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Characterization of Polyphosphates by Electrospray Mass Spectrometry

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Electrospray ionization mass spectrometry (ESI-MS) was applied for the characterization of inorganic polyphosphates [orthophosphate, pyrophosphate, tripolyphosphate, trimetaphosphate, and tetrapolyphosphate]. The high selectivity of ESI-MS allows the detection of different polyphosphate species without preseparation by ion chromatography or capillary electrophoresis. Furthermore, ESI-MS does not require the incorporation of UV-absorbing chromophores into the analytical method for the detection of phosphates, unlike conventional UV-chromatographic methods. Limits of detection by ESI-MS were estimated to range from ∼1 to 10 ng/mL. The quantification of polyphosphate samples as single-component and multicomponent mixtures was investigated. Linear signal response for single-component samples ranged from the limit of detection to ∼10 μg/mL. Quantification of polyphosphate in streamwater is demonstrated using the standard addition method. The effect of multi-polyphosphate components and salts on signal response was also studied. For concentrations less than 2.0 μg/mL, signal response from a tetrapolyphosphate−tripolyphosphate mixtures. Signal response obtained from tetrapolyphosphate in the presence of tripolyphosphate or NH_4NO_3 at higher concentrations (∼50 μg/mL and 35 μg/mL, respectively) was significantly lower relative to single-component standards (∼40%−70%).

Over the past several decades, inorganic polyphosphates have become important components for the manufacturing and production of a diverse number of products. Some form of inorganic polyphosphate can be found in numerous everyday products including processed foods, beverages, detergents, fertilizers, and dentifrice.[1−2] However, there are some environmental concerns over the wide application of polyphosphates. Phosphates in runoffs or wastewater effluents, contributed by fertilizers, detergent builders and other phosphate-containing products, are believed to significantly promote the eutrophication of lake water and other aquatic environments.[3]

A number of analytical techniques have been developed for the characterization of polyphosphates, including gravimetric and titrimetric analysis, thin-layer chromatography, X-ray diffraction, and ^{31}P nuclear magnetic resonance spectroscopy.[4−8] However, these techniques are generally not convenient for trace analysis due to the relatively large sample quantity needed or time required to perform the analysis.

Significant improvement in polyphosphate characterization methods, in reference to detection limits and analysis time, accompanied the more recent development of ion chromatography and capillary electrophoresis.[9−13] These chromatographic techniques allow the efficient separation of different polyphosphate oligomers present in a solution sample. The polyphosphate analytes in the chromatography effluent are typically detected by a number of different methods, including atomic emission spectroscopy, flame photometry, and conductivity. The most common detection method described in the current literature involves the application of the UV absorption detector. However, because phosphates do not naturally absorb UV radiation, either postcolumn derivatization with UV absorbing chromophores or indirect photometric methods are required. Capillary electrophoresis techniques utilizing indirect photometric detection were reported to have detection limits in the low microgram per milliliter levels (∼0.1−5 μg/mL).

Electrospray mass spectrometry (ESI-MS) is a highly selective and sensitive analytical technique.[14−15] The soft nature of the electrospray ionization process allows the analyte to be typically observed as intact molecular ions, with minimum fragmentation. Although a routine analytical tool for bio-organic and environ-

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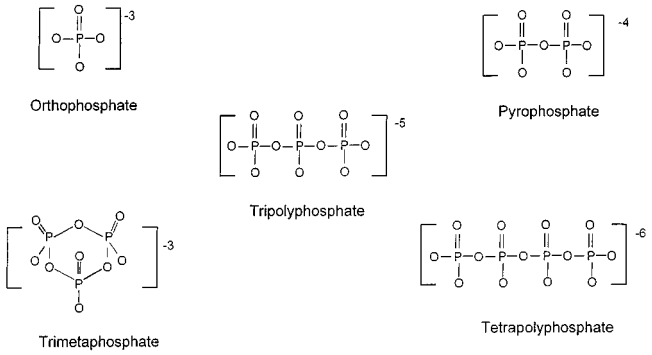

Figure 1. Structures of the phosphates used in this study.

mental research,[14−16] there has been a growing interest in the application of ESI-MS for the characterization of inorganic and organometallic compounds.[17]

Unlike ion chromatography and capillary electrophoresis, ESI-MS can detect a number of different analytes present in a sample, simultaneously, without the need for preseparation. ESI-MS is also capable of detecting a diverse number of analytes with various chemical properties. In contrast, chromatographic methods relying on UV detectors are typically limited to UV active analytes or must incorporate chromophores into the analytical method.

In this report, the application of ESI-MS for the characterization of inorganic polyphosphates was investigated. The analytes used in this study are ortho-, pyro-, tri-, trimeta-, and tetrapolyphosphates, as shown in Figure 1. Qualitative and quantitative ESI-MS analyses of single- and multicomponent polyphosphate samples were performed. Effects on signal response when characterizing a polyphosphate in the presence of a second component (i.e., polyphosphate and NH_4NO_3) were also studied.

EXPERIMENTAL SECTION

Material. $(NH_4)_2HPO_4$, $Na_4P_2O_7{\cdot}10H_2O$, $Na_5HP_3O_{10}{\cdot}6H_2O$, $Na_3P_3O_9$, $(NH_4)_6P_4O_{14}$, and ethylenediamineteraacetic acid (free acid) were obtained from Sigma (St. Louis, MO). HPLC grade solvents and ACS grade NH_4OH were obtained from Fisher Scientific (Pittsburgh, PA). Streamwater was obtained from the Rohm and Haas Co. (Philadelphia, PA).

Sample Preparation. For both qualitative and quantitative analyses, polyphosphate solution samples were prepared in 1:1 acetonitrile/water with 0.1% NH_4OH (v/v). Solutions with different polyphosphate concentrations were prepared by serial dilution of the stock solution. All samples were prepared daily and used immediately for analysis.

Standard Addition Analysis. A 2 mM EDTA/streamwater solution was prepared and adjusted to pH ∼8 with concentrated NH_4OH. To simulate water runoff, the water was fortified to a concentration of 0.1−10 μg/mL of pyrophosphate or tripolyphosphate. A sample was prepared by mixing 1 mL of the streamwater solution to a total volume of 4 mL with 0.1% NH_4OH 1:1 ACN/W (v/v). A reference sample was prepared by diluting a 1-mL streamwater sample with 1 mL of 0.1−10 μg/mL pyrophosphate or tripolyphosphate standard solution (0.1% NH_4OH 1:1 ACN/W (v/v)). Total volume was then adjusted to 4 mL with 0.1% NH_4OH 1:1 ACN/W (v/v).

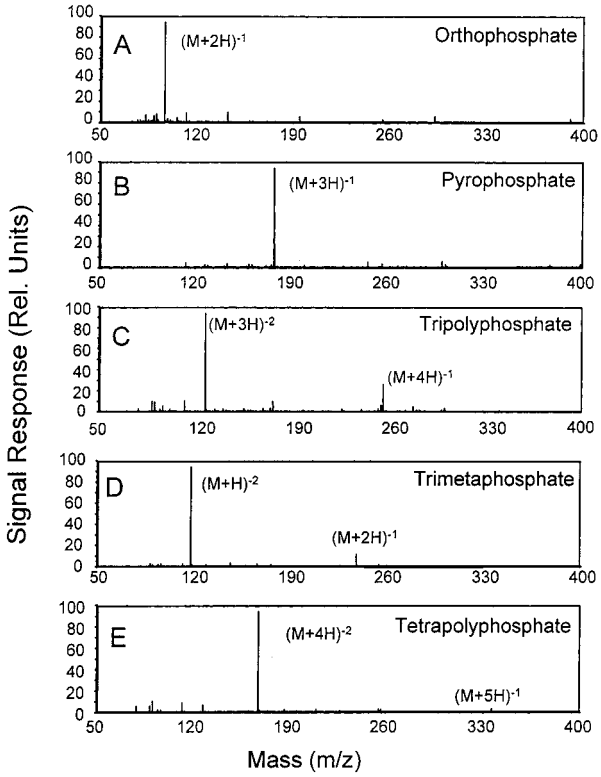

Figure 2. ESI-MS mass spectra of (A) orthophosphate, (B) pyrophosphate, (C) tripolyphosphate, (D) trimetaphosphate, and (E) tetrapolyphosphate. Concentrations are all 5 μg/mL.

Instrumentation. ESI-MS analysis was performed on a Mariner time-of-flight electrospray mass spectrometer (Perseptive Biosystems, Framingham, MA). The instrument was operated in the negative-ion mode. Spray needle and nozzle potentials were applied with ∼−2600 and ∼−30 V, respectively. Nebulizing gas and curtain gas were set at ∼0.2 and 2 LPM, respectively. Temperature of the ion source was 140 °C. Samples were infused into the ion source with a flow rate of 2 μL/min. Mass spectra were acquired for 10−100 s. For quantitative analysis, the infused standard was allowed to equilibrate for ∼5 min prior to signal acquisition. Each standard was characterized in triplicate. The average signal area was used to generate the calibration curves.

RESULTS AND DISCUSSION

Qualitative Analysis. Figure 2 shows the ESI-MS mass spectra acquired from 5 μg/mL orthophosphate, pyrophosphate, tripolyphosphate, trimetaphosphate, and tetrapolyphosphate in 0.1% NH_4OH 1:1 acetonitrile/water. In the ESI-MS spectra, orthophosphate and pyrophosphate appear predominately as singly charged species $[(M + XH)^{-1}]$, while the other polyphosphate species are observed primarily as doubly charged species $[(M + XH)^{-2}]$. The limit of detection was estimated to range from 1 to 12 ng/mL, using a signal-to-noise ratio of 3 as this limit (Table 1).

It should be noted that the species observed in the mass spectra of the polyphosphates are not those known to be present in aqueous solution. The solvent used was 1:1 acetonitrile/water

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Table 1. Estimated Limit of Detection for ESI-MS Polyphosphate Analysis (S/N = 3)

polyphosphate	concn (ng/mL)
ortho-	12
pyro-	11
tri-	6
trimeta-	1
tetra-	7

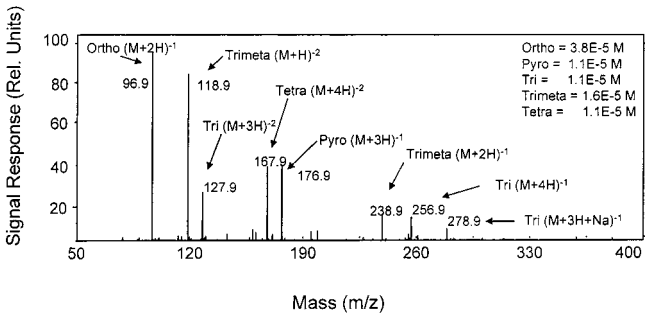

Figure 3. ESI-MS mass spectra obtained from a mixture of orthophosphate, pyrophosphate, tripolyphosphate, trimetaphosphate, and tetrapolyphosphate. Concentrations are all 5 μg/mL.

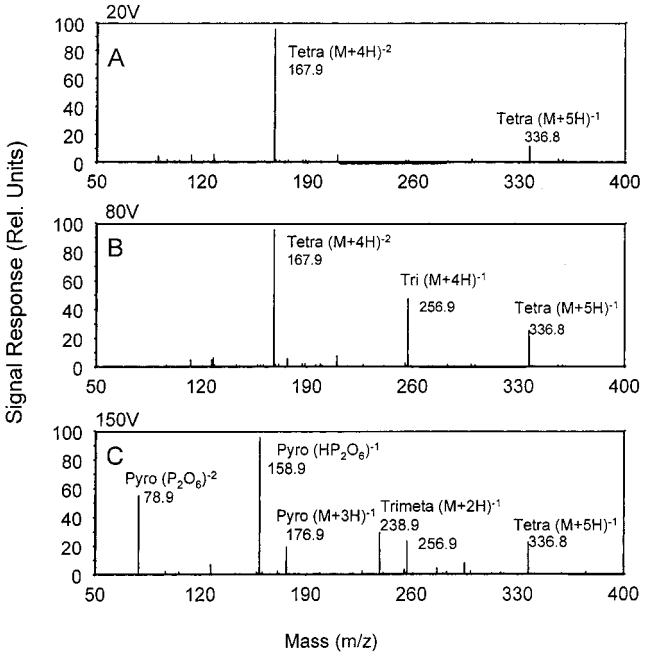

Figure 4. ESI-MS mass spectra of 5 μg/mL tetrapolyphosphate acquired at various nozzle potentials: (A) 20, (B) 80, (C) 150 V.

0.1% NH_4OH(v/v) with a measured pH of ~10. As shown in Figure 2, the major species observed for orthophosphate, pyrophosphate, and tripolyphosphate are $(H_2PO_4)^{-1}$, $(H_3P_2O_7)^{-1}$, and $(H_3P_3O_{10})^{-2}$, respectively. The species known to exist in aqueous solution with pH ~10, on the basis of known equilibrium constants, are $(HPO_4)^{-2}$, $(P_2O_7)^{-4}$, and $(P_3O_{10})^{-5}$, respectively (equilibrium constants for trimeta- and tetrapolyphosphates were not available for comparison). Similar protonation behavior has been observed for polyelectrolytes of other elements, such as Mo, W, and V.[18−20]

In order for the polyphosphate species observed by ESI-MS to exist in aqueous solution, the pH must range between ~0 and 7 (depending on the specific species). The extent of this pH change cannot be attributed to redox reactions occurring during the electrospray process.[21] However, the results do agree with the Fenn's electrospray model, which proposes that small compact species are less likely to appear as multiply charged due to the limited area which the species can occupy on a charged surface.[22] Similarly, it has been proposed that such protonation effects arise from dissociation of anion−water complexes in the gas phase.[23]

As a result of poor selectivity, chromatographic analytical techniques applying UV detectors depend on high-resolution separation procedures, in addition to UV absorbing chromophores, to characterize samples composed of multiple polyphosphate oligomers. The high selectivity of ESI-MS allows the analysis of polyphosphate mixtures without the need of a separation technique or UV absorbing chromophore. Figure 3 shows a 5 μg/mL mixture of ortho-, pyro-, tri-, trimeta-, and tetrapolyphosphates in a 1:1 solution of 0.1% NH_4OH 1:1 acetonitrile/water. Each polyphosphate component can be clearly monitored without spectral interference from the other oligomer signals.

It is important to note that the electrospray instrument parameters (e.g., spray needle and nozzle potential) can significantly affect the sensitivity and the type of polyphosphate species observed in the mass spectra. At low nozzle potential (~20 V), Figure 4A shows that a 5 μg/mL tetrapolyphosphate solution appears in the ESI-MS mass spectrum as intact oligomers with the dominant signal appearing as $(M + 4H)^{2-}$. Use of higher nozzle potential settings results in fragmentation of the analyte due to collision-induced dissociation occurring in the atmospheric pressure/vacuum interface of the mass spectrometer.[24,25] Figure 4B,C shows the mass spectra of tetrapolyphosphate acquired with the nozzle potential set at ~80 and 150 V, respectively. At ~80 V, signals characteristic of the singly charged tripolyphosphate species are observed in the ESI-MS mass spectra (Figure 4B). Figure 4C shows that the tetrapolyphosphate can undergo extensive fragmentation when the nozzle potential is increased further. With the nozzle potential set at ~150 V, the tetrapolyphosphate analyte is no longer the dominant species. Signals characteristic of trimetaphosphate, pyrophosphate, and $(P_2O_6)^{-2}$ appear in the mass spectra. This result demonstrates that, for the analysis of polyphosphate mixtures by ESI-MS, it is important to establish appropriate instrumental parameters to avoid misinterpretation of signals resulting from analyte fragmentation.

Quantitative Analysis. Quantitative analysis of polyphosphate solution samples was performed by ESI-MS. Figure 5 shows the calibration curves obtained for single-component polyphosphate samples of tetrapolyphosphate and trimetaphosphate. Linear signal response was obtained for concentrations ranging from the detection limit up to ~2.5 μg/mL. Statistical data for the analyses

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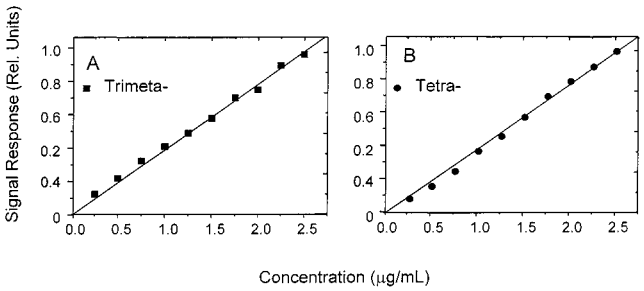

Figure 5. Calibration curves for single-component polyphosphate samples (A) trimetaphosphate and (B) tetrapolyphosphate.

Table 2. Relative Standard Deviation of Trimetaphosphate and Tetrapolyphosphate Signal Areas

concn (mg/mL)	trimeta-	tetra-
	Relative Standard Deviation (%) ($n = 3$)	
0.25	3.6	5.6
1.0	1.9	3.7
2.0	3.1	2.2

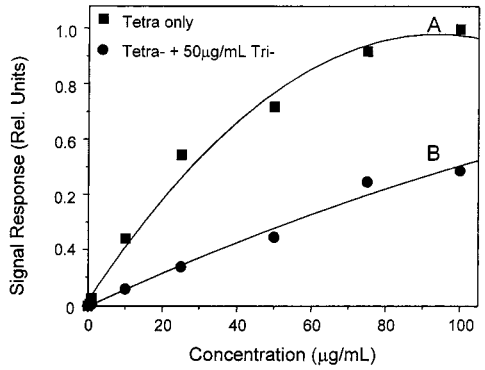

Figure 6. Calibration curves for tetrapolyphosphate as (A) a single-component solution, and (B) mixed with 50 μg/mL tripolyphosphate.

are shown in Table 2. Figure 6A shows the calibration curve for tetrapolyphosphate over a broader concentration range, from 0.5 to 100 μg/mL. Calibration becomes nonlinear for concentrations greater than ~10 μg/mL.

One difficulty associated with using ESI-MS for quantitative analysis is the susceptibility to ion suppression. The presence of excess electrolytes in the sample solution is known to have a signal-suppression or -enhancement effect on the ESI-MS signal response.[26−27] This raises some concern over the application of ESI-MS for the quantification of polyphosphates in multicomponent mixtures or environmental matrixes. Signal response obtained for the ESI-MS analysis of single-component solution samples may not be identical to those obtained when characterizing the same analyte in the presence of other polyphosphate species, salts, or buffers. The presence of the second polyphosphate component can have a signal-suppression effect on the overall ionization of the polyphosphate analytes. To determine if a polyphosphate ESI-MS signal is subject to signal-suppression

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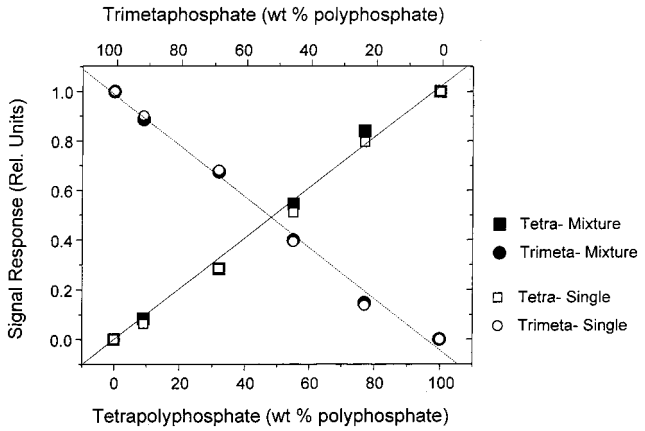

Figure 7. ESI-MS signal response from a two-component polyphosphate mixture (total concentration was 2.0 μg/mL) overlaid with those obtained from single-component polyphosphate samples.

or -enhancement effects by the presence of other polyphosphate species, an experiment was done where signal responses from tetra- and trimetapolyphosphate mixtures were compared with those obtained by single-component polyphosphate solution samples.

Figure 7 shows the ESI-MS signal response obtained from various mass percent tetra- and trimetapolyphosphate mixtures with a constant polyphosphate concentration of 2.0 μg/mL. The signal response from the two-component solution samples can be overlaid with those obtained from the single-component samples. The correlation between the signal response obtained from the mixture and that obtained from single-component samples indicates that the presence of a second polyphosphate component does not significantly affect the signal intensity of the first component at the level of concentration studied. This demonstrates that the ESI-MS signal response obtained from a polyphosphate sample mixture can correlate with a calibration curve prepared using single-component samples, provided that the total polyphosphate concentration is ~2 μg/mL or less.

Signal suppression becomes a significant issue when the concentration of the second polyphosphate component exceeds 2 μg/mL. Figure 6 shows two separate calibration curves for tetrapolyphosphate solutions in the concentration range 0.5 to 100 μg/mL. Curve A was obtained from a single-component tetrapolyphosphate solution (1:1 acetonitrile/water (v/v), 0.1% NH_4OH (v/v)). Curve B was obtained from tetrapolyphosphate in the presence of 50 μg/mL tripolyphosphate solution. The presence of the 50 μg/mL tripolyphosphate component had a significant signal-suppression effect on the tetrapolyphosphate, reducing the tetrapolyphosphate signal response by an average of ~60%.

Similar signal-suppression effects were observed when polyphosphate was measured in the presence of ions other than polyphosphates. Figure 8 shows the calibration of curves of tetrapolyphosphate alone (8A) and in the presence of 35 μg/mL NH_4NO_3 (8B). The presence of 35 μg/mL NH_4NO_3 reduced the tetrapolyphosphate signal response, relative to the single-component analysis, by an average of ~70%.

Environmental matrixes, such as streamwater, contain various ions and salts which would potentially contribute to ESI-MS signal suppression, thereby preventing correlation with standard sample analyses for external calibration. One approach that may be

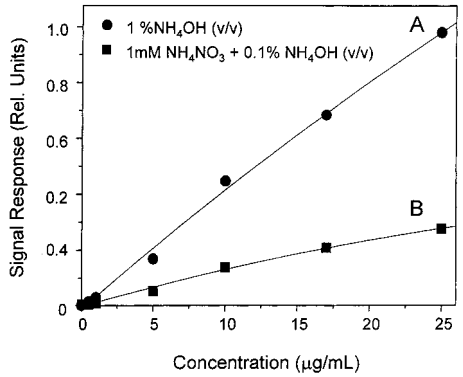

Figure 8. Calibration curves for tetrapolyphosphate in (A) 0.1% NH_4OH (v/v) and (B) 35 μg/mL NH_4NO_3 + 1% NH_4OH (v/v).

Table 3. Quantitative Analysis of Polyphosphates in Streamwater Using the Standard Addition Method

actual	pyrophosphate concn (μg/mL)	tripolyphosphate
0.100	0.0979 ± 0.0291	0.0937 ± 0.0109
1.00	1.04 ± 0.0314	1.18 ± 0.0276
10.0	9.47 ± 1.18	10.1 ± 0.966

applied to address signal suppression issues for the quantification of polyphosphates in environmental samples, such as streamwater, is the standard addition method. Table 3 compares actual and experimental data obtained for the quantification of pyrophosphate and tripolyphosphate. Average experimental results were within ~1−18% of actual polyphosphate concentration.

Although approaches, such as the standard addition method, may be used to acquire quantitative information of polyphosphates in various sample matrixes, signal suppression may still affect overall sensitivity of this analytical method. To achieve maximum sensitivity and low detection limits, the solution composition (e.g., buffer, matrix concentration, matrix contaminants, salt additives) must be controlled.

SUMMARY

The characterization of inorganic polyphosphates by ESI-MS has been demonstrated. ESI-MS is a potentially effective analytical tool for qualitative and quantitative analysis of polyphosphate solution samples. This analytical technique is capable of distinguishing between different polyphosphate oligomers without preseparation as required when using ion chromatography or electrophoretic methods. In addition, lower detection limits can be achieved by ESI-MS than UV-chromatographic techniques without the need to incorporate chromophores into the analytical method. However, sensitivity will depend on the concentration of other components accompanying the presence of the target analyte in the sample solution (e.g., polyphosphate, NH_4NO_3, buffers, matrix contaminants, etc.).

ACKNOWLEDGMENT

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