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Short Communication

Ion chromatography of polyphosphates and polycarboxylates using a naphthalenetrisulfonate eluent with indirect photometric and conductivity detection

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ABSTRACT

The potential of naphthalenetrisulfonate as a powerful displacing anionic eluent has been extended to the separation and detection of multivalent inorganic polyphosphates and organic polycarboxylates using a low-capacity polystyrene–divinylbenzene ion-exchange column. Four polyphospates (pyro-, trimeta-, tripoly- and tetrapolyphosphate) and three polycarboxylates (nitrilotriacetate, ethylenediaminetetraacetate and citrate) can be separated in about 20 min with indirect photometric and conductivity detection. Indirect photometric detection was found to be superior to conductivity detection because early-eluting anions are well resolved from the injection peak and flow programming can be done with a minimum disturbance of the baseline. Detection limits for the analytes investigated range from 2 to 100 ng which are at least an order of magnitude better than those previously reported using postcolumn derivatization methods. Linear response ranges from 500 or 75 mg/l to the limit of detection for longer and shorter retained analytes, respectively.

INTRODUCTION

Polyphosphates and polycarboxylates have long been of interest to chemists because of their multifunctional characteristics [1]. They act as color preservatives and food stabilizers and therefore are of considerable significance to the food and beverage industry. Polyphosphates, in particular, are also used as sequestering agents in various detergent formulations.

A variety of analytical methods for the determination of these anions have been explored. Some of these for carboxylic acids were gas chromatography after ester derivatization [2,3] and polarography [4]. An ion-selective electrode method has been reported [5] for the determination of polyphosphates. These methods are relatively slow and/or can give erroneous results in quantitation. A better approach is indirect flow injection analysis of polyphosphates based on their complexing ability of methylthymol blue derived from the corresponding magnesium complex [6]. For polyphosphates mixtures, gradient ion chromatography using a sodium hydroxide eluent with suppressed conductivity detection [7] has been effective.

Ion chromatography with postcolumn derivatization has been used for the determination of both polyphosphates and polycarboxylates [8];

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however, this method is more commonly applied to just the former class of compounds [9–15]. After either isocratic [11] or gradient [12,14] separation, ortho (P_1)-, pyro (P_2)-, tripoly (P_3)- and tetrapoly (P_4)-phosphate were hydrolyzed to orthophosphate and reacted with molybdate to form a molybdenum blue complex which is then detected photometrically at 830 nm. A variation of this scheme is to add molybdovanadate to the formed orthophosphate for subsequent product detection at 340 nm [13]. A mixture of P_2- and P_3-phosphate was separated in 12 min using iron(III) nitrate as a derivatizing agent but no detection limits were determined [15]. Similar approaches for the determination of P_1–P_4 polyphosphates using a low-capacity ion-exchange column with a nitric acid gradient and post-column detection with iron(III) perchlorate at 320 nm can be found in the manufacturer's literature. Ion-exchange chromatography in conjunction with phosphorous-selective detection by atomic emission spectroscopy has also been used [16,17] for the determination of polyphosphates. Chester and Smith [18] have also reported an ion-interaction chromatographic method for polyphosphates coupled to a flame photometric phosphorous-selective detector. Thus, in numerous cases, somewhat complicated postcolumn reaction methods have been adopted for these phosphorous compounds.

Naphthalenedisulfonate (NDS) and 1,3,6-naphthalenetrisulfonate (NTS) have recently been applied as pH-independent mobile phases for non-suppressed ion-exchange chromatography in which ions are separated on a polymethacrylate ion-exchange column and detected by using conductivity and indirect photometric detection (IPD). This technique has shown to offer a great deal of promise for the simple, rapid and sensitive determination of inorganic and organic anions [19,20] as well as the highly retained sulfur oxide anions [21]. Aliphatic sulfonate and sulfate surfactants have also been separated on mixed-mode reversed-phase ion-exchange columns using NDS as the eluent with IPD and conductivity detection [22,23].

The purpose of this report is to describe an analogous study for the separation and detection of multivalent polyphosphates such as P_2-, P_3-, P_4- and trimeta-phosphate as well as polycarboxylates such as nitrilotriacetate (NTA), ethylenediaminetetraacetate (EDTA) and citrate in which no chemical modification of the analyte is necessary. Using NTS as the eluent and a polystyrene–divinylbenzene (PS–DVB)-based ion-exchange column, these multivalent anions can be separated in about 20 min and then detected by IPD or non-suppressed conductivity detection. These detection methods have not been previously explored in detail for polyphosphates [24,25]. Using sodium trimesate, only P_2- and P_3-phosphates have been separated by ion-exchange chromatography with IPD [26,27]. The technique reported here provided limits of detection in the nanogram range which are at least an order of magnitude better than the most popularly used postcolumn derivatization methods.

EXPERIMENTAL

Reagents

All reagent-grade chemicals were used without further purification. NTS salt was purchased from American Tokyo Kasei (Portland, OR, USA). A 10 m*M* stock solution of mobile phase was prepared and used after further dilution with 5% acetonitrile (ACN). This percentage of ACN was found to be effective in reducing any possible lipophilic interaction between the PS–DVB stationary phase and the naphthalene moiety of the mobile phase [28]. The disodium salts of EDTA and NTA, as well as the trisodium salt of citrate were of analytical-reagent grade and obtained from Fisher Scientific (Fairlawn, NJ, USA). Tetrasodium P_2-phosphate, hexaammonium P_4-phosphate (both analytical-reagent grade), trisodium trimetaphosphate and pentasodium P_3-phosphate, (both technical grade), were all purchased from Sigma (St. Louis, MO, USA). The stock solutions (1000 mg/l) of the analytes of interest were prepared in the mobile phase and then diluted with doubly distilled deionized water for further use.

Instrumentation

The liquid chromatograph consisted of a Model 510 HPLC pump, a Model MU6K injector with 20-μl sample loop, a Model 441 fixed-

wavelength detector and a Model 430 conductivity detector, all from the Waters Chromatography Division of Millipore (Milford, MA, USA). A Model LP-21 Lo-Pulse dampener from Scientific System (State College, PA, USA) was connected between the injector and pump outlet to eliminate the noisy baseline particularly obvious with conductivity detection [20]. The analyte anions were separated using a Hamilton PRP-X100 low-capacity (0.20 mequiv./g) anion-exchange column (150 × 4.1 mm I.D.) while in some initial experiments a Hamilton PRP-X500 high-capacity (1.60 mequiv./g) anion-exchange polyether ether ketone (PEEK; 50 × 4.6 mm I.D.) column was used. Both conductivity and UV detector outputs were simultaneously displayed on two Model 1500 Fisher Recordall Chart recorders (Austin, TX, USA).

RESULTS AND DISCUSSION

Naphthalene sulfonate derivatives such as NDS and NTS were compared in a preliminary study using the PRP-X100 anion-exchange column. Although NDS can separate a mixture of polycarboxylates containing NTA, EDTA and citrate in 12 min, we choose to pursue work with NTS as this eluent seemed potentially more useful for the separation of mixtures of both polycarboxylates and polyphosphates in a shorter time with lower detection limits . In particular, the greater charge on NTS provided sharper peaks and shorter retention times for the elution of P_3-phosphate, P_4-phosphate and citrate.

Capacity factors (k') of polyphosphates such as P_2-, trimeta-, P_3- and P_4-phosphates and various polycarboxylates such as NTA, EDTA and citrate as a function of NTS concentration using the PRP-X100 column are shown in Fig. 1a and b, respectively. The capacity factors for polyphosphates (P_2-, trimeta- and P_3-phosphate) range from 0.3 to 27, with NTS concentration changing from 0.05 to 0.30 m*M*, while for polycarboxylates k' ranges from only 0.5 to 2.5 with the same range of NTS concentration. Interestingly, using the high-capacity hydrophilic PRP-X500 column, the retention order of trimeta- and P3-phosphate are reversed compared to that of the PRP-X100 column. However,

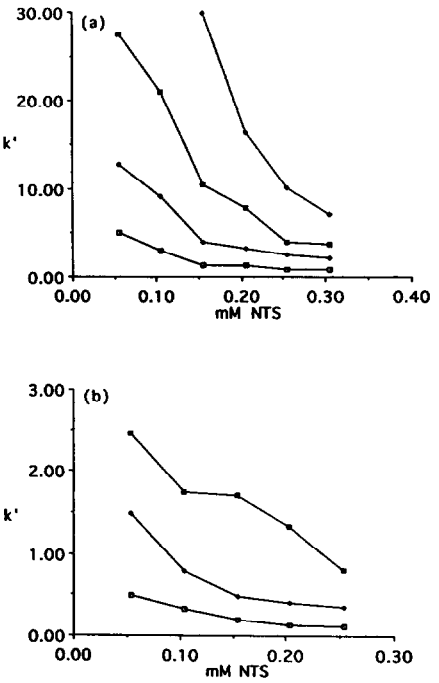

Fig. 1. Retention of polyphosphates (a) and polycarboxylates (b) as a function of eluent concentration using the PRP-X100 column. All mobile phases contained 5% acetonitrile. Flow-rate 1 ml/min for all analytes except for P_4-phosphate (2 ml/min). Analyte concentration 10 mg/l each of (□) P_2-, (◆) trimeta-, (■) P_3- and (◇) P_4-phosphate (a) and 20 mg/l each of (□) NTA, (◆) EDTA and (■) citrate (b).

peaks were broad using the PRP-X500 column and better resolution and detectability were clearly evident in the chromatograms generated using the PRP-X100 column. All future work was carried out on this column. In general, mobile phase concentrations of 0.2 and 0.1 m*M* NTS were best for the resolution of polyphosphates and polycarboxylates, respectively.

Experimental data relating log k' *versus* log [eluent] were obtained and plots were prepared using the equation $\log k' = -(y/x) \log E + \log B$, where y is the charge of the analyte anion, x is the charge of the eluent, E is the eluent concentration and B is a constant dependent on ion-exchange equilibria and resin capacity [26]. Using linear regression statistical analysis, correlation coefficients of 0.98 or higher were noted for all the polyphosphate anion plots. The observed values for P_2-, P_3- and P_4-phosphate were found to be −1.55, −1.38 and −2.14, respective-

ly which were considerably higher than the values −0.66, −1.00 and −1.33 predicted from theory. The theoretical values were obtained by calculating the predominant charge on the P_2-, P_3- and P_4-phosphate anions which were −2.0, −3.0 and −4.0, respectively, at a mobile phase pH of 5.5. One explanation for the high positive deviation of the observed slope values is that the interaction between the total charge of the analytes or NTS and the ion-exchange groups of the resin is not stoichiometric.

In non-suppressed ion chromatography, using any indirect detection mode, sensitivity is dependent on flow-rate as well as concentration of the eluent. Furthermore, the measured sensitivity using a flow-rate of 1 ml/min at an optimized mobile phase concentration of 0.20 m*M* NTS is about twice as high than that at 2.2 ml/min. As a compromise between resolution and analysis time, we have chosen flow programming for the separation of P_2-, trimeta-, P_3- and P_4-phosphate with 0.2 m*M* NTS and IPD. At a constant 2.2 ml/min, the P_2-phosphate peak overlaps with the injection peak but P_4-phosphate elutes in only 20 min (Fig. 2a). A change in flow-rate from 1 to 2 ml/min at the arrow effectively increased the retention time of P_4-phosphate to 24 min (Fig. 2b) with better retention of P_2-phosphate. However, an analogous separation with conductivity detection was more problematic. First, highly retained anions such as P_3- and P_4-phosphates produce very intense injection peaks which will obscure the P_2-phosphate anion peak. Secondly, as previously noted [20], conductivity detection causes a pronounced conductivity baseline disturbance to occur with any change in flow-rate. Therefore, a standard mixture of polyphosphates containing only P_2-, trimeta- and P_3-phosphate could be determined with conductivity detection as shown in Fig. 2c. The peak direction in conductivity detection depends on the relative ionic equivalent conductance of the eluent and the solute anions. In this case, P_2- and trimetaphosphate have higher equivalent conductance so they appear as positive peaks while the P_3-phosphate anion with a lower equivalent conductance than NTS produces a negative peak. The direction of conductivity signal is also dependent on the solute concentration of some

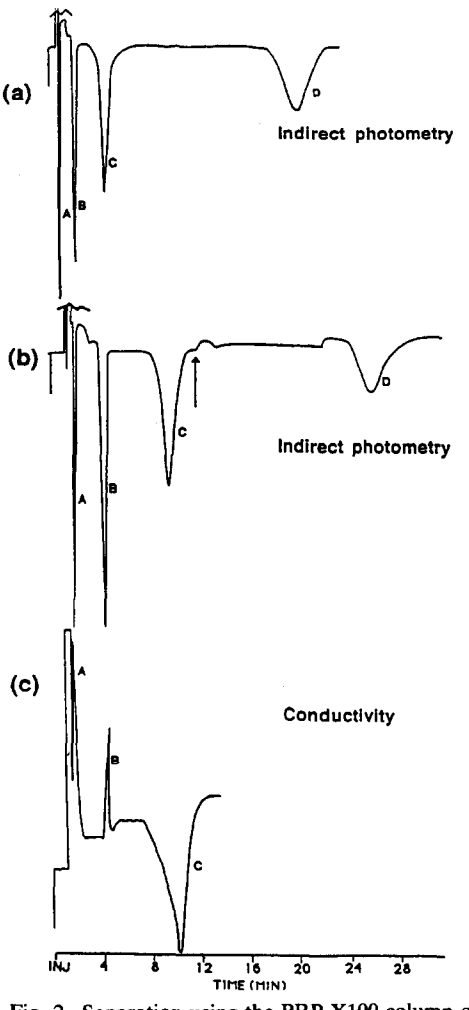

Fig. 2. Separation using the PRP-X100 column of a standard mixture of polyphosphates with (a) IPD at 280 nm, 0.05 AUFS, flow-rate 2.2 ml/min. (b) Similar conditions but flow-rate initially 1 ml/min, then changed to 2 ml/min at the arrow. (c) Conductivity detection (0.2 μS FS), flow-rate 1 ml/min. Mobile phase: 0.20 m*M* NTS–ACN (95:5), Peaks: 15 mg/l each of (A) P_2- and (B) trimetaphosphate, and 25 mg/l each of (C) P_3- and (D) P_4-phosphate.

analytes. When the concentration of P_4-phosphate was 100 ppm, a positive signal was noted while an analyte concentration below 50 ppm caused the peak to become negative.

Fig. 3 shows the separation of polycarboxylates in about 6 min with both IPD and indirect conductivity detection. The chromatogram shows no interference from the injection peak and all analyte peaks are well resolved. However, NTA

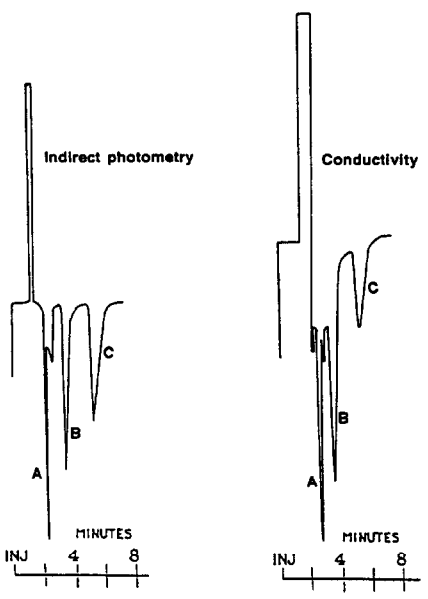

Fig. 3. Separation of a standard mixture of polycarboxylates on the PRP-X100 column with IPD (280 nm, 0.05 AUFS) and conductivity detection (0.2 μS FS). Mobile phase: 0.1 m*M* NTS–ACN (95:5), flow-rate 1 ml/min. Peaks: (A) 10 mg/l of NTA, 25 mg/l each of (B) EDTA and (C) citrate.

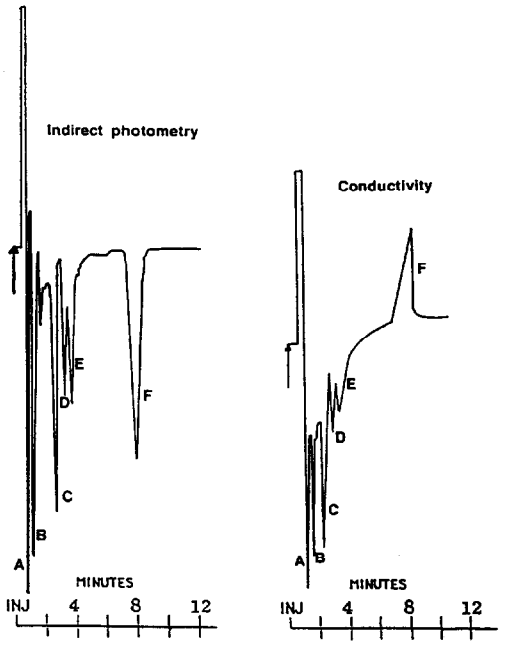

Fig. 4. Separation of a standard mixture of polyphosphates and polycarboxylates on the PRP-X100 column with IPD (280 nm, 0.05 AUFS) and conductivity detection (0.1 μS FS). Other conditions as in Fig. 3 except that the flow-rate was 1.5 ml/min. Peaks: 10 mg/l each of (A) P_1-phosphate and (B) NTA, (C) 25 mg/l of EDTA, (D) 10 mg/l of P_2-phosphate, (E) 20 mg/l of citrate, (F) 15 mg/l of trimetaphosphate.

did show a side impurity peak which was not identified. Using less competitive mobile phases than NTS in an ion-exchange mode, citrate can be retained quite long [24]. Fig. 4 shows a comparison between two chromatograms for the separation of a mixture of polyphosphates and polycarboxylates using IPD and conductivity detection. The order of elution was consistent with the trends in which retention is directly proportional to the effective charge, ionic radius and polarizability and inversely proportional to hydration energy [24,25]. At this mobile phase concentration of 0.1 m*M* NTS, the P_1-phosphate peak overlapped slightly with the injection peak. This anion can be easily separated using NDS as the mobile phase. Raising the mobile phase concentration to 0.15 m*M* NTS and using flow programming, a separation of a mixture of polyphosphates and polycarboxylates which now included P_3-phosphate instead of P_1-phosphate was possible with IPD (Fig. 5). Again, conductivity detection was troublesome because the P_2-phosphate peak becomes positive at this NTS concentration and more baseline disturbance was observed resulting in some distortion of the chromatogram.

A comparison of the detection limits of various anions using NTS as the mobile phase with both IPD and conductivity detection modes is shown in Table I. The detection limits of NTA and EDTA are the same with both detection modes. However, the citrate detection limit using conductivity detection is 40% lower than that for IPD. Conversely, the trimetaphosphate detection limit using IPD is 50% better than that for conductivity detection. These differences in detection limits are due to the fact that using a higher mobile phase concentration of 0.2 m*M* NTS for the determination of trimetaphosphate results in higher background conductance of 64 μS/cm, as opposed to only 32 μS/cm when a lower concentration of 0.1 m*M* NTS was used for citrate. P_2-Phosphate and P_3-phosphate gave essentially the same detection limits using both

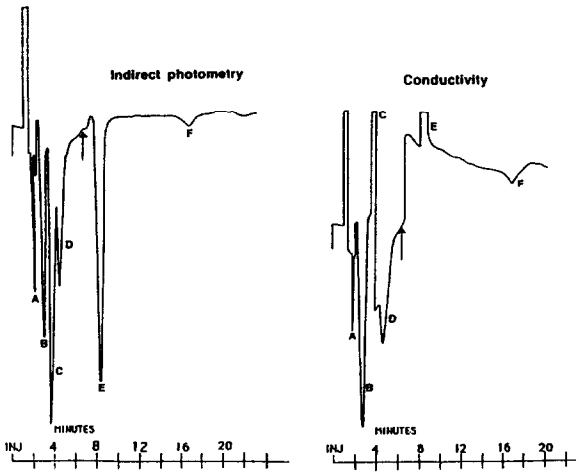

Fig. 5. Flow gradient for the separation of a standard mixture of polyphosphate and polycarboxylates on the PRP-X100 column with IPD (280 nm 0.05 AUFS) and conductivity detection (0.1 μS FS). Flow-rate of 1 ml/min changes to 2 ml/min at the arrow. Mobile phase: 0.15 m*M* NTS–ACN (95:5). Peaks: (A) 10 mg/l of NTA, 20 mg/l each of (B) EDTA, (C) P_2-phosphate, (D) citrate and (E) trimetaphosphate, (F) 10 mg/l of P_3-phosphate.

detection methods. The main reason for the higher detection limit for P_2-phosphate is the interference with the injection peak which increases in size with sample dilution. The detection limit of P_2-phosphate can be improved with a lower mobile phase concentration which causes no interference from the injection peak. P_4-Phosphate could be determined better by IPD rather than conductivity detection. This result is not surprising as P_4-phosphate is a much larger anion and has a lower ionic mobility resulting in lower conductance as compared to other members of the polyphosphate series. The detection limits obtained in our work range from 2 to 100 ng for polyphosphates and from 4 to 10 ng for polycarboxylates. Both ranges are at least an order of magnitude lower than those previously reported using other ion chromatography systems. In ion-interaction chromatography employing benzenetricarboxylic acids with indirect UV detection at 257 nm, detection limits of 60 and 100 ng for EDTA and citrate, respectively, were cited [29]. Detection limits for polyphosphates using the iron(III) postcolumn derivatization method are only in the 1–5 μg range [30].

TABLE I

DETECTION LIMITS COMPARISON FOR POLYPHOSPHATES AND POLYCARBOXYLATES ANIONS USING INDIRECT PHOTOMETRIC AND CONDUCTIVITY DETECTION MODES

Mobile phase 0.1 m*M* NTS–ACN (95:5) for nitrilotriacetate through citrate and 0.2 m*M* NTS–ACN (95:5) for pyrophosphate through tetrapolyphosphate. Detection limits: $S/N > 3$ based on peak height. Injection volume 20 μl.

Analyte	Detection limits, mg/l (ng)	
	IPD	Conductivity
Nitrilotriacetate	0.50 (10)	0.50 (10)
EDTA	0.25 (5)	0.25 (5)
Citrate	0.50 (10)	0.20 (4)
Pyrophosphate	1.0 (20)	1.25 (25)
Trimetaphosphate	0.10 (2)	0.20 (4)
Tripolyphosphate	1.0 (20)	1.0 (20)
Tetrapolyphosphate	2.5 (50)	5.0 (100)

Calibration plots starting from the detection limits for some of these polyvalent anions using NTS as the mobile phase with both detection modes are shown in Fig. 6. The average relative standard deviations of the slope of the calibration graphs were 1.0 and 0.9% for IPD and conductivity detection, respectively. The correlation coefficient varied from 0.9994 to 0.9999 for both detection methods. Reproducibility of three replicate injection of most analytes ranged from 1.1 to 1.8% R.S.D. As can be seen for all the analytes except for P_4-phosphate, the sensitivity (slope of the calibration line) obtained with conductivity detection is higher than that obtained with IPD. Fig. 6a shows the calibration runs for P_2-phosphate and trimetaphosphate. Their slope values are 16 and 2.5 times higher with conductivity detection than those by IPD, respectively. The upper limit of linearity of the P_2-phosphate anion (25 or 10 mg/l) is relatively short because of the interference with the injection peak which increases in size with solute concentration. The trimetaphosphate ion linear range extended to 75 and 50 mg/l with IPD and conductivity detection, respectively. Citrate showed a similar linear response to that of trimetaphosphate from the detection limit up to 75 mg/l. Similarly, the P_3-phosphate slope with conductivity detection is 7.6 times higher than

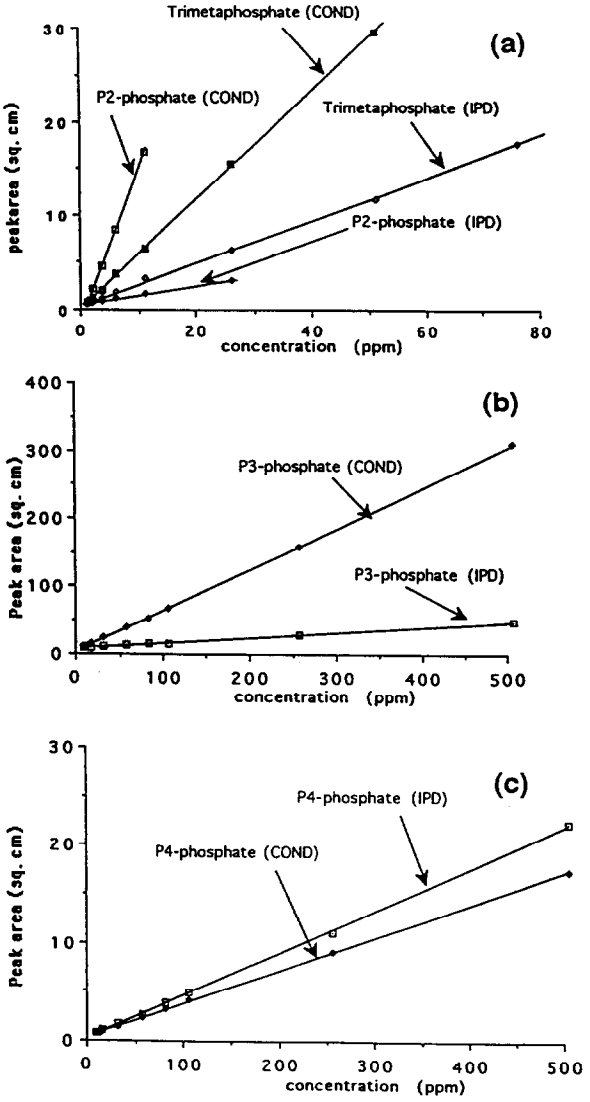

Fig. 6. Linearity comparison for (a) P_2-phosphate and trimetaphosphate, (b) P_3-phosphate and (c) P_4-phosphate using 0.2 m*M* NTS–ACN (95:5) as the mobile phase, with both IPD and conductivity detection.

that for IPD (Fig. 6b). On the other hand, the slope for P_4-phosphate is about 1.8 times higher for IPD rather than conductivity detection (Fig. 6c). Late eluting anions such as P_3- and P_4-phosphates have a much higher upper limit of quantitation with a dynamic concentration range to 500 mg/l.

Real samples such as citrate in diet Coke and P_3-phosphate in detergent can also be analyzed in less than 10 min using a NTS eluent. To expediate the IPD assay of citrate, the caffeine in Coke was removed by passing the sample through a disposable solid-phase C_{18} cartridge in tandem with a disposable 0.45-μm filter in a single operation. Caffeine did not interfere with the conductivity detection of citrate. No sample clean-up was done for the detergent sample.

The results obtained in this work are consistent with a previous comparison study of inorganic anions using naphthalenesulfonate mobile phases with both IPD and conductivity detection [20]. The low detection limits obtained in our work using both IPD and conductivity detection can be attributed to a number of useful properties associated with the use of a NTS eluent. Some of these include: (i) the large effective charge of NTS results in good elution capability of polyphosphates and polycarboxylates, (ii) high molar absorptivity and detection at a longer wavelength permits good IPD and (iii) lower background conductance allows non-suppressed conductivity detection to be more sensitive than a pH dependent eluent.

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