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Review

Application of capillary isotachophoresis and capillary zone electrophoresis to the determination of inorganic ions in food and feed samples

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Abstract

The purpose of this review is to summarise critically the possibilities of capillary isotachophoresis and capillary zone electrophoresis for the determination of inorganic ions in food and feed samples. This article covers papers published since 1977.

Keywords: Reviews; Food analysis; Isotachophoresis; Capillary zone electrophoresis; Inorganic cations; Inorganic anions

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1. Introduction

All capillary electrophoretic methods use an electric field applied on a capillary to achieve a separation. Individual methods differ in separation principles, but two CE methods which have an identical separation principle, namely, capillary isotachophoresis (cITP) and capillary zone electrophoresis (CZE). Both methods are based on the same physico-chemical property, i.e. effective mobility. The practical difference between the two techniques lies in a different arrangement of the buffering electrolyte [1]. CZE represents a separation in a continuous electrolyte (background electrolyte) whereas cITP is an example of a technique employing a discontinuous electrolyte system [2] (leading and terminating electrolyte, respectively). Only these techniques are usually used for inorganic ions determinations. Several reviews dealing with applications of CE in food analysis [3–9] have been published. The last cited paper summarises CE applications, excluding cITP, of all food compounds with more than 200 references. The main aim of our review is to summarise the possibilities of cITP and CZE for the determination of inorganic ions in real food and feed samples. This work covers papers published from 1977 to June 1998. The emphasis is on quantitative determinations in real food and feed samples. Some general problems concerning this type of analysis will be also briefly mentioned.

2. Comparison of capillary isotachophoresis and capillary zone electrophoresis if applied to inorganic ions

2.1. Separation

CZE and cITP are based on the separation of compounds according to their effective mobilities. Knowledge of electrophoretic mobilities and pK_a values is therefore of key importance for systematic method development. Most inorganic ions posses high mobilities and their dissociation degrees cannot be affected by pH changes. Selectivity enhancement of ions with similar ionic mobilities is usually achieved by forming complexes with counterions or suitable additives.

Dispersion effects accompany both the cITP and CZE separations. In the case of cITP the dispersion acts against the selfsharpening effect and causes a deformation of the zone boundaries. In CZE the dispersion effects lead to the broadening of the zones.

2.2. Instrumentation

In order to use the advantages of both methods, the instruments for CZE and cITP differ in some aspects. A detailed description of instrumentation can be found elsewhere [10,2].

2.2.1. Sample injection

The injection volume and also the process differ substantially between the methods. A typical injection volume in CZE varies between units and tens of nanoliters, because a narrow sample pulse is required. The reproducible injection of low sample volumes into the separation capillary is a key problem for both quantitative analysis and separation efficiency. With cITP about 50% of the capillary volume (tens up to hundreds of microliters) can be filled with sample. This is the reason why the concentration sensitivity of cITP is comparable with that of CZE, although the mass sensitivity of CZE is higher than that of cITP.

2.2.2. Separation capillary

The isotachophoretic equipment consists of a narrow-bore tube made of an insulating material (PTFE, FEP) with an I.D. <1 mm and with length ≈100–500 mm. In cITP analysers the outlet side of capillary is separated from the electrode chamber by a semipermeabile membrane, which does not allow the electrolyte to flow out of the capillary due to different electrolyte levels in the electrode chambers. This arrangement with closed capillary results in a reversed hydrodynamic flow in the centre of capillary with a negative effect of the zone boundaries. Particularly for anionic analysis a reduction of the EOF is required (increased viscosity, zeta potential reduction).

In the CE instruments (used for the other CE methods) open capillary tubing (I.D. 25–100 μm) made from fused silica enables the EOF to have a piston-like profile. The EOF in CZE is an efficient tool for migration time and resolution tuning [11]. On the other hand, the EOF can be also a source of insufficiently reproducible migration times and resolutions.

2.2.3. Detection

Most inorganic ions lack a chromophore and cannot be detected using direct optical detection. For cITP the absence of a chromophore is no drawback as cITP instruments are equipped as standard with universal conductivity or potential gradient detectors. In the case of CZE (run in CE instruments equipped with UV detectors) an indirect photometric detection has to be used. It is based on the application of an UV absorbing co-ion to the background electrolyte. The zones of nonabsorbing ionic species are revealed by changes in light absorption due to charge displacement of the absorbing co-ion [12]. The indirect photometric detection applied to inorganic ions has a negative effect on efficiency, because most UV absorbing co-ions used in CZE (benzoate, chromate for anions, imidazole, benzylamine for cations) exhibit significantly lower effective mobilities than some inorganic ions (chloride, potassium). Some commercial CE instruments are also equipped with conductivity detectors. For this detection in CZE the maximal conductivity difference in the sample zone and the BGE is required. For the inorganic ions a background buffer with a lower conductivity is necessary. A suitable compromise must be found which enables both sufficient sensitivity and acceptable electrodispersion.

2.3. Combination of methods (two-dimensional capillary isotachophoresis–capillary isotachophoresis, capillary isotachophoresis–capillary zone electrophoresis)

Feed or food represents a complex sample matrix. There is only a limited possibility of determining ions which occur at low concentration levels in complex ionic matrixes. Rather laborious sample pretreatment is often necessary. An on-line combination of cITP and cITP (two-dimensional isotachophoresis, cITP-cITP) can serve as a very efficient solution. In such a case, ionic macroconstituents can be determined in the preseparation capillary. With suitable timing of the column switching only zones of interest are transferred into the analytical capillary. A simultaneous determinations of macro- and microconstituents with a molar ratio up to 10^4:1 is possible with appropriately chosen running conditions.

The sensitivity (and also the selectivity) of the determination of a UV absorbing microconstituent can be increased by placing a UV detector in the analytical capillary. A limitation of cITP–cITP occurs, however, if several UV absorbing compounds migrate stacked with an ion of interest (see Fig. 1a). In such cases an application of a suitable spacer is necessary. Another solution can be to combine cITP and CZE (cITP–CZE, see Fig. 1b). A suitable electrolyte system and instrument set-up can serve as an efficient sample clean-up in the first isotachophoretic stage. Narrow cITP zone of microcomponents from sample injected to the CZE stage represents an ideal injection for the CZE if both methods are carried out in a column-coupling instrument [13,14].

3. Application to food and feed samples

Inorganic anions and cations build a group of compounds with different nutritional value found on various concentration levels in real samples. Their determination in food and/or feed samples is a

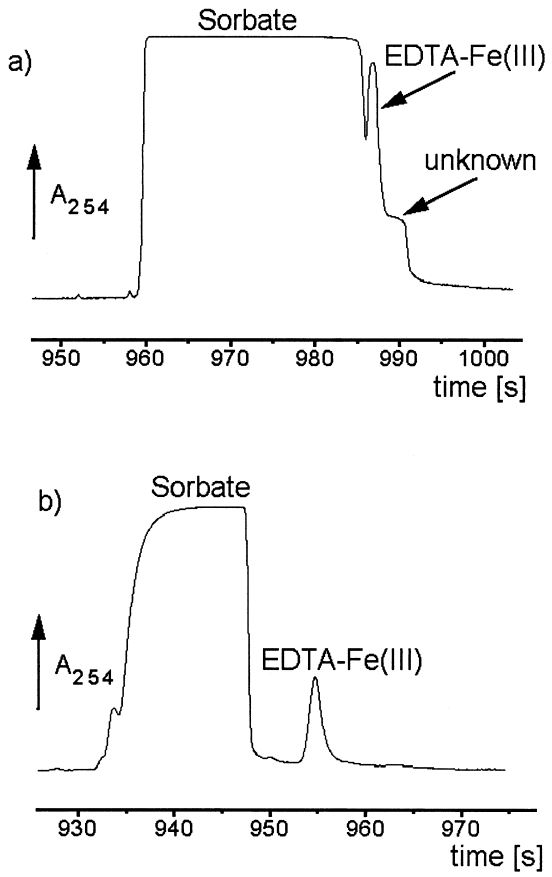

Fig. 1. Electropherograms of (a) cITP–cITP and (b) cITP–CZE. Ion of interest, (EDTA–Fe(III)) determined by cITP–cITP cannot be evaluated with an acceptable reliability, because it is stacked between the sorbate and unknown ions. Using cITP–CZE a correct evaluation is possible, as the ion of interest is destacked from other components of the sample matrix. UV records from analytical capillary are practical examples from a method development of the EDTA determination in mayonnaise.

specific area of application. It represents a determination of ions in a complex sample matrix. Very often food and feed samples exhibit appreciable heterogeneity and that is why the sample pretreatment requires more time and effort. In some cases sample pretreatment techniques (SPE, distillation, liquid–liquid extraction) have to be applied especially if a trace analysis is to be performed. Such approaches contribute a significant purification effect, which simplifies the separation requirements of the electrophoretic method.

A systematic presentation of papers dealing with determinations of inorganic ions in food and feed samples is rather complicated. A great number of published papers deal with determinations of a group of ions with different nutritional value (anions in water, cations in silages). A second group of applications are those which deal with determination of a single ion (or several ions) with a special importance in food or feed (nitrate and nitrite in vegetables, heavy metals in food, chloride in food). In our review, papers will be sorted into those in which a separation of a group of ions with different nutritional value was optimised and those in which a determination of individual ions with similar nutritional values are presented. Detailed descriptions of electrolytes used are summarised in Tables 1–3.

3.1. Determination of groups of inorganic ions with different nutritional value

Early papers mostly describe the older CE method, namely cITP. A wide application area is the analysis of ions in drinking and mineral waters. Drinking water is the simplest sample matrix in food analysis and that is why many authors apply the separations of model mixtures successfully on water samples.

3.1.1. Groups of anions

3.1.1.1. Capillary isotachophoresis

In drinking water samples the ions are found at several concentration levels. Chloride, nitrate, sulphate, bicarbonate can be considered anionic macroconstituents and their concentration in drinking water samples varies from units to hundreds of milligrams per liter. Fluoride, phosphate and nitrite are typically present at concentration levels from tenths to hundreds of micrograms per liter. From the separation point of view the critical pair of anions are sulphate and nitrate because their ionic mobilities are very similar. As pH changes cannot affect the effective mobilities of strong acids, the application of a suitable additive to the electrolyte is necessary. Boček et al. [15] used cadmium as the counterion for the selectivity enhancement. A limited dynamic concentration range (molar ratio 1:100) in a single capillary cITP instrument overcame the introduction of two-dimensional isotachophoresis (cITP–cITP) in a column-coupling device. Such an arrangement [16]

used in water analysis enabled the determination of macrocompounds (nitrate, sulphate) in the preseparation and microcompounds (nitrite, fluoride, phosphate) in the analytical capillary, respectively. The simultaneous determination of all macrocomponents (chloride, nitrate and sulphate as three visible steps on the isotachopherogram) was reported by Vacík and Muselasová [17]. Unfortunately the reaction of calcium hydroxide used as the leading electrolyte with carbon dioxide causes rather low robustness of this system which is probably unsuitable for routine analysis. An isotachophoretic method for the simultaneous determination of nitrate, sulphate, nitrite, sulphite, phosphate in sugar juices, molasses and white sugar was developed by Kvasnička et al. [18]. The method presented in that paper is an example of an application of cITP for the determinations of ions in a nonionogenic sample matrix. Even 30% (w/w) water solution of sucrose could have been injected to the cITP analyser and detection limits at ppm levels were thus achieved. cITP was also applied for the determination of inorganic (and organic) acids in wine [19,20].

3.1.1.2. Capillary zone electrophoresis

The first application of CZE to the determination of chloride, nitrate and sulphate in drinking water was with cadmium as counter-ion [21]. A laboratory-made device (closed system) with on-line potential gradient detector and isotachophoretically prepared cadmium acetate was used as the BGE. The BGE for CZE analysis of inorganic ions in CE instruments with open capillary and UV detectors consists of an UV absorbing co-ion and a suitable additive for reduction (or reversal of direction) of the EOF. Li and Li [22] used chromate as the BGE and CTAB as an EOF modifier for inorganic anion determinations in water and Pepsi Cola. Rhemrev-Boom [23] validated a CZE method for the determination of chloride, fluoride and sulphate in tap water with a commercially available BGE. A rather low linear range and high baseline noise were found to be the main drawbacks. Good correlation was found between CZE and IC if applied on drinking water samples [24]. CZE surpassed the IC in speed of analysis and ease of sample pretreatment. A wide range of real samples (milk, juice) was analysed using on-line dialysis coupled to a CE system [25]. Pyromellitic acid as UV absorbing co-ion and DETA was applied to a separation of some inorganic (and organic) acids [26]. The electrolyte system was validated for some beverages samples (wine, apple juices). Soga and Ross [27] published a method for the determination of anions in beer samples. A combination of two electrolytes was used for a determination of minor anions (chloride, sulphate and nitrate) in orange juices and pulpwash [28]. The possibility of the cITP–cITP combination for an analysis of trace anionic constituents present in a large excess of matrix ions has already been mentioned. A wide concentration span is also not acceptable for the CZE. It is, however, possible to combine cITP with CZE in order to determine both the macro- and microconstituents in a single run also when the concentration ratio of macro-/microconstituents reaches $\sim 3 \cdot 10^4$ [29]. The ions in the CZE step are detected with the conductivity detection with detection limits <1 ppb.

3.1.2. Groups of cations

3.1.2.1. Capillary isotachophoresis

Several optimisation approaches to the isotachophoretic separations of alkali and alkaline earth metals have been published. All approaches focused upon the selectivity enhancement of the two groups of cations [30] (potassium, rubidium, caesium and ammonium; calcium, strontium, magnesium and sodium) which are difficult to separate under non-complexing conditions in aqueous electrolyte systems. The concentration levels of strontium, rubidium and caesium are generally lower in comparison with other mentioned cations in many food and feed matrixes. Some of the optimisations were also aimed at mineral water samples, which can contain also significant concentration levels of these cations. Several authors used various counter- or co-counterions (see Table 1) with complexing ability to achieve a separation of cations determined in well water [17], sugar [18] and drinking or mineral water samples [31]. These approaches are, however, not efficient for the separation of ammonium and potassium cations. The solution of this separation problem can be based on the fact that ammonium hydroxide is a weaker base than the potassium hydroxide. If potassium hydroxide is acidified with a suitable weak

Table 1
Capillary isotachophoresis applied to the determination of inorganic ions in food and feed samples

Analysed ions	Electrolyte system	pH	Detection	Sample	Sample pre-treatment	Ref.
Chloride, nitrate	LE: 6 m*M* $Cd(NO_3)_2$ TE: 10 m*m* citric acid		PG PG	Drinking water	No	[15]
Chloride, nitrate, sulphate	LE: 5 m*m* $Ca(OH)_2$ TE: formic acid		CON	Well and surface water	Na_2CrO_4 used as an internal standard	[17]
Nitrate, nitrite, sulphate, sulphite	LE: 10 m*M* HCl+10 m*M* BALA +3 m*M* BTP+0.05% (w/w) HPMC TE: 10 m*M* citric acid	3.6	CON	Various water samples, sugar juices, molasses, white sugars	Liquid samples: dilution Solid samples: an aqueous extract was analysed	[18]
Phosphate, sulphate, sulphite, (and some org. acids)	LE: 10 m*M* HCl+BALA +0.05% Mowiol+0.2% HEC TE: 5 m*M* sodium propionate	2.9	CON	Wine	Dilution	[19]
Phosphate, sulphate, (and some org. acids)	LE: 10 m*M* HCl+BTP+0.2% Mowiol TE: 5 m*M* caprylic acid+Tris	6.0 8.0	CON	Wine	Dilution	[20]
Ca, K, Mg, Na	LE: 2 m*M* H_6L TE: 4 m*M* creatinine	2.4	CON	Well and surface water	Dilution	[17]
Ammonium, Ca, Mg, Na	LE: 10 m*M* KOH+50 m*M* H_3BO_3 TE: 10 m*M* lithium citrate	8.3	CON	Sugar juices, molasses, white sugars	Liquid samples: dilution Solid samples:extraction with water	[18]
Ca, K, Mg, Na	LE: 7.5 m*M* H_2SO_4 TE: 10 m*M* lithium citrate		CON	Sugar juices, molasses, white sugars	Liquid samples: dilution Solid samples: extraction with water	[18]
Ammonium, K, Ca, Mg	LE: 7.5 m*M* H_2SO_4 +7 m*M* CROWN+0.1% (w/w) HPMC: TE: 10 m*M* BTP		CON	Silages	Extraction with 0.1 *M* HCl, filtration and dilution	[34]
Iodide	LE: 10 m*M* HCl+His +0.2% HEC+6% PVP TE: 5 m*M* MES+His	6.0 6.0	UV_{254}	Mineral water	Addition of sulphate and fluoride as discrete spacers	[41]
Phosphate, (and some org. acids)	LE: 5 m*M* HCl+Gly–Gly TE: 5 m*M* caproic acid	2.8	CON	Cocoa, chocolate products	Extraction with hot water, filtration, ultrafiltration	[42]
Phosphate (and phytic acid)	LE: 10 m*M* HCl+5.6 m*M* BTP +0.1% MHEC TE: 5 m*M* MES	6.1	CON	Cereal grains, legumes, feed mixtures	Extraction with 3.5% HCl, filtration, dilution	[44]
Phosphate (and some organic acids)	LE: 10 m*M* HCl+5.6 m*M* BTP +0.1% MHEC TE: 5 m*M* caproic acid	6.1	CON	Feed additives	Extraction with 0.1 *M* NaOH in ultrasonic bath	[52]
Nitrate	LE: 8 m*M* HCl+3.5 m*M* BALA +3 m*M* BTP+0.1% HEC TE: 5 m*M* citric acid	3.55	CON	Vegetables	Homogenization with water, filtration and dilution	[57]
Fluoride	LE: 2 m*M* HCl+5 m*M* EACA +0.05% HPMC TE: 2 m*M* tartaric acid	4.25	CON	Feed mixtures, phosphates	Fluoride is released by heat-facilitated microdiffusion from 25% perchloric acid and trapped in 0.5 *M* NaOH	[58]
Al, Cd, Co, Cu, Fe, Mn, Ni, Pb, Yn	LE: 20 m*M* KOH +HAc+HIBA TE: 5 m*M* HAc	4.1	CON	Water samples	Sample enrichment on the Chelex 100 ion exchanger	[59]

acid (boric acid [18]) to a pH of ~8.3 the effective mobility of ammonium is reduced and the separation can be achieved. Another possibility is the application of a suitable nonionic additive i.e. crown ether [32] or polyethyleneglycol (PEG) [33]. PEG pseudophase in the leading electrolyte was applied for the determination of cations in water samples and aqueous extract of apple flesh. For mineral water samples the macroconstituents (K, Na, Ca) were determined in the preseparation column, removed from the separation compartment, while the microconstituents (Mg, Sr, Li, Ba) were determined under more favourable conditions in the analytical column.

Crown ether (18-crown-6) was applied for a simultaneous determination of ammonium, calcium, magnesium and potassium in silage samples [34].

3.1.2.2. Capillary zone electrophoresis

Combined complexing effect of negatively charged counterions and electroneutral 18-crown-6 [30] was utilised for the simultaneous determination of alkali, alkaline earth metals and ammonium in rain, tap and mineral water samples using an instrument with a closed capillary. The EOF results in a CZE analysis of cations which is very fast if run in a system with an open capillary. Imidazol–H_2SO_4 BGE was used to carry out the determination of K, Na, Ca and Mg in beverages [35]. The method was validated and compared with flame atomic spectrometry. For the optimisation of separation of mono-, di- and trivalent cations (see Table 2), a combination of counter-ions sulphate and 2-hydroxyisobutiric acid (HIBA) and additives 18-crown-6 and methanol was used. The electrolyte system [36] was applied to a Chinese tea infusion. Closed-vessel microwave acid digestion served as an efficient sample preparation technique for determinations of minerals in food materials [37]. The yield of the extraction was verified by analysis of reference materials. Morawski et al. [38] compared AAS, IC and CZE applied to the analysis of nutrients released from various food samples by microwave digestion. Higher potassium amounts found by CZE were probably caused by a comigration of ammonium cation with potassium. The effect of HIBA was also studied by Weston et al. [39] who determined some metal cations in tap water and orange juice. Pretswell et al. [40] determined the major cation content of an International Atomic Energy Agency (IAEA) standard reference material (powdered milk). The accuracy of this method was tested by comparison with standard flame AAS cation analysis. CZE was stated to have lower precision and accuracy and to be more susceptible to matrix effect, enabling, however, simultaneous quantitation with a wider linear range.

3.2. Determination of individual ions

3.2.1. Naturally occurring compounds

3.2.1.1. Capillary isotachophoresis

Madajová et al. developed a method for the determination of iodide in mineral water [41]. They studied the influence of linear polymer polyvinylpyrrolidone on selectivities of some inorganic ions. cITP was used for the determination of phosphoric acid in cocoa [42] and coffee [43]. Phosphate was also determined in cereal grains and legumes [44].

3.2.1.2. Capillary zone electrophoresis

Fukushi et al. [45] analysed free calcium in vegetables. Iron in drinking water can influence the organoleptic properties of water (colour, taste, turbidity). Simultaneous determination of iron(II) and iron(III) selectively complexed with 1,10-phenanthroline and CDTA was presented by Pozdniakova et al. [46]. Total iron content was determined in drinking and mineral water samples [47] and mayonnaise [48] by cITP–CZE as an EDTA–Fe(III) complex. CZE with a micellar pseudophase (MECC) was applied for the determination of thiocyanate, iodide, nitrate and nitrite in milk [49]. Selenate was analysed in selenium-rich water by Gilon and Potin-Gautier [50]. Selenate and selenite in mineral and tap waters were determined by Li and Li [51].

3.2.2. Additives

3.2.2.1. Capillary isotachophoresis

Phosphate (and some organic acids) was analysed by cITP in commercial feed additives [52]and also in feed mixtures [44].

3.2.2.2. Capillary zone electrophoresis

Sulphite is added to a wide variety of foods and beverages as an antioxidant. A method for its

Table 2
Capillary zone electrophoresis applied to the determination of inorganic ions in food samples

Inorganic ions sep.	Electrolyte buffer composition	pH	Detection	Sample	Sample pre-treatment	Ref.
Chloride, nitrate, sulphate	Cadmium acetate prepared by cITP run of LE: 4 m*M* $Cd(NO_3)_2$ and TE: 10 m*M* HAc		PG	Drinking water	Dilution	[21]
Carbonate, chloride, nitrite, sulphate	8.2 m*M* Na_2CrO_4 +0.048 m*M* CTAB	8.5	UV_{254}	Different Water samples, Pepsi Cola	Dilution	[22]
Chloride, fluoride, sulphate	2.25 m*M* PMA+6.5 m*M* NaOH +0.75 m*M* HMOH+1.6 m*M* TEA	7.7	UV_{250}	Tap water	No	[23]
Chloride, fluoride, nitrate, sulphate	4.5 m*M* Na_2CrO_4 +0.4 m*M* OFM		UV_{254}	Drinking water	No	[24]
Carbonate, chloride, nitrate, phosphate, sulphate	6 m*M* Na_2CrO_4+0.32 m*M* CTAB +3 m*M* H_3BO_3 +$Na_2B_4O_7$	8.0	UV_{372}	Tap water, fruit pulp, milk	On-line dialysis coupled to CE instrument with a specially designed interface	[25]
Chloride, nitrate, phosphate, sulphate (and some org. acids)	3 m*M* PMA +3 m*M* DETA+Tris	7.5	UV_{220}	Wine, apple juice	Dilution	[26]
Chloride, phosphate, sulphate, (and some org. acids)	5 m*M* PDC+0.5 m*M* CTAB	5.6	UV_{350}	Beer	Sonication and dilution	[27]
Chloride, sulphate	5 m*M* Na_2CrO_4+0.4 m*M* OFM anion-BT+lactic acid	8.0	UV_{254}	Orange juice,	Dilution	[28]
Nitrate	10 m*M* NaCl+0.4 m*M* OFM anion-BT+NaOH	8.0	UV_{214}	Orange pulpwash		
Ammonium, Ca, K, Mg, Na, (Ba, Cs, Li, Rb, Sr)	5 m*M* benzimidazole +tartaric acid+0.1% (w/v) HEC+40 m*M* CROWN	5.2	UV_{254}	Drinking water, mineral water	Without a pretreatment	[29]
Ca, K, Mg, Na	5 m*M* imidazole+H_2SO_4	4.5	UV_{214}	Orange and apple juice	Filtration and dilution	[35]
Ca, K, Mg, Mn, Na, (ammonium, Ba, Cu, Cr, Li, Sr, Ni, Zn)	5 m*M* imidazole+H_2SO_4 +6.5 m*M* HIBA+0.53 m*M* CROWN +20% (v/v) methanol+HCl	4.5	UV_{214}	Chinese tea infusion various food materials	Tea was infused in boiling water, filtered and diluted food materials: closed-vessel microwave acid digestion (HNO_3, H_2O_2)	[36] [37]
Ca, K, Mg, Na	1.2 m*M* UV-Cat-2 +3 m*M* Tropolone solution		UV_{185}	Various food samples	Microwave digestion (HNO_3, H_2O_2)	[38]
Ca, Cu, K, Mg, Na	5 m*M* UV-Cat-1+6 m*M* HIBA 5 m*M* UV-Cat-1+8 m*M* HIBA	4.4 4.4	UV_{214} UV_{185}	Tap water Orange juice	No	[39]
Ca	20 m*M* $Na_2B_4O_7$+2 m*M* EDTA	9.2	UV_{200}	Vegetables	Extraction with boiling water, filtration, dilution	[45]
Fe(II) and Fe(III)	100 m*M* H_3BO_3+NaOH	9.0	UV_{254}	Water	Filtration, pH adjustment (pH 2–2.4), addition of 1,10-phenathroline and CDTA	[46]

Table 2 (*continued*)

Inorganic ions sep.	Electrolyte buffer composition	pH	Detection	Sample	Sample pre-treatment	Ref.
Iodide, nitrate, nitrite, thiocyanate	50 m*M* DTAB+18 m*M* $Na_2B_4O_7$ +30 m*M* $NaHPO_4$+HCl	7.0	UV_{235}	Milk	Purification using ion-exchange technique with Sephadex A-25	[49]
Selenate	5 m*M* Na_2CrO_4+0.5 m*M* TTAOH	10.5	UV_{254}	Selenium-rich waters	No	[50]
Sulphite	5 m*M* Na_2CrO_4+0.5 m*M* OFM Anion-BT	8.0	UV_{254}	Cordial, wine, fresh vegetables, processed foods, seafood	Conversion to SO_2 (after addition of concentrated HCl), distillation into 3% H_2O_2 solution nitrate was used as an I.S.	[53]
Nitrate, nitrite	0.5 m*M* OFM Anion-BT +1000 ppm NaCl	8.0	UV_{210}	Cured meat, cheese, fruit juice, cabbage puree	For solid samples: extraction followed by a filtration and sample cleaning (SPE) For liquid samples: dilution thiocyanate was used as an I.S.	[54]
Zn	10 m*M* borate+0.1 m*M* HQS capillary was pretreated with TTMAB (reduction of EOF)	9.2	UV_{254}	Tap water	Addition of HQS to the sample	[63]
Nitrate, nitrite	10 m*M* Na_2CrO_4+ 2.3 m*M* CTAB+NaOH	11.5	UV_{254}	Vegetables	Homogenization with water, filtration and dilution	[64]

determination based on its conversion to sulphur dioxide and finally to sulphate using a Monier-Williams distillation was applied to many food samples [53] (see Table 1). The detection limit was about 5 ppm. Nitrate and nitrite salts are added to certain foods. Their determination has been published by Marshall and Trenery [54].

3.2.3. Contaminants

3.2.3.1. Capillary isotachophoresis

Determination of chromate in water by cITP–cITP at ppb levels was reported by Zelenský et al. [55]. Chromate stacked between spacers (see Table 3) was evaluated as a peak using photometric detection at a wavelength of 405 nm with detection limit 4–5 ppb. Although nitrate and nitrite are added to some samples during the food processing, they also occur in some samples as contaminants from microbial activity or nitrogenous fertilisers. A method for the determination of nitrates in milk was developed by Stránský et al. [56]. Karovičová et al. [57] analysed nitrates in vegetables. Dicalcium phosphate is generally added to animal feed mixtures to increase the nutritional value. Phosphates however, also contain fluoride, which contaminates the feed mixture. Two isotachophoretic methods [58] were presented for a determination of fluoride in feed mixtures and dicalcium phosphates (used as a phosphate source). A method for a simultaneous determination of heavy metals and aluminium in water at ppb levels was published by Everaerts et al. [59]. Samples were analysed after an enrichment on a cation exchanger Chelex 100. The possibilities of photometric detection of metal cations based on the formation of kinetically labile, light absorbing complexes during isotachophoretic separation were studied by Zelenský et al. [60]. The authors used xylenol orange as a chelating co-counterion in the cationic mode of cITP; the method was applied to a tap water sample where Zn, Cd and Mn were detected without sample pretreatment. However the authors state that further experimental work is essential before the method can be considered for routine use.

3.2.3.2. Capillary zone electrophoresis

Romano and Krol [61] presented a CZE method for the determination of inorganic contaminants (fluoride, nitrite, nitrate, sulphate, chloride) in drinking water. A similar method [62] was proposed for the determination of anionic disinfection by-products in drinking water. Timerbaev et al. [63] separated some transition and alkaline earth metals as pre-column formed chelates with 8-hydroxyquinoline-5-

Table 3
Combined electrophoretic method applied in food and feed analysis

Inorganic ions separated	Electrolyte buffer composition	pH	CE method	Detection	Sample	Sample pretreatment	Ref.
PK: nitrate, sulphate	LE_1: 8 m*M* HCl+3 m*M* BTP +1.5 m*M* BALA+0.1% HEC	3.68	cITP–cITP	CON–CON	Drinking water	No	[16]
AK: nitrite, fluoride, phosphate	LE_2: 2 m*M* HCl+1.5 m*M* BALA +0.1% HEC TE: 5 m*M* citric acid	3.54					
PK: nitrate, sulphate	LE: 8 m*M* HCl+BALA +3 m*M* BTP TE: 4 m*M* Asp+BALA	3.4	cITP–cZE	CON–CON	River water	Dilution	[29]
AK: nitrite fluoride, phosphate	BGE: 10 m*M* Asp+BALA	3.4					
AK: Ba, Ca, Li, Mg, Na Sr	LE: 10 m*M* NH_4OH+ACES +3 m*M* HIDA+0.1% HEC TE: His	6.8 6.3	cITP–cITP	CON	Drinking water, mineral water	No	[31]
AK: K, Na, Ca, Mg	LE: 10 m*M* NH_4OH+HAc +0.2% (w/v) HEC, in water–PEG (55:45, v/v) TE: 3 m*M* TBA^++ClO_4^-	5.0	cITP–cITP	CON	Drinking water apple flesh	Water: dilution apple flesh: extraction with water, filtration, dilution	[33]
PK: Ca, K, Na	LE_1: 20 m*M* NH_4OH+HAc +0.2% (w/v) HEC, in water–PEG (55:45, v/v)	5.0	cITP–cITP	CON–CON	Mineral water	Filtration, dilution	[33]
AK: Ba, Li, Mg, Sr	LE_2: 5 m*M* NH_4OH+HAc +0.2% (w/v) HEC, in water–PEG (55:45, v/v) TE: 3 m*M* TBA^++ClO_4^-						
AK: iron	LE: 10 m*M* HCl+20 m*M* His +0.1% HPMC TE: 5 m*M* MES	6.0	cITP–CZE		Drinking water, mineral water,	Water:acidification with 65% HNO_3, dilution, analysis with 0.1 m*M* EDTA	[47] [48]
	BGE: 25 m*M* MES +10 m*M* BTP	6.6			Mayonnaise	Mayonnaise: extraction with water (ultrasonic bath), separation of the fat phase (centrifugation)	
AK: chromate	LE: 10 m*M* HCl+BALA +0.1% (w/v) HEC TE: acetate	3.5	cITP–cITP	UV_{405}	Different water samples	Addition of NTS (reduction of chromate adsorption on the capillary wall and spacer from the side of leading zone) and methanesulponate (spacer from the side of termination zone)	[55]
AK: fluoride	LE_1: 8 m*M* HCl+22 m*M* EACA +1 m*M* $CaCl_2$+0.05% HPMC LE_2: 2 m*M* HCl+5 m*M* EACA+0.05% HPMC TE: 10 m*M* tartaric acid (10 m*M* Na_2HPO_4 for a determination of flouride in phosphates)	4.45 4.25	cITP–cITP	CON	Feed mixtures, dicalcium phosphates	Extraction with, 1 *M* NaCl filtration, dilution	[58]

sulphonic acid (HQS). The method applied to a tap water sample enabled the determination of zinc at a ppb level without preconcentration. CZE with indirect detection was also used for the simultaneous determination of nitrate and nitrite in vegetables [64]. The method was cross-validated with an official AOAC method and good agreement was found. Arsenic compounds were determined in water samples. On-column enrichment (100 fold) enabled to achieve detection limits on ppb levels [51].

4. Conclusion

Determination of inorganic ions in food and feed samples represents rather small application field of food and feed analysis. CZE applied to food samples can offer advantages such as very short analysis time and ultra low electrolyte and sample consumption, respectively. The main disadvantages are complications with transfer of the method to real samples (matrix effect [35,65]) and electrodispersion enforced by the use of an UV absorbing co-ion (described in more detail in Section 2.2.3).

Inorganic ion determinations in food samples can be considered one of the main application areas of cITP. The main advantages of cITP compared with CZE are higher robustness and precision. The disadvantages are longer analysis times and a requirement for higher electrolytes purity. A further drawback of cITP is that instruments available for cITP do not reach the user comfort of CE (HPLC, GC) instruments.

cITP–cITP and cITP–CZE are techniques suitable for analysis of trace levels of ions in complex ionic matrixes. The limitations of these methods can be seen in the sensitivity to ionic impurities in the chemicals used for the electrolyte preparations and in a possible sample adsorption on the capillary wall.

5. Abbreviations

AAS	Atomic absorption spectrometry
ACES	N-(2-acetamido)-2-aminoethane sulphonic acid
AK	Analytical capillary
AOAC	Association of Official Analytical Chemists
ASP	Aspartic acid
BALA	β-Alanine
BGE	Background electrolyte
BTP	1,3-Bis[tris(hydroxymethyl)methylamino]propane, bis-tris-propane
CDTA	*trans*-Cyclohexane-1,2-diaminetetraacetic acid
cITP	Capillary isotachophoresis
cITP–cITP	Capillary isotachophoresis run in column coupling device
cITP–CZE	On-line combination of capillary isotachophoresis and capillary zone electrophoresis
CON	Conductivity detection
CROWN	18-Crown-6
CTAB	Cetyltrimethylammonium bromide
CZE	Capillary zone electrophoresis
DETA	bis(2-Aminoethyl)amine, diethylenetriamine
DTAB	Dodecyltrimethylammonium bromide
EACA	ϵ-Aminocaproic acid
EDTA	Ethylenediaminetetraacetic acid
EOF	Electroosmotic flow
FEP	Fluorinated ethylene–propylene
Gly–Gly	Glycyl–glycine
H_6L	N-Oxide of nitrilotrismethylenephosphonium acid
HAc	Acetic acid
HEC	Hydroxyethylcellulose
HIBA	α-Hydroxyisobutyric acid
HIDA	Hydroxyethyliminodiacetic acid
His	Histidine
HMOH	Hexamethonium hydroxide
HPMC	Hydroxypropylmethylcellulose
HQS	8-Hydroxyquinoline-5-sulphonic acid
IAEA	International Atomic Energy Agency
IC	Ion chromatography
I.D.	Internal diameter
LE	Leading electrolyte
LE_1	Leading electrolyte in the preseparation capillary
LE_2	Leading electrolyte in the analytical capillary
MECC	Micellar electrokinetic capillary chromatography
MES	2-(N-Morpholino)ethanesulfonic acid

MHEC	Methylhydroxyethylcellulose
NTS	Naphthalene-1,3,6-trisulphonate
OFM	Anion-BT EOF modifier supplied from Waters
PDC	2,6-Pyridinedicarboxylic acid
PEG	Polyethylene glycol
PG	Potential gradient detection
PK	Preseparation capillary
PMA	1,2,4,5-Benzene-tetracarboxylic acid, pyromellytic acid
PTFE	Polytetrafluoroethylene
PVP	Poly(vinylpyrrolidone)
SPE	Solid-phase extraction
TBA	Tetrabutylammonium
TE	Terminating electrolyte
TEA	Triethanolamine
TRIS	Tris(hydroxymethyl)aminomethane
TTAOH	Trimethyltetradecylammonium hydroxide
TTMAB	Tetradecyltrimethylammonium bromide
UV_{254}	UV detection at 254 nm
UV-Cat-1	Background electrolyte for separation of non-UV absorbing cations supplied from Waters

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