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Determination of Phosphates and Polyphosphates Using Capillary Isotachophoresis

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Abstract: In this article, phosphate, di- and triphosphates are the analytes determined in commercially marketed samples with the aid of capillary isotachophoresis. After method development, the optimal electrolyte system comprised 10 mM HCl + β-alanine + 0.1% hydroxy-ethylcellulose (pH 3.6) as the leading electrolyte and 10 mM citric acid as the terminating electrolyte. The calibration / detection characteristics are as follows: linearity over the concentration range of 0–200 mg.l^{-1} P_2O_5, limit of detection *ca.* 0.3 mg.l^{-1} P_2O_5, limit of quantification of *ca.* 1 mg.l^{-1} P_2O_5. The time of analysis was from 15 to 30 minutes. Furthermore, relatively simple procedure, low operational costs, and sufficient sensitivity are further positive attributes of the method tested in analyses of selected real samples.

Keywords: Capillary isotachophoresis, Phosphate, Polyphosphate, Condensed phosphate, Determination.

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Introduction

Phosphates and polyphosphates belong to very diverse group of chemical compounds [1], and some of them are constituents of living organism. Their content in soil influences yield of agricultural crop, industrial production and even in households serves in water softening, and last but not least they are used as foodstuff additives. On the other hand, their excess in surface water causes unpleasant eutrophication. Industrially, monophosphate, diphosphate and triphosphate — all having linear chain — are widely used.

Cyclic trimetaphosphate and hexametaphosphate (*Graham´*s salt) are mixtures of various poly-metaphosphates. In foodstuffs phosphates are added due to their ability impound water (especially in meat products) and adjust proper pH [1]. The maximal allowed addition of the phosphate species is 5 g/kg P_2O_5 [2].

From analytical point of view, the dominant property of polyphosphates is relatively considerable stability which allows their mutual separation. In aqueous media and at room temperature they are quite stable, however in diluted solutions they slowly hydrolyze to monophosphate. It is possible to speed up a hydrolysis e.g. by heating of acidified solution. On contrary to this, it is possible to convert monophosphate to polyphosphates by heating to hundreds of degrees centigrade. [1]

For determination of phosphates in various matrices, procedures based on formation of phosphomolybdate are still used [3]. Individual polyphosphates can be determined by different approaches [4,5]. Most common is chromatography [6,7], primarily ionic chromatography [8-17]. Recently, electrophoresis [18-23] and isotachophoresis [2,24-29] are in the centre of interest. In special cases the use of X-ray diffraction [30-32], Fourier transform infrared spectrometry [33], nuclear magnetic resonance [29,34-36], etc. were described.

The aim of this study was to develop and optimize a new suitable electrolyte system, as well as to verify the respective procedure to determine polyphosphates in various matrices.

Experimental

Chemicals and Apparatus

All used chemicals, if not stated otherwise, were of analytical reagent grade. Sodium dihydrogenphosphate was purchased from *Lachema* (Brno, Czech Republic), hydrochloric acid from *Penta* (Chrudim, Czech Rep.), hydroxyethylcellulose from *Serva* (Heidelberg, Germany). Potassium pyrophosphate $K_4P_2O_7$ (≥97%), sodium tripolyphosphate $Na_5P_3O_{10}$ (technical grade, ≥85%), trisodium trimetaphosphate $Na_3P_3O_9$ (≥95%), sodium hexametaphosphate $(NaPO_3)_n$, metaphosphoric acid $(HPO_3)_n$, β-alanine, bis-tris propane and citric acid were then purchased from *Sigma Aldrich* (Praha, Czech Republic).

Samples were prepared in common laboratory glassware; the appropriate volumes of water and solutions have been introduced with the aid of a set of adjustable transfer-pipettes (model "Proline"; *Biohit*, Helsinky, Finland).

All solutions for measurements were made from deionized water. If needed, the individual solutions and samples were dissolved – when additionally deaerated – using a laboratory ultrasound bath (model "K-2"; *Kraintek*, Podhájska, Slovakia).

Isotachophoretic analyses were performed using the CS Isotachophoretic Analyser ZKI 01 (*URVJT*, Spišská Nová Ves, Slovakia), which is an instrument in the two-column arrangement consisting pre-separation capillary (0.8 mm ID, 160 mm length) and analytical capillary (0.3 mm ID, 160 mm length), both from FEP (copolymer of hexafluoropropylene and tetrafluoroethylene). Conductivity detectors were placed at the end of both capillaries. The sample was injected using 30 µl injection valve made from PTFE. The analytical signal and its derivation were recorded via double X-line recorder model "TZ 4620" from *Laboratorní přístroje* (Praha, Czech Republic).

The pH value of the leading electrolyte was controlled by combined pH-electrode (model "HI-1131"; Hanna Instruments Czech, Praha, Czech Rep.) connected to a portable pH meter (model "GRYF 208 L", *Elektronické přístroje Gryf*, Havlíčkův Brod, Czech Republic). If needed, samples were injected through microfilters Whatman 1.0 µm GMF-150 (Clifton, NJ, USA).

The 10 mmol.l^{-1} fresh stock solution of phosphates was prepared daily. Model samples of phosphates were prepared by diluting the freshly made stock solution. Diluted solutions were prepared directly prior to analysis due to their slow hydrolysis to monophosphate. After a week at laboratory temperature, 0.1 mmol.l^{-1} solutions of polyphosphates only contained monophosphate.

The Samples

Samples were obtained from local market and were selected in order to represent various matrices which can contain monophosphate and polyphosphates of different concentrations. *Baking powder* is a relatively simple mixture containing high concentration of diphosphate. *Dishwasher tablet* consisted of blue and white parts which were analyzed separately. Both parts contained high concentration of triphosphate. Samples of *washing powder* contained low amount of diphosphate and triphosphate. *Tooth paste* contained low amount of monophosphate. *Ham* and *frozen fish (Pangasius* sp.*)* contained low amount of mono- and diphosphate. All samples except of baking powder have complicated matrix of inorganic or organic character.

Sample Treatment

A portion of the powder samples was treated in a mortar, weighted with analytical precision and dissolved in water. In case of necessity dissolution was supported by sonication using ultrasound bath. Sample solutions were transferred into volumetric flasks and finally filled with deionized water up to the mark. Portions of finely sliced meat product samples were extracted by water using ultrasound bath and after transfer of the solution into volumetric flasks filled with deionized water up to the mark. Tooth paste was weighted to a baker and dissolved. The solution was transferred into a volumetric flask a filled with deionized water up to the mark. For standard addition method the appropriate volume of standard solution containing 0.01 M phosphates was added into sample solution before the filling of the volumetric flask up to the mark.

Experimental Conditions and Instrumental Parameters

Phosphates were determined under following conditions (unless otherwise stated): Leading electrolyte consisted of 10 mM HCl + β-alanine + 0.1% hydroxy-ethylcellulose (pH 3.6), where the lastly named served for reducing the electroosmotic flow and β-alanine was buffering counter-ion. Leading ion was chloride. As the terminating electrolyte, the 10 mmol.l^{-1} solution of citric acid was used (with citrate as terminating ion). Analysis was performed at a driving current of 200 μA flowing through pre-separation column. A driving current in analytical column, if used, was 30 μA. Two signals were recorded: (i) conductivity of solution leading through the detector and (ii) the absolute value of conductivity derivation.

Results and Discussion

The phosphate and polyphosphates are polybasic anions, which occur in several dissociative grades dependent on pH of solution (see data in Table I).

Table I: *Protonization constants of phosphate a polyphosphate anions [37]*

Aniont	A ↔ HA	HA ↔ H_2A	H_2A ↔ H_3A	H_3A ↔ H_4A
PO_4^{3-}	12.35	7.2	2.15	
$P_2O_7^{4-}$	9.4	6.7	2.2	0.8
$P_3O_{10}^{5-}$	9.25	6.54	2.5	1.0
$P_3O_9^{3-}$	2.05			

Legend: A – fully deprotonized anion PO_4^{3-}, $P_2O_7^{4-}$, $P_3O_{10}^{5-}$, $P_3O_9^{3-}$, resp.

The table points out that prevailing form of phosphates and polyphosphates anions at pH of solution in range 3 to 6 is $H_2PO_4^-$, $H_2P_2O_4^{2-}$, $H_2P_3O_{10}^{3-}$ and $P_3O_9^{3-}$. At pH 7 to 9 HPO_4^{2-}, $HP_2O_4^{3-}$, $HP_3O_{10}^{4-}$ a $P_3O_9^{3-}$ are dominant. Such data is necessary to take into account when suggesting new electrolyte system for isotachophoretic separation.

Optimization of Electrolyte System

Common leading electrolyte being used for determination of inorganic anions usually consists of 10 mM HCl + 3 mM bis-tris propane + β-alanine + 0.1% hydroxy-ethylcellulose (pH 3.6). Bis-tris propane (at analysis conditions) presenting cation carrying two positive charges is primarily added into the solution due to the ability of sulphate anion mobility decrease, which results in prevention of origin of mixed zone of sulphate and nitrate. The decrease of sulphate mobility can be assumed as a consequence of the electrophoretic and relaxation effect, during which the counter-ion with larger charge retards ("slows down") the separated anions with higher charge.

Leading electrolyte at pH 3.6 containing Bis-tris propane is also recommended for determination of polyphosphates [31]. With respect to conditions of analysis (i.e. pH of leading electrolyte 3.6) polyphosphates carry out approximately as many charges as phosphorus atoms they contain and even on their record the slowing down effect of Bis-tris propane can be observed even more by triphosphate and trimetaphosphate than by diphosphate. It results in low value of diphosphate and triphosphate ions mobility decreasing separation capacity of the system and possibly leading to mixed zone formation. (see diagrams in Fig. 1, overleaf).

When the sample is containing diphosphate and triphosphate, it is recommended to proceed the determination by using more acidic leading electrolyte with composition e.g. 10 mM HCl + 20 mM glycylglycine (pH 3.0) [2] or 10 mM HCl + glycine (pH 3.0) [31]. Other suggested possibility is to use leading electrolyte of composition 5 mM HCl + β-alanine (pH 4.5) [26]. In this system, the rather buffering ability of counter-ion is decreased, i.e. β-alanine, which has pK_A 3.55 [38].

Within this work the bis-tris propane leading electrolyte consisting of 10 mM HCl + 0.1% hydroxy-ethylcellulose + β-alanine to pH 3.6V was used. Such system offers sufficient separation of di- and triphosphate signal. (see Fig. 1). Unfortunately, the zones of sulphate and nitrate are not resolved and merge.

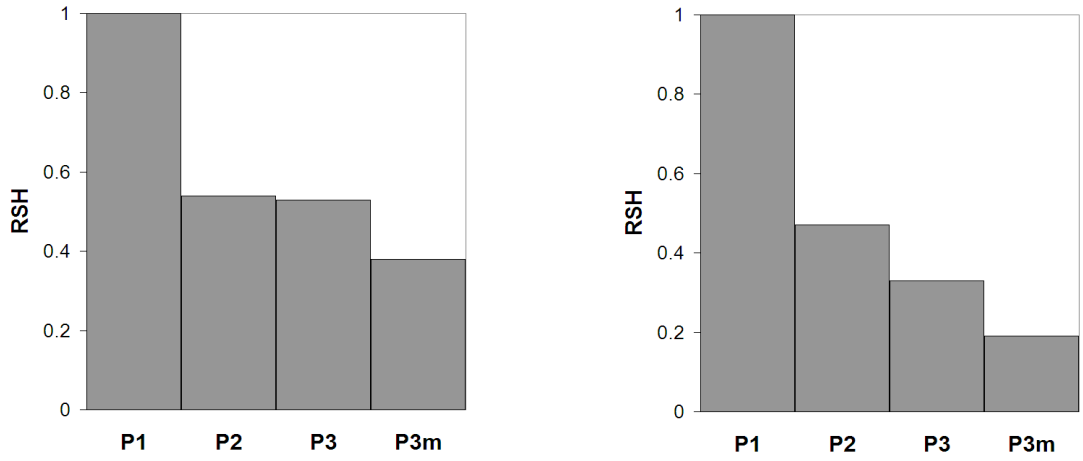

Fig. 1: *Relative step heights (RSH) of polyphosphates obtained by leading electrolyte with bis-tris propane (left) and without bis-tris propane (right), resp.* Values of RSH are relative to monophosphate signal. P1 – monophosphate, P2 – diphosphate, P3 – triphosphate, P3m – trimetaphosphate.

Hexametaphosphate and metaphosphoric acid provide complicated signal, from which it is clear that chemicals being used contain mixture of phosphates instead as a chemical individual. Some of them were already identified.

In respect to conditions at which the isotachophoretic separation is performed it is not possible to exclude partial decomposition of polyphosphates during analysis [39]. Leading electrolyte has pH 3.6 and even the pH value is rising up in following zones it is still in mild acidic range. The whole separation system is warming up because of Joule heat even more with lower conductivity of individual zones. The analysis can take up to 30 minutes. These conditions can evoke decomposition of polyphosphates. Nevertheless during analyses significant decomposition of analytes has not been observed, but this cannot be excluded and one has to keep it in mind.

Analysis of Model Samples

Calibrations of Phosphates. A calibration curves confirms a fine linearity of the dependence of interest – the zone length vs. concentration of phosphate and polyphosphates (see data in Table II, overleaf). Zones are stable and it seems that even at low concentrations the decomposition does not play significant role.

Limit of Detection and Limit of Quantification. Minimum detectable zone length is about one second [40]; the respective detection limits of phosphates were lower than 30 μmol.l^{-1} (i.e. 3 mg.l^{-1} P_2O_5) for pre-separation column and can be lowered up to 3 μmol.l^{-1} (0.3 mg.l^{-1} P_2O_5) in analytical column.

Table II: *Equations of calibration curves*

Aniont	*Equation of calibration curve*
PO_4^{3-}	$l = 33.7c + 1.2$
$P_2O_7^{4-}$	$l = 44.4c + 1.0$
$P_3O_{10}^{5-}$	$l = 49.0c + 0.9$
$P_3O_9^{3-}$	$l = 59.9c + 2.4$

Legend: l, step length in seconds; c, analyte concentration in mM. The equations correspond to a signal from pre-separation column. The slope of equations corresponding to signal from analytical column is six times higher.

The limit of determination has been evaluated as a triple value of detection limit, which is for analytical column about 10 μmol.l^{-1} of phosphate (i.e. 1 mg.l^{-1} P_2O_5).

Reproducibility of the Analytical Signal. Relative standard deviations of zone length of 5-times repeated injection of individual phosphates standard solutions at concentration 5 x 10^{-4} mol.l^{-1} were better than 1.5%.

Analysis of Real Sample

Just one real sample offered content of phosphates declared by manufacturer (without close description about type of phosphate to which the value is related). It was a tablet to dishwasher which consisted of two parts – blue and white. Each of them contained about 32% of triphosphate (calculated as $Na_5P_3O_{10}$). The manufacturer declares >30% of phosphates.

The content of phosphorus in washing powders is limited by law [41] up to max. 0.5%. Both washing powders being analyzed contained diphosphate and triphosphate their limit was in agreement with law. On record of analysis, very short zone of monophosphate has been observed, but it comes probably from decomposition of polyphosphate during analysis. Tooth paste contained only very small amount of monophosphate, which is in agreement with data given by manufacturer.

In the sample of ham, a content of triphosphate (additive E451) and diphosphate (additive E450) was declared by manufacturer. Nevertheless in contrast to this, the content of mono and diphosphate was found. In a sample of frozen fish (*Pangasius* sp.), the presence of polyphosphates (additive E452) was declared, the di- and monophosphate was found. The content of phosphates in both samples was much lower than the limit given by the legislative norm. No sample contained trimetaphosphate.

Conclusions

In the above sections, a simple and rapid determination of phosphate and polyphosphates has been presented based on the isotachophoretic separation with conductometric detection. The leading electrolyte of choice was Cl^- ion, whilst the terminating ion was citrate. The electrolyte system has utilized the leading electrolyte consisting of 0.01 M HCl + 0.1% hydroxyethylcellulose + β-alanine to pH 3.6 and the terminating electrolyte, a solution of 0.01 M citric acid.

The proposed method has been tested on practical analysis of several samples with complicated matrix containing several types of phosphates of different concentrations. Analytes has been determined by means of two methods of quantitative analysis, (i) calibration curve- and (ii) standard addition method.

It can be stated that isotachophoretic determination offers a number of advantages compared to other techniques; mainly, minimal requirements on sample treatment prior to analysis, inactivity and insensitivity against non-ionic species in the sample matrix, as well as a very small amount of the sample needed. At present, the method is in further development.

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