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Review

Use of ion chromatography in agricultural research

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ABSTRACT

During the last decade ion chromatography developed into a powerful analytical technique. Versatility, speed of operation, simultaneous multi-ion analysis, small sample size requirements and reasonable cost are some of the factors that have contributed to its popularity. One of the unique features of this technique has been the quantitative determination of various species of an ion (*e.g.*, different oxidation states) that may exist in a sample. This paper describes the applications of ion chromatography technology in the agricultural research and some future directions.

CONTENTS

1. INTRODUCTION

In the past decade ion chromatography (IC) techniques have become an increasingly important analytical methodology. Its versatility, simultaneous multi-ion analysis, speed of operation and reasonable cost contributed to its popularity. It is an analytical technique that can separate ionic species, in discrete bands in a liquid moving phase, using different separation modes and different detection technology. The modes of separation are: high-performance ion chromatography (HPIC), mobile phase ion chromatography (MPIC) and mobile phase ion chromatography exclusion (MPICE) [1]. The ionic species of interest are detected by proper selection of one of

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these detection modes: conductivity with and without suppression, amperometric or pulsed amperometric, ultraviolet–visible light absorption, fluorescence and other appropriate systems.

IC has been utilized by many agricultural scientists and soil, water and plant analysis commercial laboratories. There have been significant improvements in different areas of IC including, but not limited to, development of new stationary phases, use of different mobile phases, pre-injection sample concentration, advanced suppressor technology, use of highly sensitive detectors and column-switching techniques [2]. The impact of these progressive improvements will make IC a method of choice now and in the future. The objective of this paper is to describe some of the applications of this technique that have been used in agricultural research.

2. INORGANIC ANION ANALYSIS

Fluoride, chloride, nitrite, bromide, nitrate, sulfate and phosphate in rain, sewer water, soil pore-water and plant and soil extracts have been analyzed by several investigators using IC [3–11]. They have used single-column IC with low-conductivity eluent [12] (Fig. 1) or dual columns in which the second column suppresses the background conductivity [4,10] (Fig. 2). Some investigators used conductivity as a mode of detection [9–11] while others used direct UV detection [5]. Indirect UV detection, in which the eluent includes an ionic species that absorbs in the appropriate region and that is soluble in the eluent, has also been used. Inorganic anions which do not absorb in that region appear as throughs in the baseline [3].

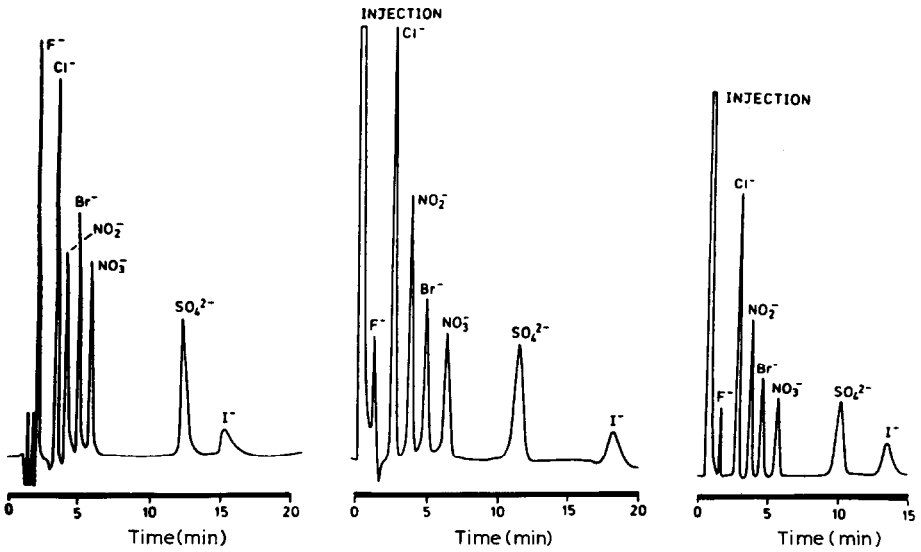

Fig. 1. Non-suppressed (single-column) IC of inorganic anions using different columns. (Left) Hamilton PRP-X100, with 1 m*M* phthalate eluent pH 5.5 at a flow-rate of 2 ml/min. (Middle) Bio-Gel TSK IC Anion PW, with 1 m*M* phthalate eluent pH 5.3 at a flow-rate of 1.2 ml/min. (Right) Waters IC Pak A, with 1 m*M* phthalate eluent pH 7.0 at a flow-rate of 1.2 ml/min. Sample, 10 μl of a mixture containing 100 ppm of the indicated anions. From ref 6.

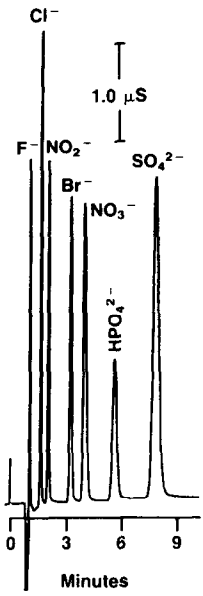

Fig. 2. Typical suppressed (dual-column) IC for inorganic anions. Column: HPIC-AS4A; eluent: 2 m*M* Na_2CO_3, 0.75 m*M* $NaHCO_3$; flow-rate: 2 ml/min; sample: anion standard; F^- (0.5 ppm), Cl^- (1.5 ppm), NO_2^- (2.5 ppm), Br^- (5.0 ppm), NO_3^- (5.0 ppm), HPO_4^{2-} (7.5 ppm) and SO_4^{2-} (7.5 ppm). Reproduced from document No. LPN 32629, Dionex Corporation, Sunnyvale, CA, November 1985.

Tabatabai and Dick [11] compared IC methods for measuring nitrate, chloride, sulfate and phosphate in natural water and soil extracts with currently available wet chemistry methods. They used steam distillation for nitrate, mercuric thiocyanate and ferric ammonium sulfate, colorimetric method for chloride, reduction and colorimetric determination using methylene blue for sulfate and colorimetric molybdenum blue method for phosphate. In all instances there was a close agreement between both techniques (Fig. 3), with the added advantage that IC was rapid and sensitive, required microgram levels of sample size, and all anions were determined in one assay rather than four separate assays.

Nitrate analysis of soil and plant extracts by directly measuring UV absorption at 210 nm suffers from the background interferences by organic compounds. Thayer and Huffaker [13] developed a method for separation of nitrate from the interfering compounds, using an anion-exchange column and then its determination by UV absorbance at 210 nm. Schroeder [14] proposed the use of a reversed-phase separation on an octadecyl column with aqueous phosphoric acid–dihydrogenphosphate as mobile phase. Both methods separated nitrate from organic chromophores. In another approach using MPIC, nitrate was separated using 2 m*M* tetrabutylammonium hydroxide in 0.05 m*M* Na_2CO_3, 10% acetonitrile [1] as mobile phase, and detected with a conductivity detector.

Poulson and Borg [15] used single-column IC for the analysis of sulfide, sulfite, thiosulfate and thiocyanate in water samples. The system used a resin-based anion-exchange column, a gluconate–borate eluent and electrochemical and UV detectors.

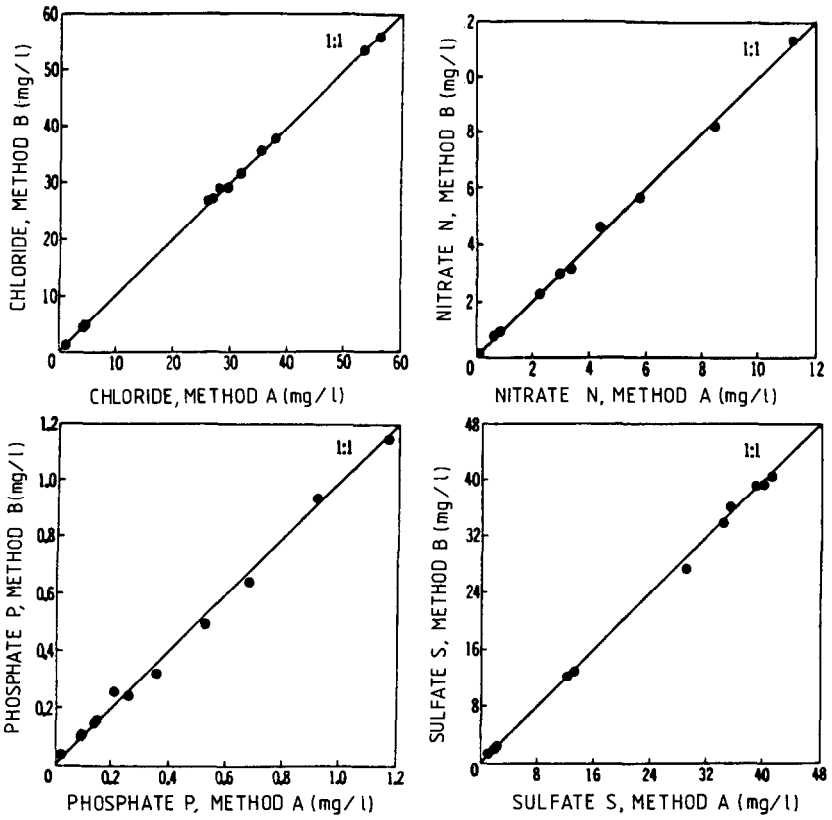

Fig. 3. Reliability of IC method as compared to wet chemistry methods. The x-axis (method A) refer to IC values and the y-axis (method B) are (I) $Hg(CNS)_2$–Fe^{3+} colorimetric method for chloride, (II) steam distillation method for nitrate, (III) molybdenum blue method for phosphate and (IV) methylene blue method for sulfate. From ref. 11.

Determination of total sulfur in plant material or soil requires conversion of all sulfur forms to SO_4^{2-} by wet digestion or to S^{2-} by reduction. Neither approach yields a sample suitable for IC because of high background ion concentration. Both Hern *et al.* [3] and Busman *et al.* [16] resorted to combustion of sample in oxygen atmosphere with no excess of ionic material. However, combustion of samples in an oxygen flask is cumbersome, time-consuming and difficult to automate. Recently, Hafez *et al.* [17] used IC for the determination of total sulfur (as sulfate) in plant materials digested with nitric and perchloric acids. The sample matrix problem (high ionic background) was solved by evaporating the acid digests to dryness. The separation of sulfate was accomplished on a multisubstrate column (Omni Pac-PAX 500) developed by Dionex [18]. The column substrate has a polymeric hydrophobic surface core with an ion-exchange polymeric colloid attached on it. This created a stationary phase suitable for simultaneous ion-exchange and reversed-phase modes of separation. It took a much longer time for perchlorate ions (relative to sulfate) to elute from a low-capacity anion-exchange column. This would make the assay time

long and render the method impractical. With simultaneous ion-exchange and reversed-phase separation, however, perchlorate ions rapidly eluted while fully separated from sulfate (perchlorate ions are less polar than other inorganic ions). Using this column with 25 m*M* sodium hydroxyde–acetonitrile (45%) as mobile phase at a flow-rate of 0.8 ml/min, the analysis time was only 10 min per sample (Fig. 4) compared to as much as 60 min on a single-mode ion-exchange separation. With this method about 98.2–101% of total sulfur was recovered from several different reference plant materials with and without addition of different sulfur compounds.

Selenite, selenate and arsenate have been measured in soil extracts using single-column IC [19–21]. For selenite analysis, interfering chloride ions were removed by treating the sample with silver-saturated cation-exchange resin [19]. The concentration limit above which there was interference of sulfate on selenate analysis was 40 mg/l, while that of phosphate on arsenate analysis was 30 mg/l. The United States Environmental Protection Agency report [22], investigating the IC technique for determining selenium species in ground water, found that for suitable selenate analysis the sample must be pretreated with $Ba(OH)_2$ to lower the sulfate interference. For lower detection limits (sub-ppb, 10^9) of selenium analysis free from sulfate interference, Yamada *et al.* [23] measured selenite in soils after derivatization with 2,3-diaminonaphthalene (DAN). The derivative, 4,5-benzopiazselenol, was extracted in cyclohexane, separated on a Cosmosil 5 SL silica gel column and detected fluorometrically at 380 nm for excitation and 525 nm for emission. Selenate can be mea-

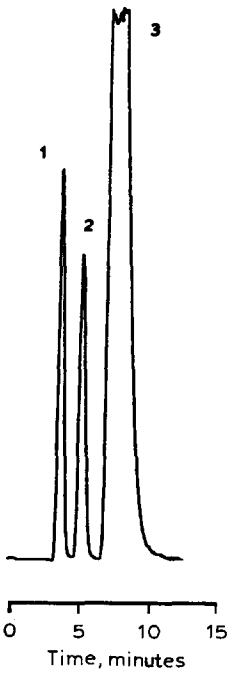

Fig. 4. Determination of sulfate in nitric, perchloric acids plant digest. Peaks: 1 = chloride; 2 = sulfate; 3 = perchlorate from ref. 17.

sured after its reduction to selenite with hydrochloric acid. Recently, Goyal *et al.* [24] reported an IC method using photometric detection for the simultaneous determination of arsenite, arsenate, selenite and selenate in water samples. A background sulfate concentration of up to 2000 mg/l did not affect the selenate analysis. However, arsenate analysis was affected by sulfate concentrations greater than 1400 mg/l.

3. INORGANIC CATION ANALYSIS

Total K, Na, Ca and Mg in plant materials have been measured after dry ashing and extraction in 5 m*M* HCl, with supressed IC and conductivity detection [25]. Exchangeable bases in soils were extracted by 1 *M* ammonium acetate followed by evaporation of the extract and ignition of the residue at 400°C to remove the ammonium acetate background. The clean residue was extracted in 5 m*M* HCl and exchangeable Na, K, Ca and Mg were measured by IC [26]. Fritz *et al.* [27] using single-column IC analyzed drinking water for Li, Na, K, NH_4, Rb and Cs ions using nitric acid as eluent. They also measured Ba, Sr, Ca and Mg ions using ethylene diammonium dinitrate as eluent.

An IC method for the simultaneous determination of urea and ammonium nitrogen in soil extracts has been available [28]. The method used a cation-exchange which retarded the migration of ammonium ions while urea molecules were not retained. An in-line catalytic solid-phase urease reactor, placed after the cation-exchange column, hydrolyzed the urea to ammonium ions. Thus, both ammonium and urea peaks were detected fluorometrically as ammonium after post-column derivatization with *o*-phthalaldehyde (OPA) and mercaptoethanol [29].

An anion- and cation-exchange column, HPIC-CS5 (Dionex) has been shown to separate NO_2^-, NO_3^- and NH_4^+ simultaneously. Nitrite and nitrate are detected by direct UV, while NH_4^+ is post-column-derivatized by OPA and detected by a fluorescence detector. The minimum detection limit was 1 ppm in 10% KCl [30].

4. HEAVY METAL ANALYSIS

Methods for heavy metal analysis such as atomic absorption or inductively coupled plasma atomic emission spectroscopy are based on total mass and do no distinghuish between the ionic species. Speciation of the ionic form is important in studying heavy metals in the environment. For instance some metals can exist in more than one oxidation form. They can also exist in both cationic or anionic form such as Cr(III) or chromate, Cr(VI). IC can be used to separate ionic species and molecules with different oxidation states. In general, separation of metals has been done through on-line metal chelating using an eluent containing complexing agent. The chelating ligands react with the coloring agent to form UV–visible-absorbing derivatives in a wide variety of metal cations including Hg, Pb, Co, Cr, Cu, Fe, Al and Zn [31]. Post-column reaction with 4-(2-pyridylazo)resorcinol results in derivatives of Fe^{2+}, Fe^{3+}, Pb^{2+}, Cd^{2+}, Ni^{2+}, Zn^{2+} and Co^{2+} that can be detected at 520 nm [32]. Heavy metals such as Cu, Zn, Ni, Co and Cd can also be measured by a coulometric detector [33].

5. ORGANIC COMPOUNDS ANALYSIS

Organic acids such as maleic, malonic, lactic, formic and propionic acids were successfully separated by an HPICE-Asl (Dionex) column using suppressed IC with conductivity detection. Amines, carbohydrates, amino acids, sugars and polysaccharides can be measured with IC using a suitable mode of separation (column) and a proper detector. For instance ion-exchange columns with pulsed amperometric detection have been used to measure organic compounds like mono- di- and tri-saccharides, alcohols and primary and secondary amines. Amino acids can be separated on an ion-exchange column and detected fluorometrically or spectrophotometrically after post-column derivatization with OPA or ninhydrin, respectively [34].

6. RECENT DEVELOPMENTS

6.1 New stationary phases

Brown and Pietrzyk [35] demonstrated the use of mixed-bed ion-exchange columns containing alumina and silica for simultaneous separation of anion and cation analytes. Manipulation of the alumina to silica ratio, pH of the mobile phase, eluent counter ions and their concentration were the major factors to improve resolution and shifting retention order. Such stationary phases can separate mono- and divalent anions and cations in single-sample injection with a single column and a single detector.

The Dionex column Omnipac-PAX500 [18] (PCX 500 for cation exchange) combines ion-exchange, reversed-phase and ion-pair separation modes on the same column. These modes of separation can be performed either simultaneously or in series depending on the composition of the mobile phase. This gives a great flexibility to the analyst to choose the best separation conditions.

6.2 Sample pre-concentration

Concentrator columns are used to trap trace levels of solutes and insure interference-free analysis. However, careful selection of eluent and concentrator substrate must be exercised to insure quantitative retention of ions of interest and their subsequent transfer to the analytical column [36].

6.3 Supressor column technology

The use of a micromembrane suppressor, to lower the background conductivity as well as enhancing the conductivity of the analytes, is based on the Donnan equilibrium. Thus in anion analysis, a satisfactory replacement of eluent cation by H ions requires a thin-wall membrane plus continuous-flowing regenerate acid. Higher operation cost due to the flowing acid can now be minimized by regenerating the acid. Tian *et al.* [37] developed an electrochemical supressor with anode and cathode made of platinum-plated titanium. For a 5 m*M* Na_2CO_3 eluent with a flow-rate of 1 ml/min, a constant current of 50 mA is applied and the voltage across the suppressor electrodes is 4 V. For such low voltage, the heating effect on the eluent solution is acceptable. The advantage of the electrochemical supressor is the elimination of the acid, and the back pressure of the suppressor is lowered. The technique shows promise and could simplify supressed IC in the future. However, the suppression efficiency of both systems appears to be similar.

6.4 Column switching

It is either a simple on-line mobile phase direction change or a chromatographic mode change which provides more resolving power as multidimensional chromatography [2]. A single-mode column with mobile phase direction switching can be used to reduce analysis time and eliminate gradient elution when using the same eluent.

Continuous development of techniques in IC for better sensitivity, matrix-free interferences, new separation modes, in-line pre- and post-column treatments and new detection techniques will increase the scope of its utilization in agricultural research.

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