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Separation of synthetic inorganic polymers of condensed phosphates by capillary gel electrophoresis with indirect photometric detection

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Abstract

Separation of synthetic inorganic polymers of condensed phosphates was carried out by capillary gel electrophoresis with indirect photometric detection. Pyromellitic acid was employed as ultraviolet (UV) absorbing background electrolyte and absorbance of UV was measured at 254 nm. The separation of condensed phosphates in 12% (w/v) linear polyacrylamide gel was found to be mainly dependent on the size of the separands, but charge-to-size ratio played a part in the migration behaviour of orthophosphate and metaphosphates. The results presented in this paper demonstrate that the combination of capillary gel electrophoresis with indirect photometric detection provides a highly efficient and universal method which should be useful for the characterization of nonabsorbing ionic polymers. © 1998 Elsevier Science B.V.

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1. Introduction

Condensed phosphates is a generic term for all phosphate polymers resulting from the elimination of water between orthophosphoric acid molecules or orthophosphate salts. Examples are linear polyphosphoric acids.

$$H_{(n-1)+2}P_{(n-1)}O_{3(n-1)+1} + H_3PO_4 \rightarrow H_{n+2}P_nO_{3n+1} + H_2O \quad (1)$$

Polyphosphates derived from linear polyphosphoric acids are linear polyanions of $P_nO_{3n+1}^{(n+2)-}$ (effective charges of polyphosphates in solution depend strongly on pH). The use of condensed phosphates can be found in many industries such as detergents, foods, fertilisers and metal treatments [1]. They are very interesting materials from the viewpoint of electrolyte solution chemistry because of negative charges on their relatively compact molecules [2]. During their manufacture as either the acid or salt, control of the final mixture is both an art and a science.

In a particular application, the usefulness and effectiveness of a typical polydisperse polymer often strongly depend on its molecular mass distribution. But characterization of chain length distributions of condensed phosphates is a challenging problem. To determine number-average chain length ($\bar{n}$) of condensed phosphates, the most common method is at present pH titration [3]. Other methods, such as ^{31}P NMR (nuclear magnetic resonance), are also reported to be useful in the determination of number-average chain length ($\bar{n}$) [4,5]. Separation methods which can determine chain length distributions of condensed phosphates include paper chromatography

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[6], ion chromatography [5,7–12], and isotachophoresis [13,14]. Nevertheless, most of the above methods can only separate polyphosphates of a range of short chain lengths much less than ten. Some ion chromatographic methods were able to separate polyphosphates of chain lengths of several tens, but they usually required long separation time and involved a time-consuming process for equilibrating the column due to the use of gradient elution [5,8,12].

Capillary electrophoresis (CE) emerged as a high-performance separation technique due to the fact that high area-to-volume ratios of small capillaries can efficiently dissipate the Joule heat generated during electrophoretic processes, and therefore high voltage can be applied to produce rapid and highly efficient separations [15,16]. Versatility of CE can be readily realised by using different electrophoretic media without changing basic experimental set-up. Use of gel-filled capillaries established another mode of CE [17,18], capillary gel electrophoresis (CGE), which renders size-dependent characteristic to the separation of ionic polymers. CGE was demonstrated to be a separation technique of extremely high separation efficiency in terms of theoretical plate number. For example, over millions of theoretical plates were achieved in CGE separation of oligonucleotides [19,20], which was attributed to the focusing of the sample plug at the injection end of gel-filled capillaries and slow diffusion of analytes in gels. To date, the applications of CGE for the separation of polydisperse polymers, with both crosslinked and linear gels, have been almost exclusively for biopolymers [18–26]. Few reports have been made on the CGE separation of synthetic polymers of other materials [27,28]. In fact, there has been very little work done so far on the separation of synthetic polymers by any other modes of CE [28–36]. The CE separation of polyphosphates were not achieved beyond tetrapolyphosphates [31–33] until most recently when Stover [36] reported that polyphosphates up to chain lengths of ca. 30 were separable in buffers containing a cationic electroosmotic flow (EOF) modifier which played a role in ion-pairing with the polyphosphates.

To detect nonabsorbing ions, either cations or anions, indirect UV detection based on the displacement of UV absorbing co-ions in the background electrolyte by the nonabsorbing ions was the most common method in use [37–42]. Our previous study showed chromate to be unsuitable as the UV absorbing co-ion for detection of polyphosphates because mismatch of high mobility of chromate with low mobilities of polyphosphates resulted in severely tailing peaks [33]. Pyromellitic acid was reported to be a better UV absorbing background electrolyte in CE for the separation and the detection of orthophosphate and polyphosphates [36,42].

To the authors' knowledge, there has been neither any studies on CGE separation of inorganic synthetic polymers, nor any reports on the feasibility of exploiting the combined advantages of the universality of indirect UV detection and the high efficiency of CGE, although each of their merits has been well recognized. In this paper we report the results of the separation of condensed phosphates by using linear polyacrylamide gel-filled capillary with indirect UV detection. These results are compared with those obtained in other types of electrophoretic media.

2. Experimental

2.1. Chemicals

Polyphosphoric acid (sodium salt), tripolyphosphate pentasodium salt, trisodium trimetaphosphate, tetrapolyphosphate hexaammonium salt, pyromellitic acid (PMA) and methylcellulose (viscosity of 1500 centipoises for 2% aqueous solution at 25°C) were products of Sigma (St. Louis, MO, USA). Cetyltrimethylammonium bromide (CTAB) and sodium pyrophosphate were purchased from Aldrich (Milwaukee, WI, USA). Tris(hydroxymethyl) aminomethane (Tris), acrylamide and *N,N,N′,N′*-tetramethylethylenediamine (TEMED) of electrophoresis purity reagent were obtained from Bio-Rad (Richmond, CA, USA). Ammonium persulfate (APS) was obtained from J.T. Baker (Phillipsburg, NJ, USA). Disodium hydrogenphosphate anhydrous and 3-(trimethoxysilyl)propylmethacrylate were supplied by Fluka (Buchs, Switzerland). Other chemicals used were of analytical grade. Deionized water used throughout the experiments was prepared with a Milli-Q system (Millipore, Bedford, MA, USA).

CE buffers consisted of 5.0 m*M* pyromellitic acid

and 20 m*M* Tris and had a pH of 7.2. To suppress cathodic EOF in bare fused-silica capillaries, 0.02 m*M* of CTAB was added into the above buffer. Polyacrylamide coated capillaries and linear polyacrylamide gel-filled capillaries were prepared by following Hjerten's procedure [43]. Solution obtained by dissolving 0.4 g acrylamide in 10 ml water was used for the preparation of the coated capillaries and solution obtained by dissolving 1.2 g acrylamide in 10 ml buffer consisting of 5.0 m*M* pyromellitic acid and 20 m*M* Tris (pH=7.2) for the linear polyacrylamide gel-filled capillaries.

2.2. Apparatus

CE was performed with a laboratory-built CE system, equipped with a Spellman CZE1000R power supply with dual polarities (Spellman, Plainview, NY, USA) and a Linear UVIS 200 detector (Linear Instruments, Reno, NV, USA). Electropherograms were recorded with a HP 3390A integrator (Hewlett-Packard, Avondale, PA, USA) connected with a switch for changing the polarity of the input signal. Polyamide coated fused-silica capillaries of an inner diameter (I.D.) of 50 μm and an outer diameter (O.D.) of 362 μm (Polymicro Technologies, Phoenix, AZ, USA) were used. The total length (L_t) was ca. 40 cm and the effective length (L_e) was 30 cm as specified in Figure captions. On-column indirect UV detection was conducted at 254 nm. Temperature was maintained at 25±1°C.

3. Results and discussion

No peak was observed within 35 min of run time when separation of polyphosphoric acid (sodium salt) was conducted at a positive polarity of 7.8 kV in an electrophoretic buffer of 5.0 m*M* PMA and 20 m*M* Tris at pH=7.2. It might be due to the fact that EOF could not adequately overcome the mobilities of the phosphate and polyphosphates under the above conditions. Then, the separation was performed in the same electrophoretic media, but in capillaries with polyacrylamide coating for suppressing the EOF and with the power supply operated at reverse polarity. A typical electropherogram is shown in Fig. 1. The peak of orthophosphate was identified by spiking the sample solution with orthophosphate standard and was found to be overlapping with the peaks of some of the slowest migrating polyphosphates. This result implies that the mobility of orthophosphate under this set of experimental conditions was very low. The fast migrating peaks were identified to be trimetaphosphate, tripolyphosphate, pyrophosphate and tetrapolyphosphate. It is worth

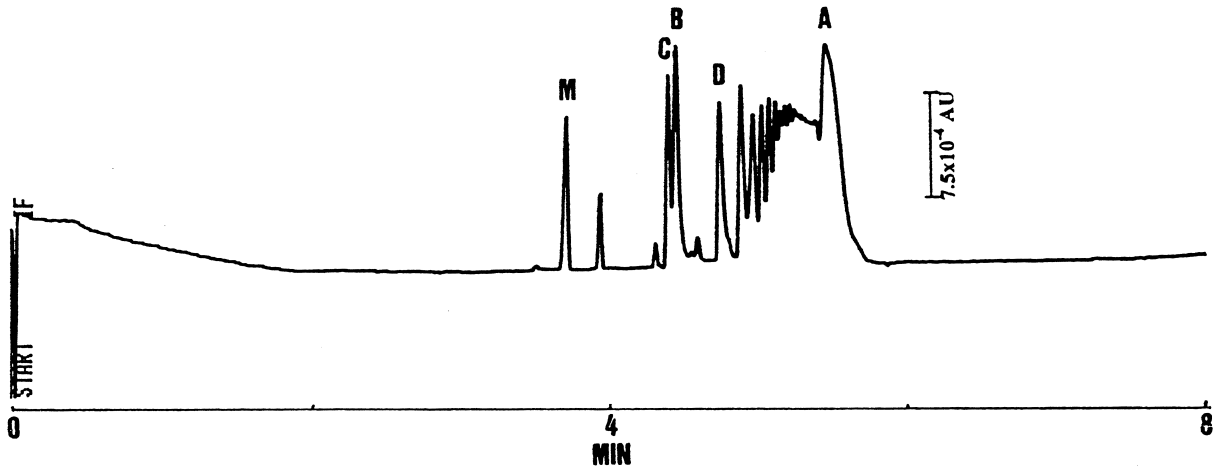

Fig. 1. Separation of polyphosphoric acid (sodium salt) in electrophoretic buffer of 5.0 m*M* PMA and 20 m*M* Tris (pH=7.2) inside a polyacrylamide coated capillary. Sample: polyphosphoric acid (sodium salt) 1.0 mg/ml; hydrodynamic injection: 5.0 cm for 10 s; detection: 254 nm; separation voltage: −7.8 kV; L_t=40.0 cm and L_e=30.0 cm. Peaks: A=orthophosphate (overlapped with some polyphosphates); B=pyrophosphate; C=tipolyphosphate; D=tetrapolyphosphate; M=trimetaphosphate. For other experimental conditions see Section 2.

noting that the migration orders of these polyphosphates of short chain lengths could be correlated with the charge-to-size ratios of the separands. The merged peaks of polyphosphates indicated the similarities in the mobilities of these polyphosphates in spite of the differences in their chain lengths. Fig. 2a and Fig. 2b show typical electropherograms for polyphosphoric acid (sodium salt) obtained in an electrophoretic buffer of 5.0 m*M* PMA and 20 m*M* Tris containing 0.02 m*M* CTAB as EOF modifier (pH = 7.2). These electropherograms are generally similar to that in Fig. 1, but the orthophosphate was better separated from polyphosphates in Fig. 2a and b. The results obtained showed that orthophosphate

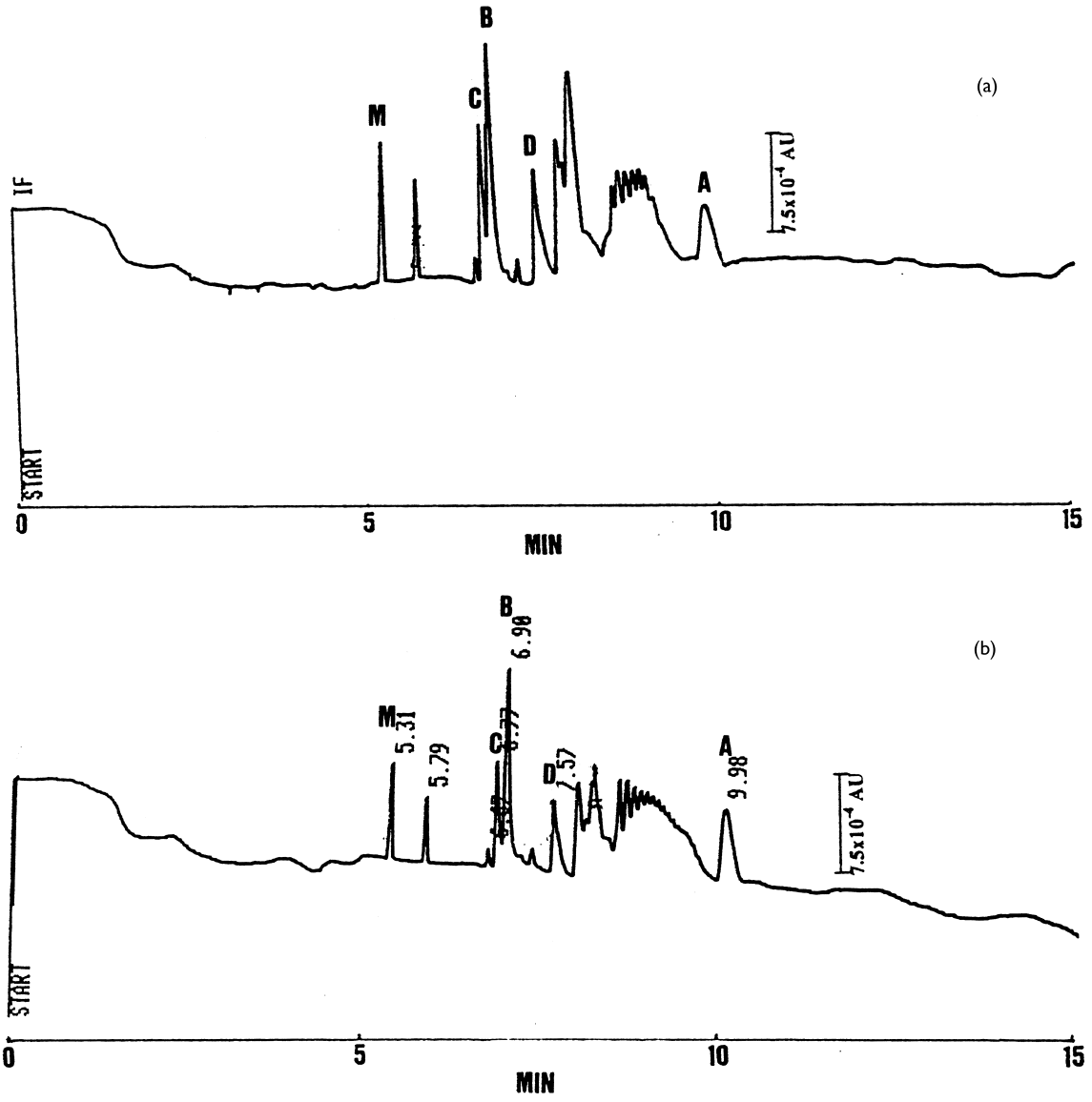

Fig. 2. Separation of polyphosphoric acid (sodium salt) in electrophoretic buffer of 5.0 m*M* PMA and 20 m*M* Tris containing 0.02 m*M* CTAB as EOF modifier (pH = 7.2). (a) Electrokinetic injection: −1.5 kV for 10 s. (b) Hydrodynamic injection: 5.0 cm for 10 s. For identification of peaks A, B, C, D and M refer to Fig. 1. Other experimental conditions as in Fig. 1.

and polyphosphates up to tetrapolyphosphate could be separated by free solution capillary zone electrophoresis, but the separation of polyphosphates of greater chain lengths could not be achieved readily by this method.

Fig. 3 shows a typical electropherogram for polyphosphoric acid (sodium salt) obtained in an electrophoretic buffer of 5.0 m*M* PMA and 20 m*M* Tris containing 1% methylcellulose (pH = 7.2). Such entangled methylcellulose solution was reported to be a useful alternative size sieving medium for electrophoretic separation of DNA fragments [44]. It is seen that there are only two narrow peaks in the electropherogram and all the other peaks are lumped together in Fig. 3. The poorer resolution might be attributed to the heterogeneity of the electrophoretic medium. No size sieving effect was observed in the separation of polyphosphates in the electrophoretic medium containing 1% methylcellulose. It implies that mesh size of 1% entangled methylcellulose was too large for separating the polyphosphates [45].

Finally, the separation of polyphosphoric acid (sodium salt) was carried out in linear polyacrylamide gel-filled capillaries. Fig. 4 shows a typical electropherogram obtained for the separation of polyphosphoric acid (sodium salt) in the linear polyacrylamide gel-filled capillaries, which were prepared by polymerisation of 12% (w/v) acrylamide solution containing 5.0 m*M* PMA and 20 m*M* Tris (pH = 7.2). It is observed that there are about 30 peaks in the electropherogram. Compared with Figs. 1–3, the best separation of the polyphosphates could be obtained by employing the conditions described in Fig. 4. It is interesting to note the migration order of trimetaphosphate, pyrophosphate, tripolyphosphate, orthophosphate and tetrapolyphosphate in the gel medium and to compare them with that in Fig. 1. In Fig. 4, migration time increased from pyrophosphate to tripolyphosphate and then tetrapolyphosphate, but orthophosphate shows longer migration time than pyrophosphate and tripolyphosphate, whereas trimetaphosphate migrates faster than orthophosphate and all linear polyphosphates. These results indicate that the separation of the linear polyphosphates is on the basis of their size and that the entire separation pattern of the condensed phosphates is not solely size dependent since the charge-to-size ratios play a significant role in the migration behaviour of orthophosphate and metaphosphates. Quantitative analysis of each component in the polyphosphates requires correction for the bias generated by the electrokinetic injection besides the consideration of differences in

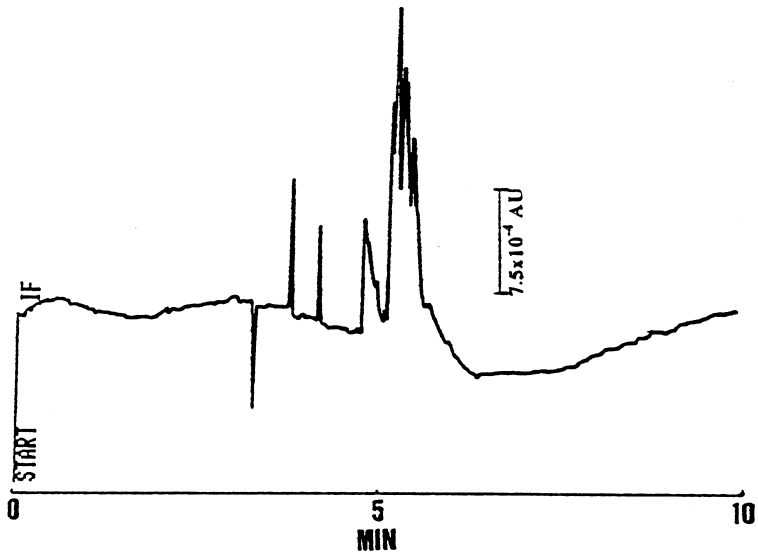

Fig. 3. Separation of polyphosphoric acid (sodium salt) in electrophoretic buffer of 5.0 m*M* PMA and 20 m*M* Tris containing 1% methylcellulose (pH = 7.2). Electrokinetic injection: −1.5 kV for 5 s; L_t = 39.0 cm and L_e = 29.5 cm. Other experimental conditions as in Fig. 1.

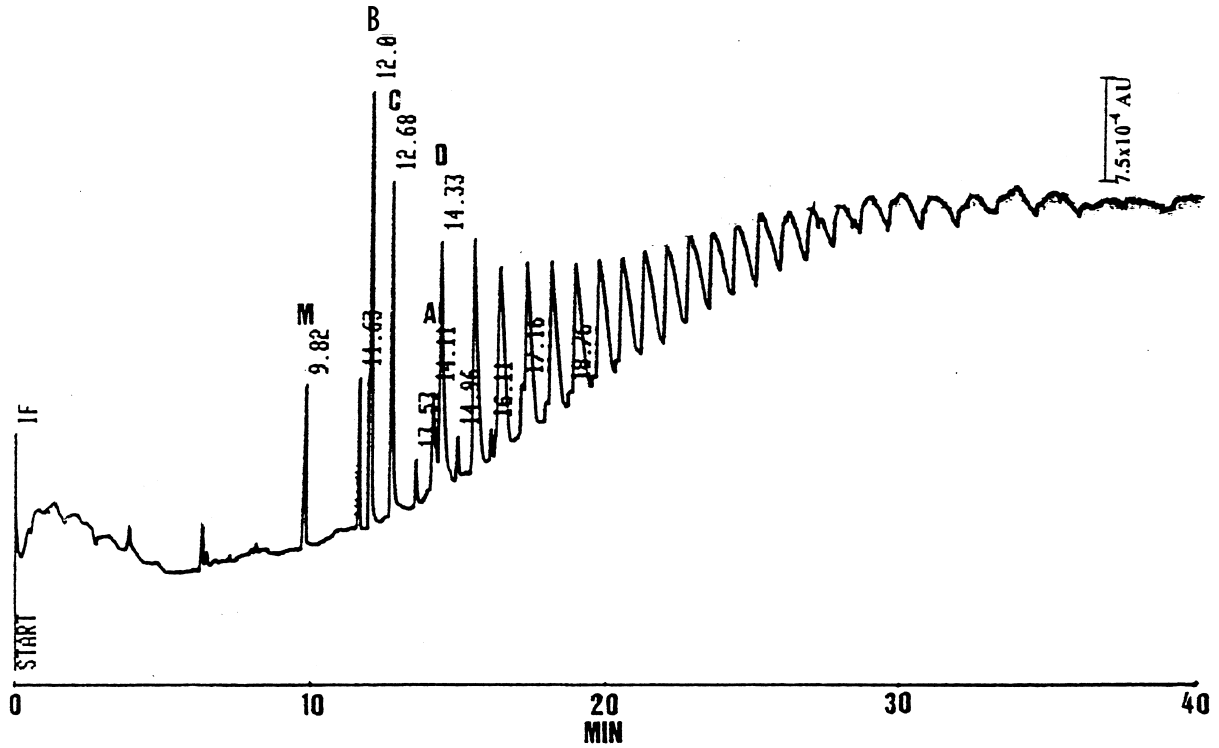

Fig. 4. Separation of polyphosphoric acid (sodium salt) in a linear polyacrylamide gel-filled capillary. The linear polyacrylamide gel was prepared by polymerisation of 12% (w/v) acrylamide solution containing 5 m*M* PMA and 20 m*M* Tris (pH = 7.2). Electrokinetic injection: −4.5 kV for 5 s. Separation voltage: −8.4 kV; L_t = 40.0 cm and L_e = 31.0 cm. For identification of peaks A, B, C, D and M refer to Fig. 1. Other experimental conditions as in Fig. 1.

the detection response for polyphosphates of different chain lengths. Study on quantitation of each component in the polyphosphates will be performed in our future work.

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References

[1] D.E.C. Corbridge, Phosphorus: An Outline of its Chemistry, Biochemistry and Technology, Elsevier, Amsterdam, 5th ed., 1995, p. 172.
[2] J.R. Van Wazer, Phosphorus and its Compounds, Interscience, New York, 1958.
[3] J.R. Van Wazer, E.J. Griffith, J.F. McCullough, Anal. Chem. 26 (1954) 1755.
[4] J.C. MacDonald, M. Mazurek, J. Magn. Reson. 72 (1987) 48.
[5] F.S. Stover, J.A. Bulmahn, J.K. Gard, J. Chromatogr. A 688 (1994) 89–95.
[6] Y. Kiso, M. Kobayashi and Y. Kitaoka, in M. Halmann (Editor), Analytical Chemistry of Phosphorus Compounds. Wiley-Interscience, New York, 1972, Ch. 3, p. 124.
[7] T. Nakamura, T. Yano, A. Fujita, S. Ohashi, J. Chromatogr. 130 (1977) 384–386.
[8] Y. Baba, N. Yoza, S. Ohash, J. Chromatogr. 350 (1985) 119–125.
[9] P. Linares, M.D. Luque de Castro, M. Valcacel, J. Chromatogr. 585 (1991) 267–271.
[10] S.A. Shimsi, N.D. Danielson, J. Chromatogr. A 653 (1993) 153–160.
[11] D.J. Halliwell, I.D. McKelvie, B.T. Hart, R.H. Dunhill, Analyst 121 (1996) 1089–1093.
[12] E.S. Baluyot, C.G. Hartford, J. Chromatogr. A 739 (1996) 217–222.
[13] T. Yagi, K. Kojima, H. Nariai, I. Motooka, Bull. Chem. Soc. Jpn. 55 (1982) 1831–1833.

[14] I. Motooka, H. Nariai, K. Nakazaki, M. Tsuhako, J. Chromatogr. 260 (1983) 377–382.
[15] J.W. Jorgenson, K.D. Lukacs, J. Chromatogr. 218 (1981) 209.
[16] J.W. Jorgenson, K.D. Lukacs, Anal. Chem. 53 (1981) 1298.
[17] S. Hjerten, J. Chromatogr. 270 (1983) 1–6.
[18] A.S. Cohn, B.L. Karger, J. Chromatogr. 397 (1987) 409.
[19] A.S. Cohn, D.R. Najarian, A. Paulus, A. Guttman, J.A. Smith, B.L. Karger, Proc. Natl. Acad. Sci. USA 85 (1988) 9660.
[20] A. Guttman, A.S. Cohn, D.N. Heiger, B.L. Karger, Anal. Chem. 62 (1990) 137–141.
[21] D.N. Heiger, A.S. Cohn, B.L. Karger, J. Chromatogr. 516 (1990) 33–48.
[22] A. Widhalm, C. Schwer, D. Blaas, E. Kenndler, J. Chromatogr. 549 (1991) 446–451.
[23] H.F. Yin, J.A. Lux, G. Schomburg, J. High. Resolut. Chromatogr. 13 (1990) 624.
[24] T. Wang, G.J. Bruin, J.C. Kraak, H. Poppe, Anal. Chem. 63 (1991) 2207.
[25] K. Ganzler, K.S. Greve, A.S. Cohn, B.L. Karger, A. Guttman, N.C. Cooke, Anal. Chem. 64 (1992) 2665.
[26] V. Dolnik, M. Novotny, J. Microcol. Sep. 4 (1992) 515.
[27] R.A. wallingford, Anal. Chem. 68 (1996) 2541–2548.
[28] F. Garcia, J.D. Henion, Anal. Chem. 64 (1992) 985–990.
[29] L.N. Amankwa, J. Scholl, W.G. Kuhr, Anal. Chem. 62 (1990) 2189–2193.
[30] J.B. Poli, M.R. Schure, Anal. Chem. 64 (1992) 896.
[31] F.S. Stover, S.S. Keffer, J. Chromatogr. A 657 (1993) 450–454.
[32] S.A. Shamsi, N.D. Danielson, Anal. Chem. 67 (1995) 1845.
[33] T. Wang, S.F.Y. Li, J. Chromatogr. A 723 (1996) 197.
[34] J. Bullock, J. Chromatogr. 645 (1993) 169–177.
[35] J.Y. Gao, P.L. Dubin, T. Sato, Y. Morishima, J. Chromatogr. A 766 (1997) 233–236.
[36] F.S. Stover, J. Chromatogr. A 769 (1997) 349–351.
[37] S. Hjerten, K. Elenbring, F. Kilar, J. Liao, A.J.C. Chen, C.J. Siebert, M. Zhu, J. Chromatogr. 403 (1987) 47.
[38] F. Foret, S. Fanali, A. Nardi, P. Boeck, Electrophoresis 12 (1990) 780.
[39] W.R. Jones, P. Jandik, J. Chromatogr. 546 (1991) 445.
[40] P. Jandik, W.R. Jones, A. Weston, P.R. Brown, LC·GC 9 (1991) 634.
[41] W. Beck, H. Engelhardt, Chromatographia 33 (1992) 313.
[42] M.P. Harrold, M.J. Wojtusik, J. Riviello, P. Henson, J. Chromatogr. 640 (1993) 463–471.
[43] S. Hjerten, J. Chromatogr. 347 (1985) 191–198.
[44] M. Zhu, D.L. Hansen, S. Burd, F. Gannon, J. Chromatogr. 480 (1989) 311.
[45] P.D. Grossman, D.S. Soane, Biopolymers 31 (1991) 1221–1228.