification of the separated species difficult.

3.4. Dependence of polyphosphate retention on the number of phosphorus atoms in the molecule

The retention of phosphates increases with increasing number of phosphorus atoms, n_P, in the molecule. Linear relationships between logarithms of capacity ratios and n_P have been found with correlation coefficients $r > 0.998$ (Fig. 3). It is possible to use this behaviour for the identification of long-chain condensed phosphates, as standards are not available for them. Alkaline eluents are more suitable for these
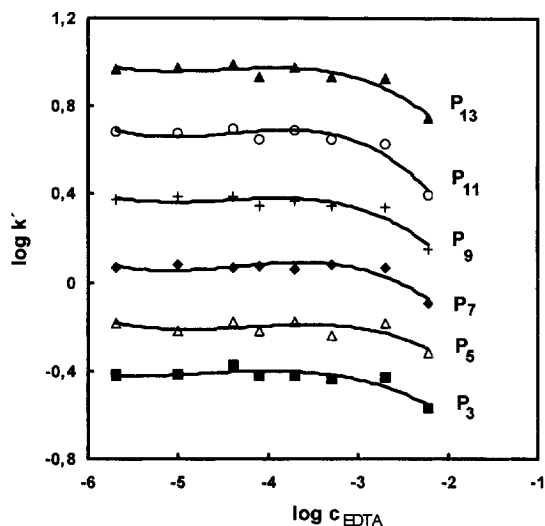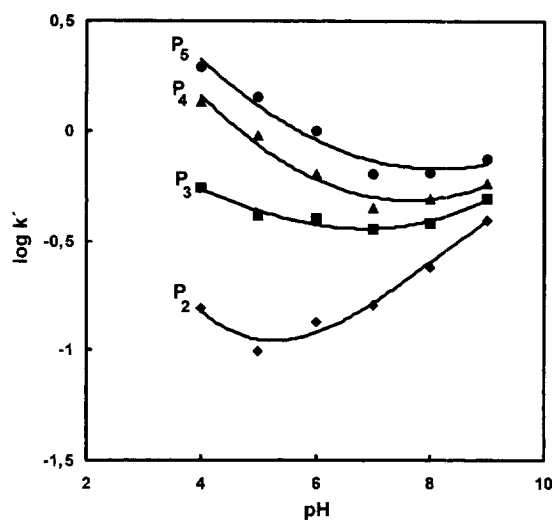

Fig. 1. Dependencies of the retention of polyphosphates on the molar concentration of EDTA in the mobile phase. Pyromellitic acid concentration in the mobile phase, c_{PA}, 1.10^{-2} M; eluent pH, 9. Curves: P_3, P_5,...P_{13} = tri- to tridecaphosphate.
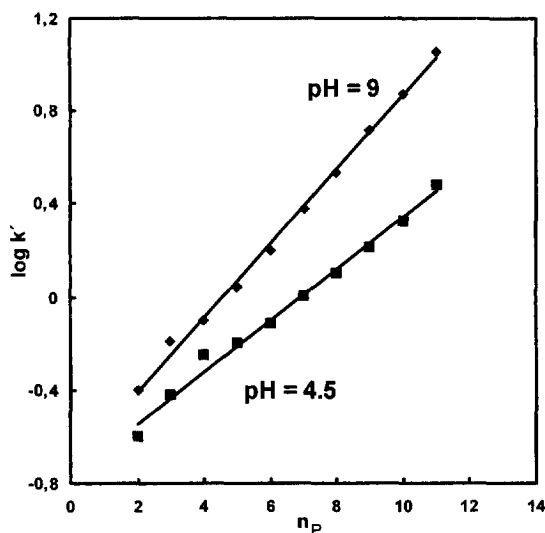
Fig. 3. Dependencies of the retention of polyphosphates on the number of phosphorus atoms in the molecule. The concentration of pyromellitic acid in the mobile phase was $c_{PA} = 2 \cdot 10^{-2}$ M (pH=4.5) and $c_{PA} = 1 \cdot 10^{-2}$ M (pH=9), respectively. The concentration of EDTA in the eluent, $c_{EDTA} = 8 \cdot 10^{-5}$ M.

purposes because of a better separation efficiency, good peak symmetry and shorter time of analysis.

3.5. Quantitative analysis

The dependencies of peak areas, A_p, on the molar concentrations, c, of pyrophosphate and triphosphate in solutions injected were linear in the studied range (0.02–0.1 mol/l) for calibration equation $A_p = a + bc$, calculated by linear regression, and are suitable for the determination of polyphosphates by IC in the chromatographic system used. The correlation coefficients were better than 0.999 for both phosphates, with values of the coefficients of the calibration curves of $a = 18.6$ ($s_a = 6.33 \cdot 10^3$), $b = 4.86 \cdot 10^6$ ($s_b = 9.54 \cdot 10^4$) for pyrophosphate and $a = 77.3$ ($s_a = 6.83 \cdot 10^3$), $b = 6.11 \cdot 10^6$ ($s_b = 1.03 \cdot 10^5$) for triphosphate. The calibration curves of the peak heights were non-linear. Detection limits of 0.02 mM, i.e. 3–5 mg/l ($S/N = 3$) were estimated for these anions under optimal chromatographic conditions ($1 \cdot 10^{-3}$ M PA, $8 \cdot 10^{-5}$ M EDTA, pH=9) and with an injected volume of 20 μl. Detection limits for polyphosphates using the iron(III) post-column de-

rivatization method [11] and indirect UV detection mode with NTS eluents [9] in the low ppm-range were reported by previous workers.

The IC analyses of solutions containing the same pyro- and triphosphate molar concentrations proved that the areas of the elution peaks are not proportional to the phosphate anion charges. Therefore, it is not possible to apply the theoretical principle of indirect photometric detection for the determination of polyphosphates under the chromatographic conditions and detection mode used in this work; calibration standards for phosphates or a special phosphorus detector must be used.

3.6. Applications

Examples of polyphosphate anion separations are shown in Figs. 4 and 5. The first demonstrates the separation of the phosphate components of a commercial washing agent, the second presents the isocratic separation of a mixture of sodium–potassium glassy phosphates with an average chain length, $\bar{n} = 6.9$.
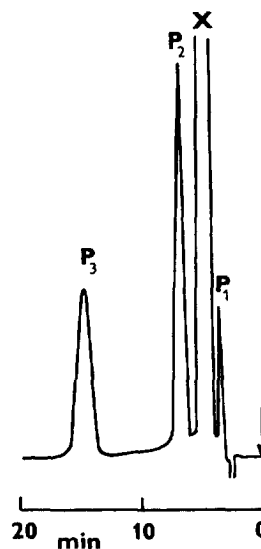

Fig. 4. Analysis of a phosphate washing agent. Eluent conditions: $2 \cdot 10^{-3}$ M pyromellitic acid; $8 \cdot 10^{-5}$ M EDTA; pH=9; flow-rate, 0.4 ml/min; sample, washing agent, aqueous solution 0.5% (m/V); injection volume, 20 μl; detection, indirect UV, $\lambda = 296$ nm, 0.16 AUFS. Peaks: P_1 = orthophosphate, P_2 = pyrophosphate, P_3 = triphosphate, X = unidentified components of the washing agent.
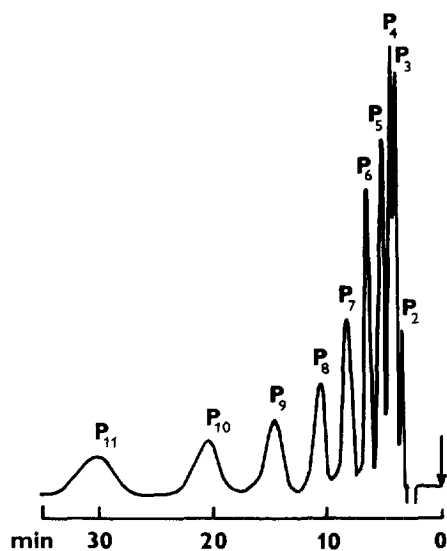
Fig. 5. Analysis of a glassy phosphate mixture. Eluent conditions: $1 \cdot 10^{-2}$ M pyromellitic acid; $8 \cdot 10^{-5}$ M EDTA; pH = 9; flow-rate, 0.4 ml/min; sample, sodium–potassium glassy phosphate mixture, aqueous solution 1% (m/V); injection volume, 20 µl; detection, indirect UV, $\lambda = 312$ nm, 0.08 AUFS. Peaks: P_2, P_3,...P_{11} = pyro- to undecaphosphate.

4. Conclusions

It is possible to achieve optimum isocratic separations of P_2–P_5 and P_6–P_{10} linear phosphates by IC analysis in the chromatographic system tested with concentrations of pyromellitic acid of $2 \cdot 10^{-3}$ M and $1 \cdot 10^{-2}$ M, respectively, an EDTA concentration of $8 \cdot 10^{-5}$ M and an eluent pH of 9. These conditions warrant good peak symmetry, polyphosphate stability and desirable elution strength of the mobile phase. To eliminate the presence of system peaks in the chromatograms, it is useful to inject the solutions of samples in mobile phase onto a column or to adjust the acidity of the sample solution to the pH of the eluent. Analyses of phosphate glasses with $\bar{n} > 7$ containing long-chain compounds with $n_P > 10$ are possible in the system described. To elute all compounds, a concentration of pyromellitic acid in the mobile phase that is too high would be necessary, which is not acceptable because of the high UV absorption of the eluent and the low sensitivity of indirect photometric detection.

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