Journal of Chromatography, 516 (1990) 473–477
Elsevier Science Publishers B.V., Amsterdam

CHROM. 22 538

Note

Determination of trimetaphosphate in pyrophosphate by capillary isotachophoresis

PAVEL JANOŠ
Research Institute of Inorganic Chemistry, 400 60 Ústí nad Labem (Czechoslovakia)
(First received December 19th, 1989; revised manuscript received May 7th, 1990)

Pyrophosphates, like other phosphates, have many applications, including uses in the foodstuffs industry. On the other hand, condensed cyclic polyphosphates are considered to be potentially detrimental to health. Hence a requirement has arisen for the selective determination of cyclic triphosphate (trimetaphosphate) in foodstuff-grade pyrophosphate.

A number of procedures for the determination of phosphates have been described, including gravimetric, titrimetric and spectrophotometric[1] methods. These, however, do not permit individual types of phosphates to be determined selectively. Paper[1,2], thin-layer[1,3], ion-exchange[1,4,5] and ion chromatography[6] make it possible in some instances to separate and identify the phosphates but they are inadequate when a small amount of one type of phosphate is to be determined in the presence of a large excess of other phosphates.

Papers dealing with the determination of various phosphates by capillary isotachophoresis have been published (*e.g.*, refs. 7–9).

Yagi *et al.*[10] have described the separation of different phosphorus oxo acids, together with trimetaphosphate and pyrophosphate, using capillary isotachophoresis. The separation and determination of trimetaphosphate in a mixture with other phosphates has been reported[11,12].

In the above studies chloride served as the leading ion in most instances and a histidine buffer solution was used to maintain the required pH value. In this system the effective mobility of the trimetaphosphate anion is close to that of the leading ion, which makes detection and identification difficult. Motooka *et al.*[13] were successful in altering the effective mobility of condensed cyclic phosphates by the addition of bivalent inorganic cations (cations of alkaline-earth elements). In this study we have attempted to influence the effective mobility of the separated ions by addition of organic cations (quaternary ammonium bases) with the objective of developing a method for the determination of trimetaphosphate in pyrophosphate.

0021-9673/90/$03.50

EXPERIMENTAL

Apparatus

The measurements were carried out on an Agrofor isotachograph (JZD Odra, Krmelín, Czechoslovakia) equipped with a PTFE separation capillary (300 × 0.4 mm I.D.), a 20-μl sampling valve and a conductimetric detector connected to a TZ 4620 line recorder (Laboratorní přístroje, Prague, Czechoslovakia). The pH of the solutions was checked with an OP-211/1 pH meter (Radelkis, Budapest, Hungary) with an Orion 81-02 combined electrode.

Chemicals

The leading electrolytes were prepared by combining the following stock solutions in appropriate proportions: 0.1 mol/l hydrochloric acid (Merck, Darmstadt, F.R.G.), 0.1 mol/l hydrobromic acid (Lachema, Brno, Czechoslovakia), 0.1 mol/l tetrabutylammonium hydroxide (Fluka, Buchs, Switzerland) and 0.01 mol/l cetyltrimethylammonium bromide in methanol, 1% poly(vinyl alcohol) (purified on a mixed ion exchanger). The solutions containing cetyltrimethylammonium bromide were always prepared a day in advance and were filtered prior to use.

As terminating electrolytes 0.1 mol/l solutions of 2-thio-6-azathymine, caproic acid (Serva, Heidelberg, F.R.G.) and potassium hydrogentartrate (Lachema) were used.

Histidine (Reanal, Budapest, Hungary) was adopted for adjusting the pH value of solutions.

All the chemicals used [except poly(vinyl alcohol)] were of analytical-reagent-grade. Water deionized on a mixed-bed ion-exchange column served for preparing the solutions.

RESULTS AND DISCUSSION

Trimetaphosphoric acid is a comparatively strong acid. At pH > 3 it is dissociated almost completely to the third degree of dissociation. Therefore, it is not possible to influence the effective mobility of the trimetaphosphate ion by the method that is most commonly applied in isotachophoresis, *i.e.*, by a change in the pH of the leading electrolyte. This may be demonstrated with published isotachophoretic indices[14]. Within the pH range 3–10 the relative detector response ($R_E = R_X/R_{Cl}$) ranges from 1.088 to 1.061, *i.e.*, it differs from the R_E value of the chloride ion less than required for a successful separation[14] ($\Delta R_E > 0.15$).

In spite of this, it has been demonstrated[10–12] that trimetaphosphate can be determined in the normal HCl–histidine system, provided that the content of the analyte in the sample is not too high. According to Hirokawa *et al.*[15], the effective mobility of the trimetaphosphate anion decreases owing to the formation of an ion pair with the histidine cation. In spite of this, however, difficulties were encountered when working with this system, as proper functioning of the Agrofor instrument requires that there should be a sufficiently large difference between the effective mobilities of the leading ion and of the first of the separated ions.

The effective mobility of cyclic polyphosphates can be influenced by addition to the leading electrolyte of, *e.g.*, Ca^{2+}, Mg^{2+}, Sr^{2+} or Ba^{2+}[13]. If, however, in addition

to the trimetaphosphate that is being determined, the sample also contains a large amount of pyrophosphate, there is a risk of precipitation of sparingly soluble salts of these elements[16].

We examined the effect of the presence of quaternary ammonium bases in the leading electrolyte on the isotahophoretic behaviour of trimeta-, pyro- and orthophosphate anions. Tributylammonium cation (TBA^+) and cetyltrimethylammonium cation ($CTMA^+$) were selected as they are frequently used in ion-pair reversed-phase chromatography (see, *e.g.*, ref. 17). The measurements with TBA^+ were carried out in an operating system employing Cl^- as the leading ion and those with $CTMA^+$ in a system with Br^- as the leading ion in 25% (v/v) methanol (because of the limited solubility of CTMA-Br in water).

It was found that TBA^+ does not affect the migration of ortho- and pyrophosphates; only in with the trimetaphosphate anion at concentrations of TBA^+ higher than about 3 mmol/l was a certain decrease in effective mobility observed (Fig. 1).

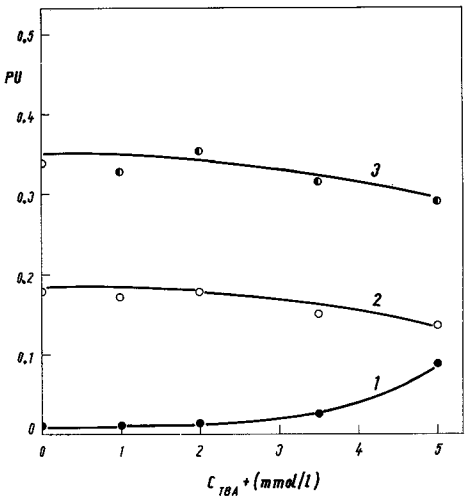
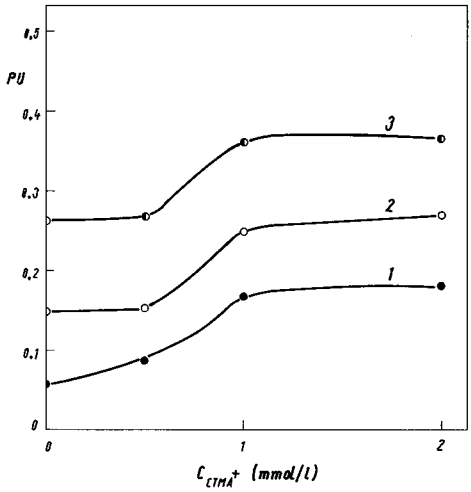


Fig. 1. Effect of TBA^+ concentration on the *PU* values of separated ions. Leading electrolyte (LE): 0.01 mol/l Cl^- + TBA^+ + histidine + 0.05% poly(vinyl alcohol), pH = 5.5. Terminating electrolyte (TE): 0.01 mol/l 2-thio-6-azathymine. *PU* = potential units[14] calculated from responses of the conductimetric detector; $PU = (R_X - R_L)/(R_T - R_L)$. R_X, R_L and R_T are zone heights of the analyte, leading and terminating ions, respectively. 1 = Trimetaphosphate; 2 = pyrophosphate; 3 = orthophosphate.

It is probable that the trimetaphosphate anion, $P_3O_9^{3-}$, forms an ion-pair with TBA^+ in a similar manner to the histidine cation[15], whereas ortho- and pyrophosphate anions do not form ion-pairs.

It can be seen in Fig. 2 that trimeta-, pyro- and orthophosphates can be separated effectively in the HBr–histidine system in 25% (v/v) methanol. The influence of $CTMA^+$ is more pronounced than that of TBA^+ and manifests itself at $CTMA^+$ concentrations as low as 0.5–1 mmol/l. Fig. 3 shows the isotachophoretic separation of trimeta-, pyro- and orthophosphate in the presence of TBA^+. It can be seen in Fig. 4



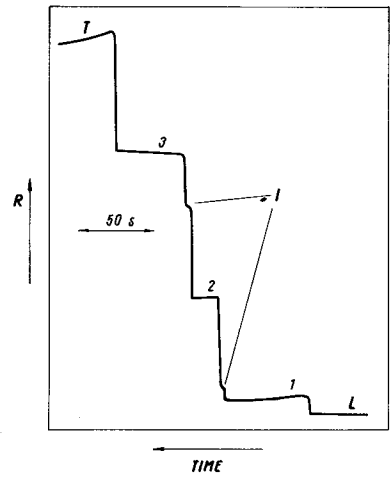
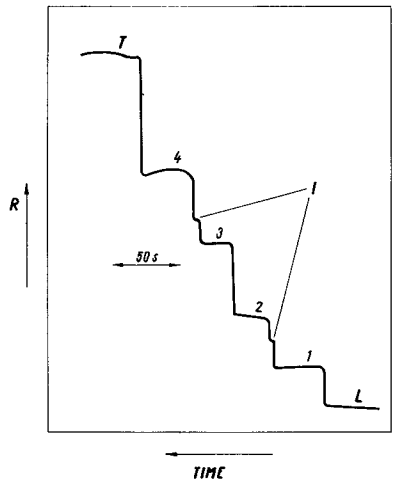


Fig. 2. Effect of $CTMA^+$ concentration on the *PU* values of separated ions. LE: 0.01 mol/l Br^- + $CTMA^+$ + histidine + 0.05% poly(vinyl alcohol) in 25% (v/v) methanol, pH = 5.3. TE: 0.01 mol/l 2-thio-6-azathymine. 1 = Trimetaphosphate; 2 = pyrophosphate; 3 = orthophosphate.

Fig. 3. Isotachopherogram of the separation of a model mixture of trimeta-, pyro- and orthophosphates (system with TBA^+). LE: 0.01 mol/l Cl^- + 0.004 mol/l TBA^+ + histidine + 0.05% poly(vinyl alcohol), pH = 5.5. TE: 0.01 mol/l caproic acid. Driving current intensity during recording = 60 μA. 1 = Trimetaphosphate; 2 = pyrophosphate; 3 = orthophosphate; I = unidentified impurities. R = Resistance.



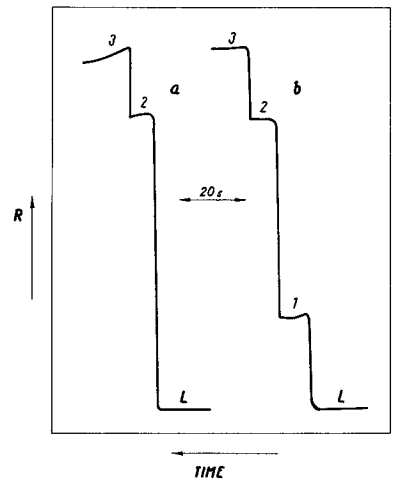


Fig. 4. Isotachopherogram of the separation of a model mixture of sulphate and trimeta-, pyro- and orthophosphates (system with $CTMA^+$). LE: 0.01 mol/l Br^- + 0.001 mol/l $CTMA^+$ + histidine + 0.05% poly(vinyl alcohol) in 25% (v/v) methanol, pH = 5.3. TE: 0.01 mol/l caproic acid. Driving current intensity during recording = 60 μA. 1 = Sulphate; 2 = trimetaphosphate; 3 = pyrophosphate; 4 = orthophosphate; I = unidentified impurities.

Fig. 5. Analysis of commercial pyrophosphate. LE: as in Fig. 3. TE: 0.01 mol/l potassium hydrogentartrate. Sample: (a) $Na_2H_2P_2O_7$ (Ladensburg, F.R.G.), 0.2 g per 100 ml; (b) same as (a), spiked with *ca.* 0.5% of trimetaphosphate. Driving current intensity during recording = 60 μA. 1 = Trimetaphosphate; 2 = tartrate; 3 = terminating zone, mixed zone of tartrate and pyrophosphate.

that trimeta-, pyro- and orthophosphates are separated very effectively in a system with $CTMA^+$ in a mixed aqueous–methanolic medium. This system can be considered optimum for separating the above phosphates together with sulphates (which can serve as an internal standard). In this instance probably both the influence of the mixed solvent and the formation of ion-pairs of the ions to be separated with $CTMA^+$ are combined to give this final effect. A disadvantage of this system for practical use in the limited stability of the leading electrolyte solution.

Therefore, we adopted the system with TBA^+ for routine determinations of trimetaphosphate in pyrophosphate. Tartrate was chosen as the terminating electrolyte. Its effective mobility in the given system is close to that of pyrophosphate and hence the terminating zone during an analysis is formed by a mixed zone of tartrate with pyrophosphate. The selection of the faster moving tartrate (compared with hexanoate) makes it possible to use a higher intensity of the driving current and thus shorten the duration of analysis. The composition of the leading electrolyte is 0.01 mol/l Cl^- + 0.004 mol/l TBA^+ + 0.05% poly(vinyl alcohol), pH = 5.5, adjusted with histidine.

An example of the analysis of commercial pyrophosphate used in the foodstuffs industry as an additive to baking powders is shown in Fig. 5. Under the conditions specified trimetaphosphate in pyrophosphate can be determined at concentration levels down to 0.1%, which satisfied the given application.

The equation of the linear calibration graph for the determination of trimetaphosphate is $y = 0.0340x - 0.0089$, where y (s) is the zone length (at a driving current of 60 μA) and x (mg/l) is the concentration of trimetaphosphate for an injection of 20 μl (correlation coefficient = 0.9996, $n = 9$). The detection limit, established under the conditions specified in Fig. 5 and defined according to ref. 18 as the absolute amount of the analyte that will give a zone passing through the detector during 1 s, is *ca.* 0.5 μg.

REFERENCES

1 S. Greenfield and M. Clin, *Analytical Chemistry of the Condensed Phosphates*, Pergamon Press, Oxford, 1975.
2 T. Ando and J. Ito, *Bull. Chem. Soc. Jpn.*, 25 (1952) 78.
3 N. Takai, T. Iida and J. P. W. Houtman, *Fresenius' Z. Anal. Chem.*, 244 (1969) 38.
4 J. Beukenkamp and W. Rieman, *Anal. Chem.*, 26 (1954) 505.
5 D. Lúčanský, *Chem. Prům.*, 26 (1976) 514.
6 F. C. Smith and R. C. Chang, *The Practice of Ion Chromatography*, Wiley, New York, 1983, p. 46.
7 D. Lúčanský, V. Bátora, J. Polonský and J. Garaj, in F. M. Everaerts (Editor), *Analytical Isotachophoresis (Analytical Chemistry Symposium Series*, Vol. 6), Elsevier, Amsterdam, 1981, p. 147.
8 F. Mikkers, W. van Gils and W. Roos, in F. M. Everaerts (Editor), *Analytical Isotachophoresis (Analytical Chemistry Symposium Series*, Vol. 6), Elsevier, Amsterdam, 1981, p. 91.
9 P. Boček, B. Kaplanová, M. Deml and J. Janák, *J. Chromatogr.*, 153 (1978) 287.
10 T. Yagi, K. Kojima, H. Nariai and I. Motooka, *Bull. Chem. Soc. Jpn.*, 55 (1982) 1831.
11 T. Hirokawa, S. Kobayashi and Y. Kiso, *J. Chromatogr.*, 318 (1985) 195.
12 I. Motooka, K. Nakazaki, H. Nariai and M. Tsuhako, *J. Chromatogr.*, 367 (1986) 271.
13 I. Motooka, H. Nariai, K. Nakazaki and M. Tsuhako, *J. Chromatogr.*, 295 (1984) 533.
14 T. Hirokawa, M. Nishino, N. Aoki, Y. Kiso, Y. Sawamoto, T. Yagi and J. Akiyama, *J. Chromatogr.*, 271 (1983) D1.
15 T. Hirokawa, S. Kobayashi and Y. Kiso, *J. Chromatogr.*, 410 (1987) 279.
16 J. R. van Wazer, *Phosphorus and its Compounds*, Vol. 1, Interscience, New York, 1958, pp. 628–630.
17 P. R. Haddad and A. L. Heckenberg, *J. Chromatogr.*, 300 (1984) 357.
18 I. Valášková, I. Zelenský, V. Zelenská, A. Komarnicki and D. Kaniansky, *Collect. Czech. Chem. Commun.*, 53 (1988) 515.