Application Note

Environmental, Food & Beverage Testing

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Analysis of Phosphate and Other Phosphorus Anions Using the Agilent 7100 CE

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Abstract

This application note describes the determination of phosphates and other phosphorus anions using the Agilent 7100 CE and commercially available materials in different sample matrices.

Introduction

The analysis of phosphates and other phosphorus anions is vital for many reasons. For example, the contamination of surface waters with phosphorus-containing chemicals is largely due to industrial wastewater, agricultural influence, and household wastewater. Appreciating this fact is critical for maintaining a stable ecosystem.

An excess of phosphorus leads to eutrophication, an over-enrichment with nutrients, resulting in a bloom of algae and other plants. For this reason, in the last few decades, stricter exposure limits have been set, and continual adjustments are being made to laws (e.g., for industrial wastewater).

Various phosphorus anions are also used in food processing as emulsifiers, stabilizers, acidity regulators, and raising agents. Therefore, an application of these different phosphorus anions is useful in the food industry, especially for highly processed foods.

Because the nomenclature of phosphorus anions is not uniform and trivial names are often used for historical reasons, Table 1 summarizes possible names and which name is used in this application note.

From an analytical point of view, certain gravimetric or spectroscopic methods are suitable for the analysis of phosphorus anions. However, with these methods, only sum parameters can be obtained. Differentiation between individual phosphate species is not possible.

Ion chromatography can be used to separate the individual phosphates, but complex matrices often require complicated sample preparation to preserve the chromatographic column. By contrast, the use of capillary electrophoresis (CE) results in fewer matrix influences, since free capillaries are used instead of packed columns.

Because the phosphorus anions do not absorb UV light, an electrolyte system that enables the indirect detection of anions must be used. Agilent offers various electrolyte systems that are suitable for the analysis of different anionic analytes and application fields. The advantage of these ready-to-use electrolytes is that, in addition to the UV-absorbing main component, they also contain an electroosmotic flow (EOF) modifier that enables rapid separation of anions by reversing the EOF.

Experimental

This section summarizes the consumables, chemicals, and method parameters used in this application.

Table 2. Chemicals.

Position	Material	Supplier	Order Number
1	Sodium dihydrogen phosphate dihydrate	Merck	1.06342
2	Sodium pyrophosphate tetrabasic	Sigma-Aldrich	P8010
3	Sodium tripolyphosphate	Sigma-Aldrich	238503
4	Sodium phosphite dibasic pentahydrate	Sigma-Aldrich	04283
5	Sodium hypophosphite monohydrate	Sigma-Aldrich	S5012
6	Sodium chloride	Merck	1.06404
7	Sodium nitrate	Merck	1.06537
8	Sodium sulfate	Merck	1.06649
9	Water (LC/MS grade)	Merck	1.15333

Table 3. Agilent consumables.

Position	Material	Supplier	Part Number
1	Capillary: fs, 50 µm, 72 cm	Agilent	G1600-62211
2	Plating bath buffer	Agilent	5064-8236
3	PP vials, 1 mL	Agilent	5182-0567
4	Alignment interface, green	Agilent	G7100-60210
5	Snap caps, PU	Agilent	5181-1512

Table 1. Names of the phosphorus anions.

Position	Material	Formula of the Anion	Alternative Names for the Anion	Name Used in This Application
1	Sodium dihydrogen phosphate dihydrate	PO_4^{3-}	Phosphate, orthophosphate	Phosphate
2	Sodium pyrophosphate tetrabasic	$P_2O_7^{4-}$	Pyrophosphate, diphosphate, TSPP	Diphosphate
3	Sodium tripolyphosphate	$P_3O_{10}^{5-}$	Tripolyphosphate, triphosphate, polygon, STPP, TPP5	Triphosphate
4	Sodium phosphite dibasic pentahydrate	PO_3^{3-}	Phosphonate, phosphite	Phosphite
5	Sodium hypophosphite monohydrate	PO_2^{3-}	Hypophosphite, phosphinate	Hypophosphite

The experimental conditions of the acquisition method are shown in Table 4.

Table 4. Experimental conditions.

Parameter	Value
Device	Agilent 7100 Capillary Electrophoresis System
Firmware	B.07.021
Electrolyte	Plating bath buffer
Capillary	Fused silica (fs), 50 µm ID, 72 cm
Injection	30 s, 50 mbar, followed by 5 s, 50 mbar electrolyte solution
Detection	Indirect UV, signal: 350, 80 and reference: 245, 30
Voltage	–30 kV, ramp: 0.3 min
Temperature	20 °C
Preconditioning	1 bar, 180 s flush using the electrolyte solution

The electrolyte solution was filled into three autosampler vials (Agilent product number 5182-0567) by pipetting 350 µL into each vial. Two of these vials were used as the "Inlet Home" and "Outlet Home" vials, and the third vial was only used for the preconditioning step.

Preparation of the standard solutions

For the preparation of the stock solutions, the single salts (see Table 2, positions 1 to 8) were weighed and dissolved using purified water, so that a final concentration of 1,000 mg/L for each anion was obtained. Using these stock solutions, any mixed standard solutions can be prepared as needed for the respective analysis of interest.

Results and discussion

Applying the conditions summarized in the Experimental section, the separation shown in Figure 1 can be obtained.

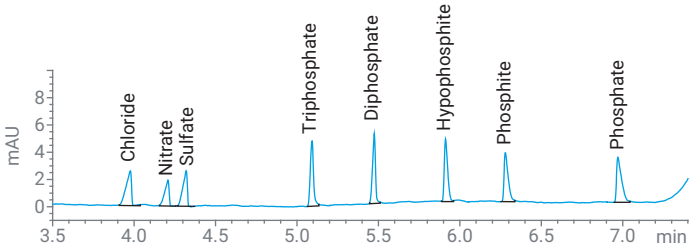

Figure 1. Standard mix in migration order: chloride (10 mg/L), nitrate (10 mg/L), sulfate (10 mg/L), triphosphate (30 mg/L), diphosphate (10 mg/L), hypophosphite (10 mg/L), phosphite (20 mg/L), and phosphate (10 mg/L).

The reproducibility of the method was tested by six consecutive injections of the standard solution without refilling the electrolyte vials. Automatic integration of all six injections was possible without the need for manual correction. An overlay of the six electropherograms demonstrates the excellent reproducibility of migration times.

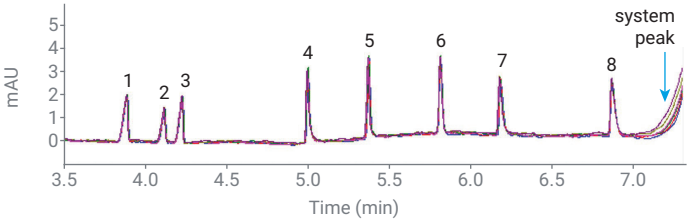

Figure 2. Reproducibility of six injections. Composition of standard solution and peak order as shown in Figure 1.

This reproducibility is also reflected in the very low relative standard deviations (RSD) of 0.05% of the migration times. Good RSD of 1 to 3% were also obtained for the reproducibility of the peak areas.

The method can be applied to the analysis of surface water, such as lakes or rivers. A surface water sample (shown in Figure 3) was taken behind the drain of a wastewater pumping station.

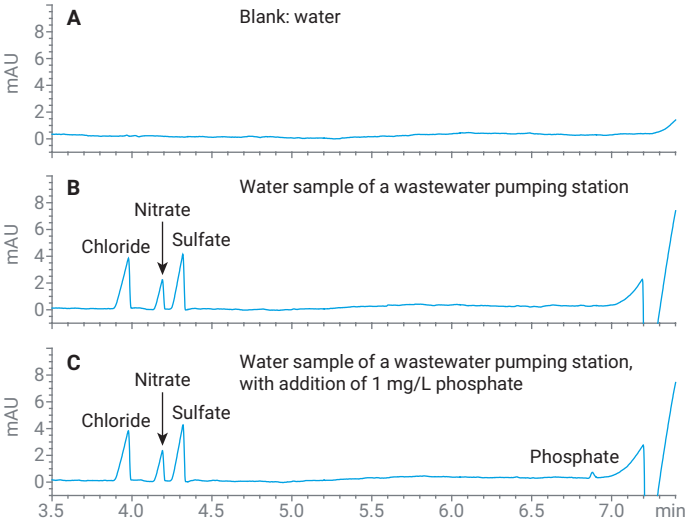

Figure 3. Determination of surface water.

Figure 3B shows that chloride, nitrate, and sulfate can be detected in the sample. Figure 3C proves that, by addition of 1 mg/L phosphate, phosphate could be detected. The limit of detection was approximately 1 mg/L.

A sample of industrial wastewater is shown in Figure 4.

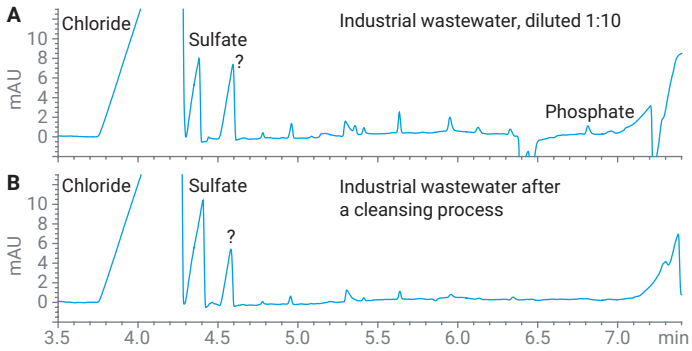

Figure 4. Industrial wastewater samples: (A) without cleansing; and (B) after cleansing processes.

In addition to the three identified ions, several unassignable peaks can also be detected. These peaks could be other inorganic anions and organic acids. Figure 4 indicates that industrial cleansing processes reduce the number of peaks, so purification is achieved through the process. Also, it is evident that phosphate is removed by the cleansing process.

Other sources of contamination of surface waters are detergents used in industry and private households, and the use of plant fertilizer in agriculture. Figure 5 shows the analysis of a liquid laundry detergent and of a plant fertilizer.

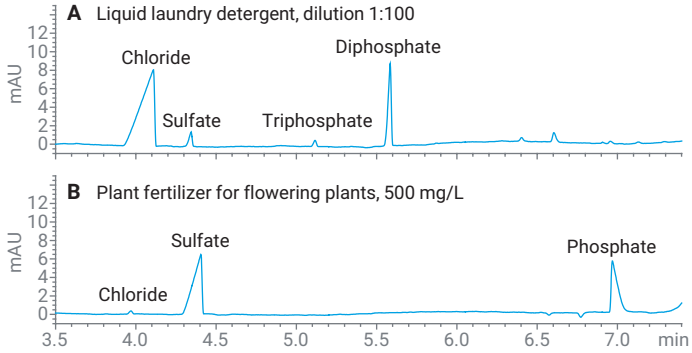

Figure 5. Laundry detergent and plant fertilizer.

For the two samples shown in Figure 5, only a sample dilution with water was performed. Although the detergent also contains nonionic and ionic surfactants, analysis of the anions of interest, such as triphosphate and diphosphate, is possible without any problems. This straightforward analysis is a clear advantage over chromatographic methods, where a surfactant matrix could lead to coating of the separation column.

Various phosphorus anions can be found in the food processing industry for several reasons. For example, diphosphates are used as emulsifiers, stabilizers, acidity regulators, and raising agents. Diphosphate and phosphate can be detected in baking powder, cake, and soft cheese, as shown in Figure 6.

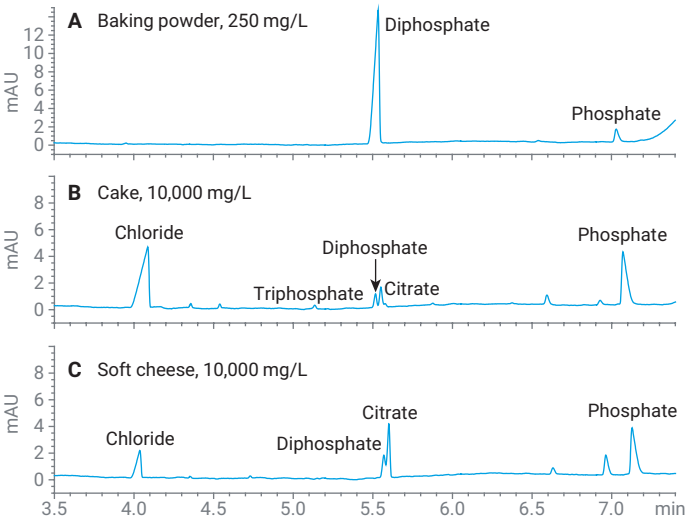

Figure 6. Analysis of food samples.

Although these sample matrices were complex, sample preparation with water in an ultrasonic bath was sufficient. Even the protein matrix contained in the cheese did not affect the analysis, and so precipitation of the proteins before analysis was not necessary.

To verify quantification, linearity was tested using a five-point calibration from 10 to 100 mg/L. In Figure 7, the results for triphosphate, diphosphate, and phosphate are summarized.

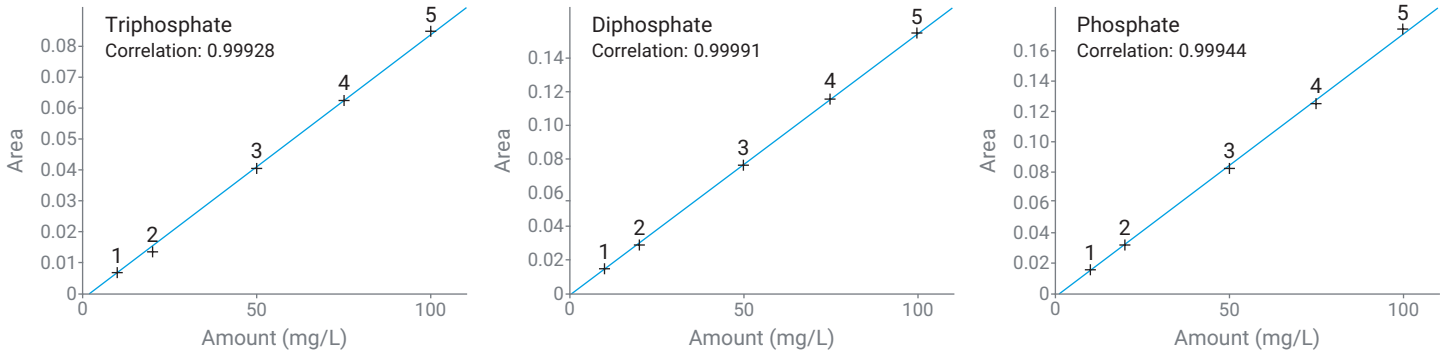

Figure 7. Calibration results of triphosphate, diphosphate, and phosphate, calculated using Agilent ChemStation software.

For each anion, a correlation of >0.999 was achieved. This result ensures that an accurate quantification can be performed. Due to this good correlation, it should be sufficient for routine analysis if only a two-point calibration or one external standard is used.

Conclusion

The method described in this application note can be used for the simultaneous determination of phosphate and other phosphorus anions as well as for other anions of interest. The analysis can be performed using currently available chemicals and materials. The reproducibility of the migration times and peak areas was investigated by a six-fold injection, and very good repeatability was obtained.

Quantification was tested by a five-point calibration, and correlations of >0.999 were obtained for all anions. The applicability of the method was demonstrated by sample measurements from a wide range of fields, such as wastewater samples, plant fertilizer, and food samples.

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