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Journal of Chromatography A, 688 (1994) 89–95

JOURNAL OF CHROMATOGRAPHY A

Polyphosphate separations and chain length characterization using minibore ion chromatography with conductivity detection

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First received 2 August 1994; revised manuscript received 15 September 1994

Abstract

The separation of linear and cyclic polyphosphates with chain lengths of 1 to ca. 30, using 2 mm I.D. polymeric ion-exchange columns and suppressed conductivity detection, is described. The separations are characterized by good resolution of phosphate species and analysis times < 30 min. To assess the accuracy of the chromatograms obtained, average chain lengths of sodium hexametaphosphate and glassy phosphates in the range 5–25 are determined and compared with results obtained by ^{31}P NMR and potentiometric titration. General agreement among the three techniques is seen. An example is presented using ion chromatography to follow the hydrolysis of sodium hexametaphosphate.

1. Introduction

One of the more challenging problems in the chemical analysis of phosphate salts is the total characterization of chain length distributions of polyphosphates. Common methods relying on end-group analysis for determining number-average chain lengths ($\bar{n}$) include ^{31}P NMR [1], pH titration [2], electrophoresis [3] and elemental analysis [4]. Polymeric phosphates with chain lengths (n) < 50, such as industrially important sodium hexametaphosphate and sodium phosphate glasses, generally are not amenable to normal polymer characterization techniques such as viscosity and light scattering.

Separation methods which have been applied to determining polyphosphate chain length distributions include classical ion-exchange chromatography [5] and paper chromatography [6]. These methods are time consuming and are only partially successful in analyzing chain lengths > 10.

While several systems for the HPLC determination of polyphosphates have been proposed [7,8], the most successful separations [9–12] used anion-exchange HPLC with post-column molybdate colorimetric detection to determine polyphosphates with chain lengths < ca. 35. We have found that similar separations can be obtained on commercially available ion chromatography (IC) systems.

We report here the separation of linear (and cyclic) polyphosphates with chain lengths < ca. 30 using minibore (2 mm I.D.) polymeric columns and suppressed conductivity detection. Advantages of this system, vs. strong anion

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0021-9673/94/$07.00
SSDI 0021-9673(94)00906-6

exchange with post-column reaction and colorimetric detection, include reduced analysis times and the ability to simultaneously detect non-phosphate anions. We also show that phosphate distributions obtained with this system give average chain lengths comparable to those obtained using other techniques.

2. Experimental

2.1. Apparatus

Polyphosphate separations were performed using IC components obtained from Dionex (Sunnyvale, CA, USA). The system consisted of a Model ASM autosampler, a Model AGP gradient pump with microbore heads, an ATC (50 × 2 mm) trap column, a CSI column stand/injector with a Rheodyne 9126 microbore valve and 25-μl sample loop, a 2-mm anion self-regenerating suppressor (ASRS) with controller module and a CDM-I conductivity detector and cell. The analytical column, a 250 × 2 mm Dionex PAX-100, contains 60-nm latex functionalized with quaternary alkanol amine and bonded to a microporous 8.5-μm ethylvinylbenzene–divinylbenzene resin substrate [13]. In certain cases, samples also were run on a standard-bore IC system, Model 4000i, using 50 × 4 mm AS5G and 250 × 4 mm AS5A columns, and a 4-mm ASRS suppressor. Data acquisition and instrument control was accomplished using a Dionex AI-450 data system.

Potentiometric titrations were performed using a Metrohm (Herisau, Switzerland) Model 636 automatic titrator equipped with a Fisher Scientific (Pittsburgh, PA, USA) Model 13-620-90 combination electrode and 20-ml buret.

^{31}P NMR spectra were collected on a Varian (Palo Alto, CA, USA) VXR-300S Fourier transform spectrometer with a phosphorus operating frequency of 121.42 MHz, at 25°C. A Varian robotic sample changer with a 50-sample tray was resident on the system. The spectrometer was equipped with a Sun Microsystems (Mountain View, CA, USA) Sparc 1+ computer, operating on Varian VNMR software, version 4.1a, and SunOS 4.1.3. Customized software developed by Monsanto was developed previously using specially designed operating environments [14].

2.2. Chemicals

Sodium hexametaphosphate was a commercial sample produced by Monsanto (St. Louis, MO, USA). Sodium phosphate glasses of nominal average chain lengths 5, 15 and 25 (type 5, type 15 and type 25) were obtained from Sigma (St. Louis, MO, USA). Sodium salts of ortho-, pyro-, tripoly- and trimetaphosphate and the hexammonium salt of tetrapolyphosphate also were obtained from Sigma. Potassium hydrogenphthalate (KHP) was a titrimetric standard obtained from NIST (Gaithersburg, MD, USA), and was dried at 105°C for 2 h. Deuterium oxide ($^2H_20$), 99.9%, was obtained from Cambridge Isotope Labs. (Woburn, MA, USA). Methanol was HPLC grade from Fisher Scientific. All water was deionized and obtained from a Millipore Milli-Q 4-bowl plus analytical purification system (Bedford, MA, USA). Other chemicals were reagent grade and used without further purification. Cation-exchange cartridges (OnGuard-H, 2 mequiv. capacity) in the acid form were obtained from Dionex.

2.3. Methods

For IC analysis, 25 ± 5 mg of sodium hexametaphosphate or sodium phosphate glass was dissolved in 100 ml deionized water. If the samples were not injected immediately, the pH was adjusted to 9.5 ± 0.5 with NaOH and stored at 4°C. Comparison of chromatograms from samples with and without pH adjustment showed no discernible effect of added base, other than minor NaOH impurity peaks eluting near the column void volume at 2 min. In certain cases, further dilution of samples was necessary to keep peaks on scale. Polyphosphate IC was performed at a flow-rate of 0.25 ml/min with a linear or No. 4 curved gradient (see Results) from 40 to 300

m*M* NaOH and a constant 5% (v/v) methanol content. The gradient was run from 0 to 20 min and held at the final eluent composition for 10 min. The suppressor was operated in the external water mode (deionized water supplied as suppressor regenerant) at a flow-rate of 3 ml/min. The conductivity detector output range was 30 μS full scale.

Conventional IC separations of ortho- through tetrapolyphosphates, when needed, were performed with linear gradients from 20 to 70 m*M* NaOH at 0 to 5 min and 70 to 100 m*M* NaOH at 5 to 15 min. Column flow-rate was 1.5 ml/min and the suppressor was operated in the recycle mode (eluent outflow supplied as suppressor regenerant).

Prior to pH titrations for determining polyphosphate chain length, sample solutions were passed through a cation-exchange resin to convert sodium salts to the acid form. Cation-exchange cartridges were prepared as per the manufacturer's instructions (4 ml water wash at 2 ml/min). A 50 ± 10 mg amount of sample was dissolved in 5 ml water, which was then passed through the cartridge at 2 ml/min using a disposable plastic syringe. Two subsequent washes of the cartridge with 5-ml aliquots of water were combined with the original eluate, and diluted to ca. 25 ml. Titrant was 0.05 *M* NaOH standardized in duplicate vs. KHP.

Samples for ^{31}P NMR analysis were prepared by adding 4 ml water and 0.5 ml $^{2}H_2O$ to 55 ± 5 mg sample, and the pH was adjusted to 9.5 ± 0.5 by addition of 0.1 *M* NaOH (ca. 0.7 ml) to minimize hydrolysis. Samples were analyzed in 5-mm NMR tubes using parameters optimized for aqueous polyphosphate solutions expected to contain significant amounts of ortho- and metaphosphates, species which have long relaxation times. Use of a 15° pulse, 7.0-s repetition rate, 40 000 data points and 0.8 s acquisition times have been shown [14] to result in >97.5% of the observable magnetization being determined for the longer-relaxing species and >99.5% for faster relaxing species such as tripoly- and pyrophosphate. Samples were signal averaged for 900 transients.

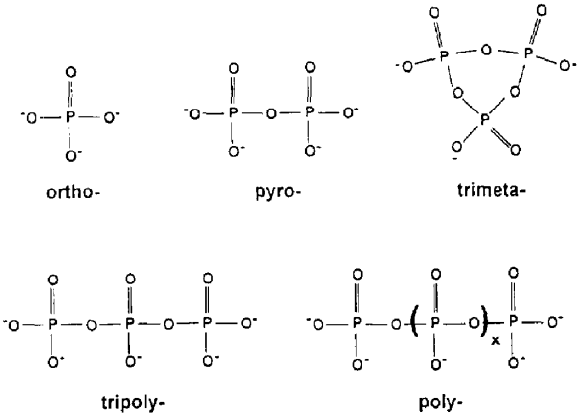

Fig. 1. Phosphate species separated by minibore ion chromatography.

3. Results

3.1. Polyphosphate separations

Phosphate species found in sodium hexametaphosphate (SHMP) and sodium phosphate glasses are shown in Fig. 1. A typical separation of SHMP using the minibore IC system is given in Fig. 2. The significant feature of this chromatogram is the improved speed of separation seen *vs.* previous analysis [9]. Comparison of

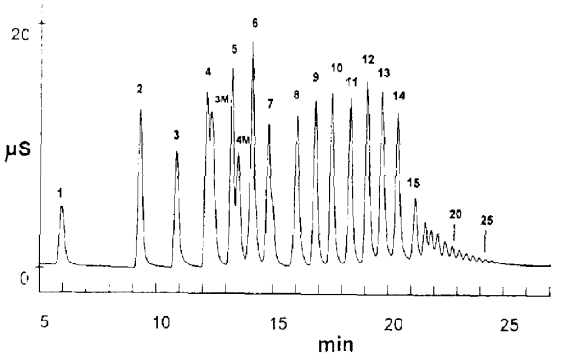

Fig. 2. Typical separation of polyphosphate species in sodium hexametaphosphate (SHMP). Peaks identified by number of phosphorous atoms for linear species, and by number of phosphorus atoms + "M" for meta-, or cyclic, phosphates. Concentration SHMP injected = 0.20 mg/ml. Gradient = linear.

peak areas with injections of standard materials indicates that minibore IC detection limits for smaller phosphates are on the order of 50 ng/ml, or ca. 1 ng by mass. Another feature of this separation is that the cyclic metaphosphates elute in order of their phosphorus numbers, instead of the inverse order seen previously [11].

Resolution acceptable for many applications is seen for phosphate species with chain lengths up to ca. 30. The poorest resolution is seen between the tetrapoly-/trimeta- and pentapoly-/tetrameta- peaks. Resolution of these pairs can be lost depending on sample loading and poly-/meta- ratios. Previous studies [9,11] indicated that an optimum separation of polyphosphates can be obtained using convex gradient elution. The effect of gradient curvature on separation quality of SHMP was tested by running the two least-curved convex and concave gradients available on the AGP pump. The most significant feature of these separations is changes in relative retention of meta- vs. linear polyphosates. While some flexibility in tailoring separations for specific samples is offered by the curved gradients, no single gradient gives complete baseline resolution of the phosphate species in SHMP. Convex gradient No. 4 is of the form $C = C_0 + [(t - t_0)/(t_f - t_0)]^a \cdot (C_f - C_0)$, where $a = 0.7$, C is the eluent concentration at time t, C_0 is the initial eluent concentration at time t_0, and C_f is the final eluent concentration at time t_f. This gradient shows improved tetrapoly-/trimeta- resolution but somewhat poorer pentapoly-/tetrameta- separation. Subsequent separations were performed with either linear or No. 4 gradient.

A typical application of the minibore IC system in our laboratory is assessment of hydrolytic stability of SHMP solutions under various conditions. The analysis of 40% SHMP solutions after 7 days storage at different temperatures is shown in Fig. 3. At room temperature, the original SHMP phosphate distribution remains intact. At 49°C, significant amounts of $n > 5$ polyphosphates are hydrolyzed. At 60°C, essentially all condensed phosphates are hydrolyzed to orthophosphate. Advantages of IC for this type of analysis include (1) obtaining a graphic description of changes in chain length distributions and (2) the capability of studying hydrolysis kinetics of individual P species.

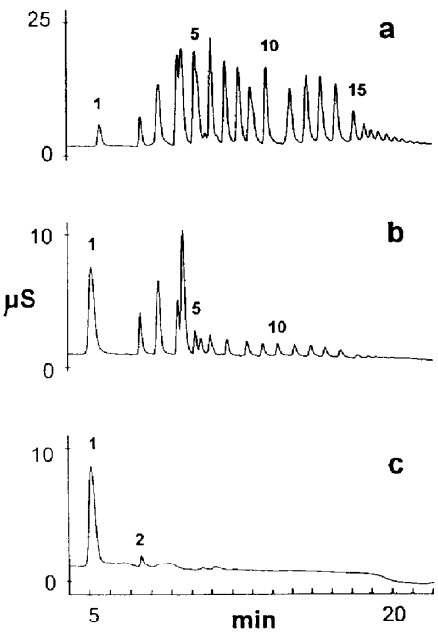

Fig. 3. Analysis of 40% SHMP solutions after 7 days storage at (a) room temperature, (b) 49°C and (c) 60°C. Peak labels as in Fig. 1. Gradient No. 4.

The ability of the minibore IC to analyze other polyphosphates is shown in Fig. 4, where separations are shown for sodium phosphate glasses with different average chain lengths. The shift toward higher phosphate species with increasing chain length is clear, and reasonable separations are obtained for nominal $\bar{n}$ up to 25. Close inspection of the highest $\bar{n}$ sample shows that peaks for n approaching 40 can be detected.

3.2. Average chain length determination

The major difficulty in using suppressed conductivity detection for the quantitative analysis of polyphosphates involves the lack of standards for determining concentration-based response factors. For the chromatograms shown here, questions remain about how well IC peak area distributions correlate with actual chain length distributions. To assess the quantitative potential of the current IC system, comparisons were

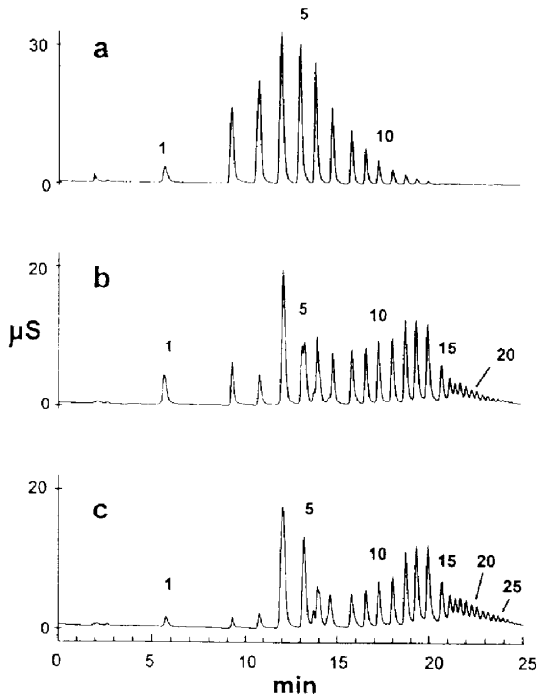

Fig. 4. Chromatograms of sodium phosphate glasses, (a) $\bar{n} \approx 5$, (b) $\bar{n} \approx 15$ and (c) $\bar{n} \approx 25$. Concentration injected = 0.25 mg/ml for (b) and (c), 0.125 mg/ml for (a). Peak labels as in Fig. 1. Gradient linear.

made between average chain lengths determined by IC and by other techniques.

Peak area-average chain lengths, $\bar{n}$(IC), were calculated from chromatograms shown in Figs. 2 and 4 using

$$\bar{n}(\mathrm{IC}) = \sum(\mathrm{area}_i \cdot n_i)/\sum(\mathrm{area}_i) \quad (1)$$

where n_i is the number of phosphorus atoms in the ith species. Average chain length calculations used all linear polyphosphate peaks (including ortho) but did not include metaphosphates. Trimetaphosphate content of the samples was verified by conventional 4-mm IC, where baseline separations of trimeta- and tetrapolyphosphate were obtained.

Typical determinations of average chain length by ^{31}P NMR and potentiometric (pH) titration are shown in Fig. 5. For NMR, the average chain length, $\bar{n}$(NMR), can be calculated from the integrated areas of the ortho (O), end-group (E) and internal (I) phosphorus resonances by

$$\bar{n}(\mathrm{NMR}) = (\mathrm{I} + \mathrm{E} + \mathrm{O})/(\mathrm{E}/2 + \mathrm{O}) \quad (2)$$

For pH titrations [2,3], the average chain length, $\bar{n}$(pH), can be calculated from the strong acid titer (A), weak acid titer (B), and an independent determination of the titers due to ortho (O) and meta (M), by

$$\bar{n}(\mathrm{pH}) = 2(\mathrm{A} - \mathrm{M})/(\mathrm{B} + \mathrm{O}) \quad (3)$$

A summary of the average chain lengths found for SHMP and sodium phosphate glasses is given in Table 1. M and O values used in Eq. 3 were taken from NMR data. Reasonable agreement among methods is seen for SHMP and the types 5 and 15 sodium phosphate glasses. Also, very good precision is seen for replicate analyses of SHMP by IC. This indicates that IC is well suited to comparative analysis of different polyphosphates. For type 25 glass, disagreement between IC/titration and NMR is pronounced. Investigations are continuing in order to assess the source of this bias. Potential sources of error include hydrolysis during sample preparation and unknown response factors for conductivity detection. However, the comparison in Table 1 shows that IC results correlate with chain lengths determined by other methods up to $\bar{n} = 15$, and gives an indication of the accuracy to be expected at different $\bar{n}$ values.

4. Discussion

End-group methods such as NMR and pH titration yield number-average, rather than mass-average, chain lengths. Polyphosphates with $n <$ 5 show roughly equal conductivity mass-response factors, as determined by injecting known amounts of standard compounds. However, if equal mass response is assumed over the entire chain length range, conversion from an area- (hence mass-) average chain length to a number-average chain length for SHMP gives $\bar{n} \approx 6$. Clearly, $\bar{n}$ values calculated from raw peak areas more closely approximate number average chain lengths.

From the known counterion binding properties

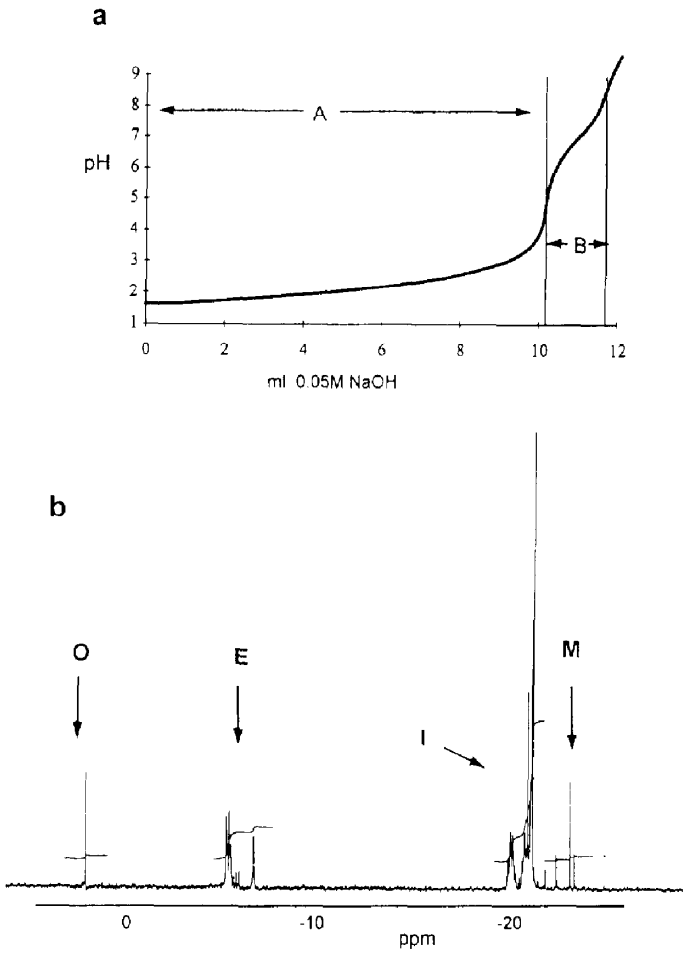

Fig. 5. Typical analysis for chain length by (a) pH titration and (b) ^{31}P NMR. (a) A = strong acid (internal, ortho and meta) region, B = weak acid (end-group and ortho) region. (b) O = ortho, E = end-group, I = internal and M = metaphosphate resonances.

Table 1
Comparison of average chain lengths obtained by different techniques

	$\bar{n}$(IC)	$\bar{n}$(pH)	$\bar{n}$(NMR)
SHMP	9.20 ± 0.06[a]	9.04 ± 0.11[a]	8.68 ± 0.26[a]
Sodium phosphate glasses			
Type 5	5.08	4.21	3.97
Type 15	11.6	12.6	10.1
Type 25	14.5	16.5	26.8

[a] Standard deviation, $n = 4$ for SHMP analyses.

of polyelectrolytes [15], counterion condensation and site-specific charge neutralization occur in longer-chain polyphosphates. Conductivity response factors for polyphosphates thus should decrease from a nearly equal-mass basis at low chain lengths to a more equal-molar response at higher chain lengths. Since standards are available for linear phosphates with $n < 5$, a preliminary assessment of response factors for $n \geq 5$ species was performed by calculating total masses and total areas of these species from SHMP chromatograms. The relative response factor (relative to $n < 5$) was found to average ca. 0.5 for all $n \geq 5$ components.

One unusual feature of the polyphosphate separations shown in Figs. 2–4 is the appearance of an almost bi-modal chain length distribution. Literature separations [9] of similar polyphosphates, using hydrolysis/colorimetry to give uniform P-based detection, have shown monomodal distributions. With conductivity detection, a minimum in the distribution appears near $n = 7$. It is likely that this is the point where conductivity response factors begin to decrease due to counterion binding. Consistent with this is the observation [3] that isotachophoretic response factors of polyphosphates show a change in the $n = 5–7$ region.

Despite the uncertainty in response factors discussed above, minibore IC with conductivity detection is shown to be a useful technique for rapid characterization of common polyphosphates. To develop the technique further as a quantitative tool, polyphosphate response factors for conductivity detection need to be determined. The most direct approach would be development of a polyphosphate standard that is well characterized by HPLC using post-column detection with constant-P response. Alternative approaches include (1) fraction collection, hydrolysis and P-content analysis by re-injection or colorimetry, and (2) solubility fractionation [16] of the polyphosphates to obtain narrow distributions for calibration.

Acknowledgements

The authors wish to thank David R. Gard, Helen M. Thompson and Edward E. Remsen for helpful discussions of polyphosphate chemistry and polymer characterization methods.

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