High-Performance Chromatographic Separation of Inositol Phosphate Isomers on Strong Anion Exchange Columns

Erika Skoglund,* Nils-Gunnar Carlsson, and Ann-Sofie Sandberg

Department of Food Science, Chalmers University of Technology, SE-402 29 Göteborg, Sweden

Inositol phosphates are focused on because of their functions in the cell, as well as their effects on the bioavailability of certain minerals. To improve the separation of inositol hexakis- to monophosphates ($InsP_6$−$InsP_1$) and their positional isomers, six different strong anion exchange columns (OmniPac PAX-100, CarboPac PA-100, CarboPac PA-10, IonPac AS11, Mini Q PC 3.2/3, and ION-120 anion column) were compared in two ion chromatographic analysis systems. $InsP_2$−$InsP_6$ were acidic gradient eluted, postcolumn derivatized, and UV detected in system 1, and $InsP_3$−$InsP_1$ were alkali gradient eluted and detected using chemically suppressed conductivity detection in system 2. Differences in retention data were shown depending on the column characteristics for inositol phosphates with various numbers of phosphate groups attached to the inositol ring. In system 1 the anion exchange columns PA-10 and PA-100 were best suited for the separation of isomers of $InsP_2$ and $InsP_6$−$InsP_3$, respectively. Mobile phase optimization was used to successfully separate inositol phosphate isomers, as illustrated in a food sample. In system 2, PAX-100 and AS11 were the only columns useful to separate isomers of $InsP_3$−$InsP_1$ and $InsP_3$−$InsP_2$, respectively, using the current eluent (NaOH).

Keywords: *Ion chromatography; anion exchange columns; inositol phosphate isomers*

INTRODUCTION

Inositol hexakisphosphate (phytate, $InsP_6$), the most abundant form of phosphorus in plants, has the ability to form insoluble complexes with multivalent metal ions and thereby cause poor bioavailability of minerals (Cheryan, 1980; Cosgrove, 1966). During food processing and digestion, $InsP_6$ can be degraded to inositol pentakis-, tetrakis-, tris, bis-, and monophosphates ($InsP_5$−$InsP_1$). Several degradation products of phytate have shown important physiological functions both in animals and in plants (Sirén et al., 1991; Streb et al., 1983). A number of reviews concerning methods for analysis of inositol phosphates have been published (Oberleas and Harland, 1986; Reddy et al., 1989; Xu et al., 1992). A high-performance ion chromatographic (HPIC) technique has been used as an important tool in the search for reliable methods to quantitatively and qualitatively determine inositol phosphates. Modern ion chromatography began in 1975 when Small and coworkers (Small et al., 1975) presented a system composed of a conductivity detector in conjugation with an ion exchange column and a second suppressor column. The suppressor column reduced the background conductivity by an ion exchange reaction with the eluent. This caused greatly increased sensitivity in detection of the sample ions. Today, membrane suppressors are used, allowing continuous regeneration. Small et al. (1975) also introduced the use of low-capacity pellicular resins, that is, ion exchange columns with low porosity and only surface modification. Ten years later, Phillippy and Johnston (1985) developed the first ion chromatographic method for the determination of inositol hexakisphosphate. Since then, several methods have been presented using HPIC for the separation of inositol phosphates and their different isomers (Mayr, 1988; Phillippy and Bland, 1988; Skoglund et al., 1997a,b). These methods use gradient elution, which allows ions of widely different retention behaviors to be eluted in the same run. For detection in ion chromatography electrical conductivity is still most commonly used (Walton and Rocklin, 1990) but is no longer the sole detection method. In our previous studies (Skoglund et al., 1997a,b) we described HPIC methods for separation and quantitative determination of the whole spectrum of inositol phosphates ($InsP_6$−$InsP_1$) and their isomers. We used anion exchange columns, OmniPac PAX-100 and CarboPac PA-10, available from Dionex (Sunnyvale, CA). The columns are composed of aminated latex particles agglomerated to sulfonic acid groups on polystyrene/divinylbenzene substrate. For efficient ion chromatography it is essential to have fast mass transfer between the stationary phase and the mobile phase. The structure and properties of the separator columns can be optimized for specific analytical problems. Little attention, however, has been paid to the use of column selectivity in chromatographic method development (Antle et al., 1985; Glajch et al., 1985).

In this paper, we discuss the retention and separation of inositol phosphate isomers on six different strong anion exchange columns (OmniPac PAX-100, CarboPac PA-100, CarboPac PA-10, IonPac AS11, Mini Q PC 3.2/3, and ION-120 anion column) using the method of Skoglund et al. (1997b) with its two analysis systems. Our approach is to describe the columns best suited for the separation of isomers of $InsP_6$−$InsP_1$.

MATERIALS AND METHODS

Instrumentation. The chromatographic systems used are referred to as systems 1 and 2, respectively, developed by Skoglund et al. (1997b). System 1 consisted of a biocompatible

* Author to whom correspondence should be addressed (telephone +46 313 35 13 46; fax +46 31 83 37 82; e-mail es@sik.se).

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Table 1. Column Characteristics

column	OmniPac PAX-100	CarboPac PA-100	CarboPac PA-10	IonPac AS11	Mini Q PC 3.2/3	ION-120 anion column
dimensions (length × i.d., mm)	250 × 4	250 × 4	250 × 4	250 × 4	30 × 3.2	120 × 4.6
pH range	0−14	0−14	0−14	0−14	3−11[a]	0−14
max operating pressure (MPa)	35	35	27.6	27.5	10	15.2
solvent stability (%)	95	100	90	100	100	not specified
capacity[b] (μeq)	40	90	100	45	14.4−21.6	100[c]
particle diameter (μm)	8.5	8.5	10	13	3	9
cross-linking[d] (%)	55	55	55	55	not specified	not specified
type of column packing	alkanol quaternary amine	alkyl quaternary amine	alkyl quaternary ammonium	alkanol quaternary ammonium	quaternary ammonium	quaternary ammonium
latex diameter (nm)	60	280	400	85		
latex cross-linking[e] (%)	4	6	5	6		

[a] pH stability 1−14 for short-term use. [b] Per column. [c] Per gram. [d] Polystyrene cross-linked with divinylbenzene (DVB). [e] Vinylbenzyl chloride (VBC) cross-linked with DVB.

HPLC pump (Waters model 626, Waters Associates, Milford, MA) equipped with a 50 μL injector loop and an analytical column. Inositol phosphates were detected, after postcolumn reaction, using UV detection (Waters 486, tunable absorbance detector). Absorbance was monitored at 290 nm. A gradient of HCl (1 mol/L, 1−92%) in conjugation with water (99−8%) was used for elution of inositol phosphates. The eluants were mixed, in a postcolumn reactor, with 0.1% $Fe(NO_3)_3 \cdot 9H_2O$ in a 2% solution of $HClO_4$, according to the method of Phillippy and Bland (1988). A mixing tee (Jour Research, Onsala, Sweden) and a homemade reactor coil consisting of a crocheted Teflon tube (i.d. = 0.2 mm, 4.5 m) were applied to avoid peak broadening, to get enough reaction time, and to achieve a high blending rate. The combined flow rate was 1.2 mL/min.

System 2 consisted of a gradient pump (Dionex series 4500 i) equipped with a 25 μL injector loop and an analytical column. An anion micromembrane suppressor (AMMS) was applied with conductivity detection. As regenerant for the AMMS, 25 mM sulfuric acid (5 mL/min) was used. For separation of various isomers of $InsP_1$, $InsP_2$, and $InsP_3$ the gradient elution was generated by mixing 200 mmol/L NaOH (8−65%), 50% 2-propanol (6%), and water (86−29%). To avoid interference of higher inositol phosphates, a fraction from system 1 containing $InsP_3$−$InsP_1$ was collected, evaporated, and diluted before injection. The NaOH eluant was prepared from 50% liquid NaOH (J. T. Baker, Deventer, Holland) to give a low carbonate amount in the solution.

All eluants were sparged with helium. Detector signals were processed by Borwin laboratory data system (Chromatography Software, JMBS Developpments, Grenoble, France).

Columns. The ion exchange columns used were OmniPac PAX-100, CarboPac PA-100, CarboPac PA-10, and IonPac AS11 available from Dionex, Mini Q PC 3.2/3 available from Pharmacia Biotech (Sollentuna, Sweden), and ION-120 anion column available from Interaction Chromatography Inc. (San Jose, CA). Column characteristics are shown in Table 1. All of the separator columns from Dionex are 250 × 4 mm i.d. These columns are latex-coated pellicular anion exchange columns. The Mini Q PC 3.2/3 column is 30 × 3.2 mm i.d., and the ION-120 anion column is 120 × 4.6 mm i.d. These two latter columns are directly functionalized at the surface.

Chemicals. *myo*-Inositol 2-monophosphate, dicyclohexylammoniumsalt, D-*myo*-inositol 1-monophosphate, cyclohexylammonium salt, D-*myo*-inositol 1,4-bisphosphate, potassium salt, l-*myo*-inositol 5,6-bisphosphate, cyclohexylammonium salt, D-*myo*-inositol 1,4,5-trisphosphate, hexasodium salt, D-*myo*-inositol 1,5,6-trisphosphate, ammonium salt, D-*myo*-inositol 3,4,5,6-tetrakisphosphate, ammonium salt, D-*myo*-inositol 1,3,4,5-tetrakisphosphate, ammonium salt, and *myo*-inositol 1,3,4,6-tetrakisphosphate, ammonium salt, were obtained from Sigma Chemical Co. (St. Louis, MO). $Ins(1,2,4)P_3$, $Ins(1,2,3)P_3$, $Ins(1,2,6)P_3$, $Ins(1,3,4)P_3$, $Ins(1,2,3,4)P_4$, $Ins(1,2,5,6)P_4$, and $Ins(1,2,4,5,6)P_5$ were received as a gift from Perstorp Pharma (Perstorp, Sweden). *myo*-Inositol 2,4-bisphosphate, tetraammonium salt, was obtained from Calbiochem Corp. (La Jolla, CA), and sodium phytate was obtained from BDH Chemicals Ltd. (Poole, England). Type 1 deionized water for HPIC was purified by a Millipore water system to a specific resistance of 18 MΩ·cm or greater. Sodium hydroxide 50% solution was purchased from J. T. Baker B.V. All other reagents used were of analytical grade.

Sample Preparation. A reference sample was prepared by dissolving 1.5 g of sodium phytate in 100 mL of 0.5 mol/L HCl. The solution was reflux boiled for 12 h and evaporated to dryness, and 100 mL of water was added to the hydrolyzed sample. A fermented wheat roll sample with a low content of $InsP_5$ and $InsP_6$ but a high content of $InsP_3$ and $InsP_4$ was analyzed for inositol phosphate isomers. The sample was extracted with HCl and purified on anion exchanger according to the method of Skoglund et al. (1997b).

Peak Identification. The elution order of different isomers of inositol phosphates was established on the basis of commercially available inositol phosphate isomers and isomers obtained as a gift from Perstorp Pharma as described above. Furthermore, the isomers $Ins(1,2,3,4,5)P_5$ and $Ins(1,2,4,5,6)P_5$ were prepared by enzymatic hydrolysis of sodium phytate (Türk et al., 1996). The identification of isomers was also performed by determination according to the method of Phillippy and Bland (1988) [$Ins(4,5,6)P_3$, $Ins(1,2,4,5)P_4$, $Ins(1,2,4,6)P_4$, $Ins(2,4,5,6)P_4$, $Ins(1,2,3,4,6)P_5$, $Ins(1,3,4,5,6)P_5$] and on Dionex AN65 [Ins(1)P, Ins(4)P, $Ins(1,4)P_2$, $Ins(4,5)P_2$, $Ins(2,4,5)P_3$]. The retention of eight of the nine possible $InsP_4$ isomers (discounting enantiomers) was established in system 1. The ninth peak was accordingly assigned the ninth $InsP_4$ isomer, that is, $Ins(1,2,3,5)P_4$. Some peaks are unidentified because of the lack of commercially available standards of the inositol phosphate isomers.

RESULTS AND DISCUSSION

In the present study our attempt was to demonstrate the separation afforded by different anion exchange columns for various inositol phosphates using the same mobile phase gradient. The solvent strength was set for elution of the sample components at reasonable retention times: $1 < k' < 20$ (Snyder et al., 1988). The separation was further optimized (in system 1) by varying the solvent strength, illustrated in a food sample for a promising column.

System 1. The analyses of the reference sample in system 1 using the columns PAX-100, PA-100, PA-10, AS11, Mini Q, and ION-120 are shown in Figure 1. Table 2 illustrates differences in retention of inositol phosphate isomers on the columns by comparing the relative retention times (RRT) for various peaks, as expressed relative to $InsP_6$. $InsP_1$ was eluted on the solvent front on all columns, in the first system. The PAX-100 separator column, initially developed for the analysis of ionic species, was previously used in

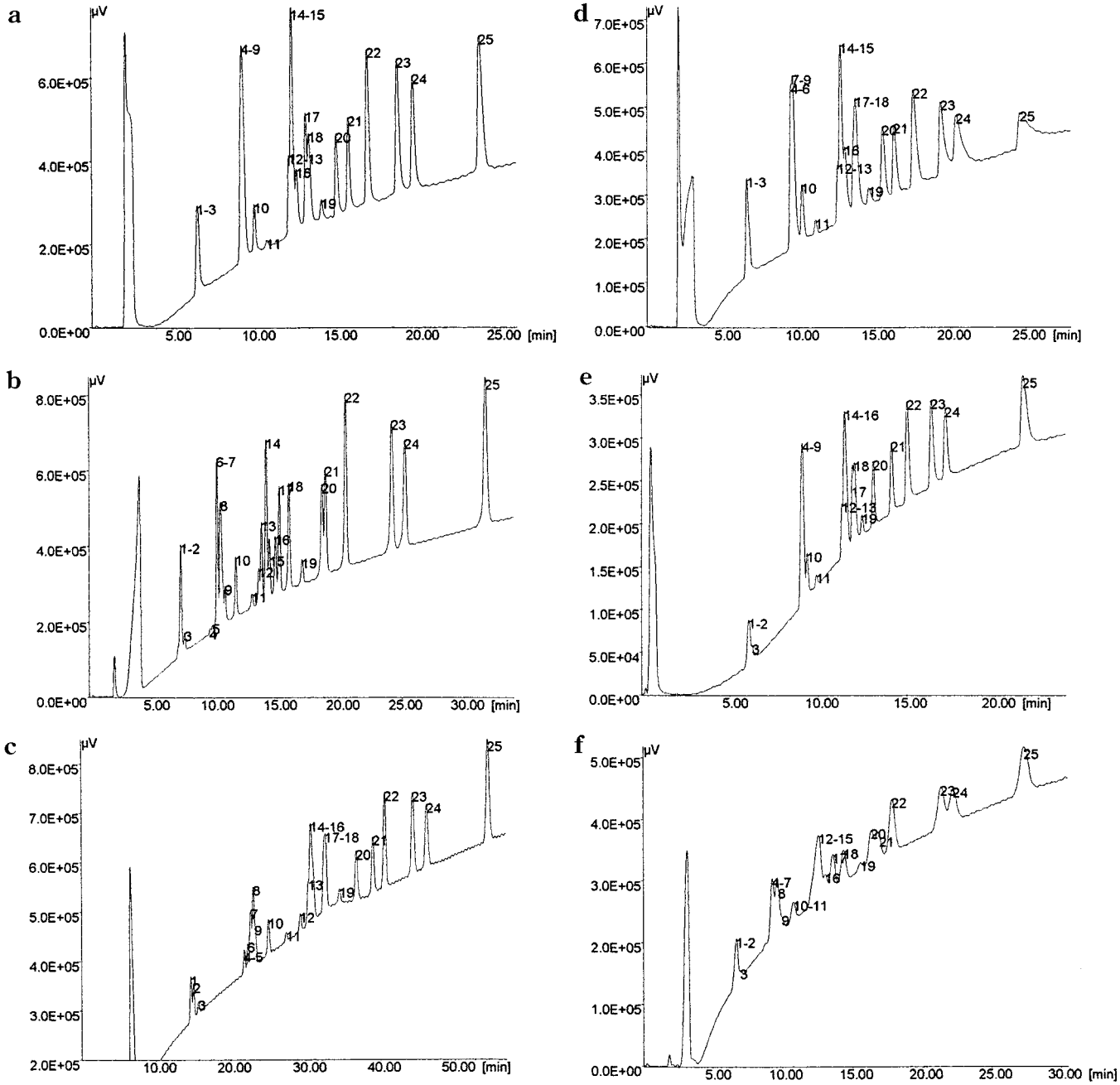

Figure 1. Analysis of a reference sample in system 1 using the columns (a) OmniPac PAX-100, (b) CarboPac PA-100, (c) CarboPac PA-10, (d) IonPac AS11, (e) Mini Q PC 3.2/3, and (f) ION-120 anion column. Peaks (unidentified peaks are indicated by an asterisk): (1) *; (2) DL-Ins(1,4)P_2, DL-Ins(2,4)P_2; (3) DL-Ins(4,5)P_2; (4) *; (5) *; (6) DL-Ins(1,2,4)P_3, [DL-Ins(1,3,4)P_3]; (7) DL-Ins(1,3,4)P_3, [Ins-(1,2,3)P_3]; (8) DL-Ins(1,2,6)P_3, Ins(1,2,3)P_3; (9) DL-Ins(1,4,5)P_3; (10) DL-Ins(1,5,6)P_3; (11) Ins(4,5,6)P_3; (12) Ins(1,2,3,5)P_4; (13) DL-Ins(1,2,4,6)P_4; (14) DL-Ins(1,2,3,4)P_4; (15) Ins(1,3,4,6)P_4; (16) DL-Ins(1,2,4,5)P_4; (17) DL-Ins(1,3,4,5)P_4; (18) DL-Ins(1,2,5,6)P_4; (19) Ins(2,4,5,6)P_4; (20) DL-Ins(1,4,5,6)P_4; (21) Ins(1,2,3,4,6)P_5; (22) DL-Ins(1,2,3,4,5)P_5; (23) DL-Ins(1,2,4,5,6)P_5; (24) Ins(1,3,4,5,6)P_5; (25) InsP_6.

Skoglund et al. (1997b) in system 1 for the determination of InsP_6−InsP_2 in foods and intestinal contents. This anion exchanger is able to separate 16 of the 25 established isomers of inositol hexakis- to bisphosphates (Figure 1a). Longer analysis time is obtained with the PA-100 separator column (Figure 1b) due to increased degree of cross-linking and latex size, that is, increased ion exchange capacity. Some of the InsP_4 isomers (peaks 12−13 and 14−15) and InsP_3 isomers (peaks 4−9) that are not separated on the PAX-100 column are better separated on the PA-100 anion exchanger. As can be seen in Figure 1b, the InsP_4 isomer DL-Ins-(1,4,5,6)P_4 interferes with the InsP_5 isomer Ins(1,2,3,4,6)-P_5 on the PA-100 column. In comparison to the anion exchanger PA-100, the functional group of the PA-10 column is more hydrophobic, which, in turn, yields a higher retention for inositol phosphates. The retention time is, as well, affected by the capacity of the resin, being higher for PA-10 (Figure 1c). Especially later eluting compounds (InsP_6−InsP_4) elute as sharper signals on the anion exchanger PA-100, due to a much shorter retention. A higher selectivity for InsP_6−InsP_4 on the PA-10 column may be obtained by optimizing the eluting gradient. A change in retention order between the column PA-10 and the column PA-100 for inositol trisphosphate isomers was demonstrated. DL-Ins(1,3,4)-P_3 was eluted in peak 6 on PA-100 and in peak 6−7 on PA-10, whereas Ins(1,2,3)P_3 was eluted in peak 8 on PA-

Table 2. Relative Retention Times (RRT) for Inositol Phosphate Isomers in System 1, Expressed Relative to $InsP_6$

inositol phosphate	peak	RRT PAX-100	PA-100	PA-10	AS11	MiniQ	ION-120
$InsP_2$	1	0.273	0.231	0.268	0.267	0.281	0.289
$InsP_2$	2	0.273	0.231	0.275	0.267	0.281	0.289
$InsP_2$	3	0.273	0.240	0.287	0.267	0.290	0.298
$InsP_2$	4	0.388	0.305	0.398	0.385	0.421	0.374
$InsP_3$	5	0.388	0.310	0.398	0.385	0.421	0.374
$InsP_3$	6	0.388	0.322	0.408	0.385	0.421	0.374
$InsP_3$	7	0.388	0.322	0.415	0.389	0.421	0.374
$InsP_3$	8	0.388	0.331	0.421	0.389	0.421	0.387
$InsP_3$	9	0.388	0.342	0.426	0.389	0.421	0.396
$InsP_3$	10	0.419	0.369	0.459	0.414	0.434	0.427
$InsP_3$	11	0.451	0.410	0.502	0.450	0.458	0.427
$InsP_4$	12	0.508	0.427	0.537	0.513	0.525	0.491
$InsP_4$	13	0.508	0.433	0.559	0.513	0.532	0.491
$InsP_4$	14	0.515	0.445	0.563	0.517	0.532	0.491
$InsP_4$	15	0.515	0.453	0.563	0.517	0.532	0.491
$InsP_4$	16	0.527	0.468	0.563	0.529	0.532	0.508
$InsP_4$	17	0.551	0.479	0.598	0.557	0.553	0.526
$InsP_4$	18	0.559	0.503	0.598	0.557	0.558	0.553
$InsP_4$	19	0.591	0.536	0.633	0.593	0.578	0.597
$InsP_4$	20	0.630	0.586	0.675	0.630	0.607	0.622
$InsP_5$	21	0.661	0.595	0.716	0.660	0.655	0.639
$InsP_5$	22	0.710	0.646	0.745	0.712	0.696	0.674
$InsP_5$	23	0.787	0.763	0.814	0.785	0.759	0.795
$InsP_5$	24	0.828	0.796	0.849	0.827	0.796	0.824
$InsP_6$	25	1.000	1.000	1.000	1.000	1.000	1.000

100 and in peak 7 on PA-10. The differences in elution profiles of $InsP_3$ on the two columns provide means to identify these anion isomers in various samples. The retention of $InsP_2$ isomers was best illustrated on the PA-10 column. The two anion exchange separator columns PA-10 and PA-100 are developed especially for the analysis of carbohydrates. Both CarboPac columns tested (PA-10 and PA-100) possess comparatively high exchange capacity, necessary since eluents with high ionic strength are required for the analysis of carbohydrates. IonPac AS11 (Figure 1d) possessed chromatographic properties similar to those of the anion exchanger PAX-100, in system 1. However, increased tailing of $InsP_5$ and $InsP_6$ peaks was obtained on the AS11 anion exchanger. The comparatively smaller particle diameter (3 μm) of the Mini Q column increases the separation efficiency and decreases the loading capacity. Reduced analysis time is as well an effect of the small particles (Figure 1e). The Mini Q anion exchanger can be operated only with a pressure of 10 MPa, due to the considerably higher back pressure. Similar to Mini Q, the ION-120 column (Figure 1f) is functionalized directly at the surface. The substrate particle diameter is almost equal to that of the latex-coated columns PA-100 and PAX-100. There is little difference between the analysis times of these columns, whereas the peak shape and separation are better illustrated on the latex-coated column. The elution order of the inositol phosphate isomers in system 1 does not differ among all tested columns, except for $InsP_3$ isomers on the anion exchanger PA-10.

System 2. In system 2 the PAX-100 column separated $InsP_1$−$InsP_3$ isomers and the AS11 column separated $InsP_3$−$InsP_2$ isomers with satisfaction (Figure 2). Table 3 illustrates the relative retention times for various peaks on the columns PAX-100 and AS11, as expressed relative to DL-Ins(1,2,6)P_3. The anion exchanger PAX-100 was in Skoglund et al. (1997b) shown to be useful to separate $InsP_3$−$InsP_1$ isomers in foods and intestinal contents in system 2. Inositol monophosphates are on AS11 eluted in the solvent front. The

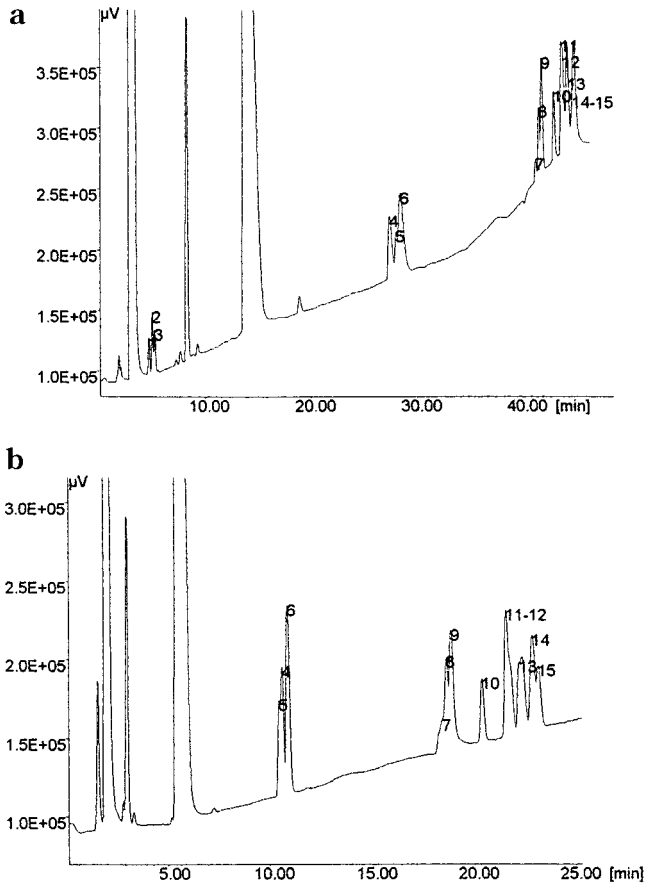

Figure 2. Analysis of a reference sample in system 2 using the columns (a) OmniPac PAX-100 and (b) IonPac AS11. Peaks (unidentified peaks are indicated by an asterisk): (1) Ins(2)P; (2) DL-Ins(1)P; (3) DL-Ins(4)P; (4) DL-Ins(1,4)P_2; (5) DL-Ins(2,4)-P_2; (6) DL-Ins(4,5)P_2; (7) *; (8) DL-Ins(1,5,6)P_3; (9) DL-Ins(1,2,6)-P_3, Ins(1,2,3)P_3; (10) *; (11) *; (12) DL-Ins(1,3,4)P_3; (13) DL-Ins(1,4,5)P_3; (14) DL-Ins(2,4,5)P_3, DL-Ins(1,2,4)P_3.

Table 3. Relative Retention Times (RRT) for Inositol Phosphate Isomers in System 2, Expressed Relative to DL-Ins(1,2,6)P_3

inositol phosphate	peak	PAX−100	AS11
$InsP_1$	1	0.111	0.105
$InsP_1$	2	0.119	0.105
$InsP_1$	3	0.124	0.105
$InsP_2$	4	0.656	0.562
$InsP_2$	5	0.670	0.553
$InsP_2$	6	0.680	0.575
$InsP_3$	7	0.986	0.963
$InsP_3$	8	0.994	0.987
$InsP_3$	9	1.000	1.000
$InsP_3$	10	1.028	1.079
$InsP_3$	11	1.045	1.148
$InsP_3$	12	1.047	1.148
$InsP_3$	13	1.059	1.185
$InsP_3$	14	1.074	1.214
$InsP_3$	15	1.074	1.227

elution orders of inositol diphosphates (peak 4 and 5) diverge between the two columns. Inositol phosphates are more strongly held on PAX-100, as compared to AS11. The remaining columns tested were inappropriate for the elution of $InsP_3$−$InsP_1$ in the second system. PAX-100 and AS11 were the only columns useful in the alkaline system, due to their ion exchange group, alkanol. An alkanol group is an ROH compound, where R is an alkyl group. For OH^--based eluent systems the k' data of all analytes are lower on the alkanol ammonium latexes than on the alkyl ammonium latexes

Table 4. Summary of the Columns on Which the Different Inositol Phosphates Are Separated Most Appropriately[a]

column	system	$InsP_1$	$InsP_2$	$InsP_3$	$InsP_4$	$InsP_5$	$InsP_6$
PAX-100	1	−	−	+	+	++	++
PA-100	1	−	+	++	++	++	++
PA-10	1	−	++	++	−	+	+
AS11	1	−	−	+	+	−	−
Mini Q	1	−	+	+	+	++	++
ION-120	1	−	+	+	+	−	−
PAX-100	2	++	++	++	na	na	na
PA-100	2	−	−	−	na	na	na
PA-10	2	−	−	−	na	na	na
AS11	2	−	++	++	na	na	na
Mini Q	2	−	−	−	na	na	na
ION-120	2	−	−	−	na	na	na

[a] ++ Indicates most suitable for the separation in the present system. −Indicates that the column is not recommended for this application in the present system. na indicates not analyzed.

(Slingsby and Pohl, 1988). Lower k' values indicate higher selectivity for the eluting ion compared to the analyte ion, giving powerful elution of anions with OH^- eluents from alkanol ammonium latexes. Increased selectivity of these latexes for OH^- may be considered in terms of the extraordinary interaction of OH^- with water. The high electrical conductivity of OH^- in water relative to other monovalent anions is the cause of this interaction. Every anion is surrounded by a large, tightly held hydration sphere that hinders their approach to the anion exchange sites (Smith et al., 1993). The anions must lose their waters of hydration to bind to an anion exchange column. It is also possible that steric hindrance prevents the negatively charged phosphate groups of inositol phosphates from coming very close to the anion exchange sites on the column, thus minimizing retention.

Because hydroxide is weakly held by most of the strong anion exchangers, high concentration is necessary to elute common anions, giving a high background conductivity. Since sodium hydroxide is suppressed to water, it is very useful in gradient elution. The highest concentration of NaOH eluent used was 130 mM, which is near the upper limit of the AMMS. Stronger eluents may be used to elute inositol phosphates on the columns PA-10, PA-100, Mini Q, and ION-120, in system 2. We examined the stronger eluent $Na_2CO_3^{2-}$ on these columns, giving elution of $InsP_1$ but not $InsP_2$ or $InsP_3$. Isomers of $InsP_1$ were not separated when using $Na_2CO_3^{2-}$ as eluent.

Table 4 summarizes the columns on which the different inositol phosphates are separated most appropriately. For optimizing the separation on each column a change in gradient eluent composition is needed. Most likely, a gradient curve will separate the isomers with better resolution than a linear gradient. A fermented wheat roll sample is shown in Figure 3. The sample has been analyzed using the PA-100 column in system 1, with optimization of the mobile phase gradient.

Conclusions. CarboPac PA-10 and PA-100 columns, respectively, were the anion exchange columns best suited for the separation and determination of $InsP_2$ and $InsP_3$−$InsP_6$ isomers in the system using acidic eluent, postcolumn reaction, and UV detection. In the system using alkaline eluent and suppressed conductivity detection, optimal separation of $InsP_1$−$InsP_3$ isomers was obtained using the OmniPac PAX-100 anion exchanger. The results obtained in the present study can be used for improvement in the separation and determination of inositol phosphate isomers.

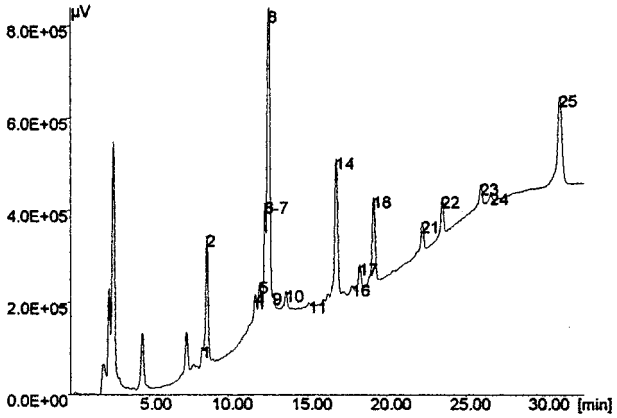

Figure 3. Chromatographic profile of a fermented wheat roll sample using the PA-100 column with optimization of elution gradient. Peaks are numbered according to Figure 1.

ABBREVIATIONS USED

AMMS, anion micromembrane suppressor; DVB, divinylbenzene; HPIC, high-performance ion chromatography; $InsP_1$−$InsP_6$, inositol mono- to hexakisphosphate; Ins, an accepted NC−IUB abbreviation for *myo*-inositol with the numbering of the D configuration unless the prefix L is explicitly added; VBC, vinylbenzyl chloride.

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