ION CHROMATOGRAPHY OF ANIONS IN CANE SUGAR MILL PRODUCTS

SN WALFORD

Sugar Milling Research Institute, University of Natal, Durban, 4041

Abstract

Ion chromatography (IC) is a simple, reliable technique that enables the simultaneous separation and determination of inorganic and organic ions in complex mixtures. Separated ions are detected using either suppressed or non-suppressed techniques. Advantages and disadvantages of each are discussed. It is shown that whilst suppressed detection offers lower detection limits for individual ions both procedures offer excellent linearity for all ions. Choice of column packing, eluent composition and pH are shown to be useful variables when analysing multivalent ions (e.g. phosphate and aconitic acid) in complex mixtures such as molasses. Good agreement with conventional methods of analysis such as potentiometric titration (chloride) and atomic emission (phosphate) are described. IC shows excellent repeatabilities and recoveries. Examples of IC analysis include clarification, and degradation of molasses.

Introduction

Ion chromatography was first introduced in 1975 for the determination of inorganic anions and cations and water soluble organic acids and bases. It has since been widely used for the analysis of waste waters, process streams, rain waters, air samples and food products (Haddad and Jackson, 1990a), gaining acceptance as the standard method of water analysis (Table 1). It's use as an analytical technique in the beet industry has increased in recent years (de Bruijn and Heringa, 1992; Puke *et al*, 1996; Rearick and Little, 1990; Van der Poel, *et al*, 1990), mainly due to the importance of alkalinity measurements for process control. Its use has been limited in the cane sugar industry where the inorganic content of factory streams is measured and reported as total ash and used as such as a process tool. Ion chromatography is a powerful research analysis tool for the sugar technologist with speciation of the ions of interest being the main advantage of this analysis technique. This paper serves as an introduction to the technique describing the principles and some applications relevant to the industry.

Methods and Materials

Equipment

A Metrohm 761 Compact Ion Chromatograph was used in this study. It consists of a double piston pump, pulsation damper, injection valve, suppressor and associated two channel peristaltic pump and thermostated conductivity detector. All the components are housed inside an insulated housing to provide thermally stable conditions and controlled from a PC. The system can be used in both suppressed and non-suppressed modes (see Results and Discussion - *Detection*). Where eluent pH had to be adjusted (phthalic and para-hydroxybenzoic acid eluents), a Crison micropH2001 pH meter was used in conjunction with a Mettler Toledo InLab 412 pH electrode. This was standardised before use with pH 7 and 4 buffers. A Metrohm 665 Dosimat and silver electrode were used for potentiometric chloride titrations.

Reagents

All eluents and buffer solutions were made from Analar reagents (phthalic acid, sodium bicarbonate, sodium carbonate, para-hydroxybenzoic acid (pHBA), tris (hydroxymethyl)-aminomethane (TRIS)). Standard stock solutions of anions were made from Analar reagents (sodium chloride, potassium

Table 1. **Officially approved ion chromatographic methods.**

Method	Title
EPA 300.1 part 1 & 2	Determination of inorganic anions in drinking water by ion chromatography
DIN 38413-8	Determination of the dissolved complexing agents NTA, EDTA and DTPA by liquid chromatography
ISO 14911	Water quality - determination of dissolved lithium, sodium, ammonium, potassium, manganese(II), calcium, magnesium, strontium and barium by ion chromatography - methods for water and wastewater
DIN 38405 D 7	Determination of cyanides in slightly polluted water by ion chromatography
ISO 10304-1 DIN 38405 D 19	Water quality - determination of the dissolved anions fluoride, chloride, nitrite, orthophosphate, bromide, nitrate and sulphate by liquid chromatography - part 1. Methods for slightly polluted wastewater
ISO 10304-2 DIN 38405 D 20	Water quality - determination of dissolved anions by LC - part 2. Determination of bromide, chloride, nitrate, nitrite, orthophosphate and sulphate in wastewater
ISO 10304-3 DIN 38405 D 22	Water quality - determination of dissolved anions by LC - part 3. Determination of chromate, iodide, sulphite, thiocyanate and thiosulphate in wastewater
ISO 10304-4 DIN 38405 D 25	Water quality - determination of dissolved anions by LC - part 4. Determination of chlorate, chloride and chlorite in slightly polluted wastewater
AOAC Method 993.30	Determination of inorganic anions in water by ion chromatography
NIOSH 4110	Determination of anions by ion chromatography
EPA 218.6	Determination of dissolved hexavalent chromium in drinking water, ground water and industrial wastewater effluents by ion chromatography

dihydrogen phosphate, sodium sulphate, potassium nitrate, potassium oxalate). Chromatographically pure *trans*-aconitic acid (Riedal de Haan) and *cis*-aconitic acid (96%, Sigma) were used to prepare stock solutions of these acids. Eluent preparation is described in Appendix 1.

Columns

A Hamilton PRP-X100 anion column was used with pHBA eluent in the non-suppressed mode. A Metrosep Anion Dual 1 glass cartridge column was used with both phthalic acid eluent (non-suppressed mode) and carbonate-bicarbonate eluent (suppressed mode).

Sample preparation

Samples were prepared by dilution with distilled water and filtration through a 0,45 μm membrane. The dilution used was dependent on the sample type (Table 2). A 20 μl injection volume was used for all analyses.

Results and Discussion

Ion chromatography (IC) is a liquid chromatographic analytical technique for the separation and determination of ionic solutes. The two important factors influencing an ion chromatographic separation are the actual separation technique (ion exchange) and mode of conductivity detection used. Each will be discussed briefly.

Ion exchange

The ion exchange process can be illustrated by considering an anion exchange material which can be represented as M^+. To preserve electroneutrality it will have an associated counterion E^- from the eluent which is passing through the column. When a solution containing a solute anion A^- is brought into contact with the ion-exchanger, an equilibrium is established between the two mobile ions E^- and A^- as follows:

$$M^+E^- + A^- \rightleftharpoons M^+A^- + E^-$$
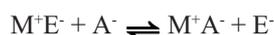
As the eluent passes through the column of ion-exchange material, the solute anion will be retained according to the selectivity of A^- compared to E^- resulting in retention on the column. As different solute anions have different selectivities toward the ion-exchange material, a separation can be effected. Generally the monovalent anions will elute first, followed by the divalent and finally trivalent anions. The separation will be influenced by choice of packing material, eluent composition and eluent concentration.

Both inorganic and polymeric materials are used as the matrix for the manufacture of ion-exchange packings. The separation

Table 2. **Dilutions used for suppressed and non-suppressed ion chromatography analysis of sugar factory samples.**

Sample type	g / 200 ml	
	Non-suppressed	Suppressed
DAC	8,0	4,0
MJ and CJ	4,0	2,0
Syrups	0,7	0,3
Final molasses	0,2	0,1
Refinery jets	20,0	
Raw sugar		5,0

columns used in this study both contained synthetic polymeric materials (poly(styrene-divinylbenzene) and hydroxylethyl methacrylate), both of which are functionalised by the addition of a quaternary amine group. Advantages of resin based material include a tolerance to a wide pH range (typically 1 to 12) and greater separation efficiency compared to inorganic materials. The main drawback of the methacrylate materials is pressure limitations, restricting column length and flow rates.

The role of the eluent is to compete with solute ions for the fixed ionic sites on the stationary phase, thus separating the mixture into well defined bands. Important eluent characteristics include:

- Compatibility with the detection mode.

The detection mode to be used is the major factor determining the type of eluent. Sensitivity of analysis is determined by the natural, background conductance of the eluent (see next section).

- Nature and concentration of the competing ion (E^-).

This is determined by the selectivity coefficient which describes the extent to which the solute ion is able to displace the competing ion from the stationary phase. Retention of a solute increases with increasing selectivity values and decreases with increasing concentration.

- Eluent pH.

Eluent pH influences the charge present on both the eluent and solute ions. The charge on polyprotic weak acids will be governed by eluent pH. Examples of solutes showing this effect include carboxylate anions, F^-, CO_3^{2-}, PO_4^{3-} and SiO_3^{2-}. When these ions are present in mixtures with other solute ions showing no pH effects (e.g. Cl^-, SO_4^{2-}), the control of eluent pH is an important variable that can be manipulated to effect a suitable separation.

- Buffering capacity.

The effect of eluent pH on the retention behavior of the solutes requires that the eluent show some buffering capacity. This enables the eluent to maintain a stable pH and provide reproducible retention times even when acidic or basic samples are injected.

Non-suppressed anionic IC methods are characterised by a wide range of eluent compositions that can be used. These include aromatic carboxylic acids and their salts (benzoic acid, phthalic acid), aliphatic carboxylic acids and their salts (citric acid, tartaric acid), sulphonic acids and potassium hydroxide (Haddad and Jackson, 1990b). Only para-hydroxy benzoic acid and phthalic acid were used in this study.

Detection

Conductivity, as a method for the detection of ions in the eluent from a chromatographic column, was reported by James *et al*. (1951). This mode of detection has two major advantages for ionic solute analysis:

- It is a universal detector since all ions are electrically conducting.

- Conductivity detectors are relatively simple to construct, maintain and operate.

The conductance of an eluent solution flowing through a conductivity cell arises from the presence of both anions and cations. As an anionic solute passes through the cell, the change in conductance that is observed will be due to the difference between the eluent and solute anions. In the case of anions, the difference is generally positive and is known as *direct* conductivity detection. Separations described in this paper using phthalate and benzoate salts as eluents use direct conductivity detection. Sensitive detection can only occur provided there is sufficient difference between the conductance's of the solute and eluent ions. The conductance change occurring on elution of a 10 ppm chloride solution has been calculated to be between 0,01 and 0,69 μS depending on the eluent used (Haddad and Jackson, 1990c). These small changes place stringent requirements on temperature control and associated cell electronics. In the 761 Compact IC, the conductivity detector is housed in an insulated, thermostated compartment. Temperature control is to within 0,01° C and full scale sensitivity can be adjusted between three ranges to cover possible eluent conductivites.

The direct conductivity detection of anions using high background ionic conductance eluents such as carbonate/bicarbonate are found to be insensitive (background conductivity > 650 μS). A novel system to suppress the background conductance and thereby increase sensitivity of the system was first described by Small *et al.* (1975). Exchange of the eluent cations (sodium) for hydrogen ions will result in the carbonate and bicarbonate being converted to the weakly conducting carbonic acid (H_2CO_3). The resulting background conductance will be very low (typically 15 - 20 μS) and is said to be *suppressed*. In the 761 Compact IC this is achieved by passing the eluent through a small cation exchange column in the hydrogen form before passing through the detector. The reactions that take place in the suppressor column are shown in equations 1 and 2 using the chloride ion as solute of interest.

$$Resin\text{-}H^+ + Na^+HCO_3^- \rightleftharpoons Resin\text{-}Na^+ + H_2CO_3 \qquad (1)$$

$$Resin\text{-}H^+ + Na^+ + Cl^- \rightleftharpoons Resin\text{-}Na^+ + H^+ + Cl^- \qquad (2)$$

Table 3. Linearity regression data for the non-suppressed analysis of selected anions in the range 0,5 - 100 ppm (20 μl injection volume; n=7).

Ion	Slope	R^2	Standard Error of Estimation
Chloride	0,49 ± 0,002	0,999	0,16
Nitrate	0,24 ± 0,005	0,999	0,40
Phosphate	0,21 ± 0,006	0,997	0,49
Sulphate	0,50 ± 0,010	0,998	0,41

Table 4. Repeatability for chloride and sulphate in molasses by non-suppressed ion analysis (n=10).

	Anion (ppm)	
	Chloride	Sulphate
Mean	14810	13341
Standard error of mean	26	126
95% confidence limits	58	285
Standard deviation	82	182
RSD (%)	0,6	1,4

The resultant carbonic acid (H_2CO_3) is weakly conducting whilst the sodium ion ($\lambda_+ = 50$ S.cm^2.equiv^{-1}) is replaced with a greatly conducting hydrogen ion ($\lambda_+ = 350$ S.cm^2.equiv^{-1}). The net result of these processes is that the eluent conductance is reduced whilst the conductance of the sample is increased enhancing solute detectability. Eluent cations accumulate on the suppressor column requiring periodic regeneration. This is achieved by passing 20 mM sulphuric acid through the suppressor column followed by a water rinse to remove excess acid. The 761 Compact IC is equipped with three suppressor columns. Each suppressor undergoes a sequence of eluent suppression, regeneration and rinsing. To ensure reproducible results a freshly regenerated suppressor is switched into the chromatographic flow stream at the beginning of each analysis. Simultaneously the other two suppressors are being regenerated and rinsed in turn. Switching between each function is automatically controlled from the IC software. Regenerant and wash solutions are pumped through the suppressor columns using a two channel peristaltic pump. Other patented suppressor devices using membranes are commercially available.

Carbonate and bicarbonate eluents used either alone or mixed are the most common for suppressed systems. The pH of this system cannot be reduced below approximately 9 making the use of pH as an eluent variable difficult. Consequently different column packings are promoted to achieve efficient separations of different matrices. This can be an economic drawback with a dedicated suppressed system. Non-suppressed systems do not have this drawback making them more versatile in certain analysis areas.

Linearity, repeatability, recovery and minimum detection limit

Non-suppressed system:

A para-hydroxybenzoic acid eluent (pH 8,5; background conductivity of approximately 300 μS/cm) was used to evaluate these method parameters.

- The system was linear over a wide range of concentration for chloride, phosphate, nitrate and sulphate (Table 3). These results are considered acceptable for the range covered.

- The precision of the method was checked by repeatedly injecting a suitably diluted molasses sample (Table 4) and found acceptable. Molasses was chosen as it can be considered the most complex of all sugar factory streams.

- Recovery experiments were employed to evaluate the method quantitatively. Known amounts of chloride, phosphate and sulphate were added to final molasses. Recovery of the added ions was calculated from the initial and final analyses (Table 5) and found acceptable for all ions.

- A minimum detection limit was determined by injecting consecutively more dilute solutions of chloride, phosphate and sulphate. For a 20 μl injection it was found to be 0,1 ppm for chloride, 0,8 ppm for phosphate and less than 0,2 ppm for sulphate in solution.

Table 5. Recovery of chloride, phosphate and sulphate added to diluted final molasses and analysed by non-suppressed ion chromatography.

	Chloride			Phosphate			Sulphate	
Added (ppm)	Recovered (ppm)	Recovery (%)	Added (ppm)	Recovered (ppm)	Recovery (%)	Added (ppm)	Recovered (ppm))	Recovery (%)
2,1	2,2	105	2,0	4,2	210	0,9	0,9	100
3,2	3,4	106	3,0	4,3	143	1,4	1,3	93
4,2	4,4	105	4,0	4,4	110	1,8	1,8	100
5,3	5,4	102	5,0	5,4	108	2,3	2,3	100
6,4	6,4	100	6,0	6,3	105	2,7	2,9	107
7,4	7,6	103	7,1	7,6	107	3,2	3,2	100
8,5	8,5	100	8,1	9,2	114	3,7	3,5	95
9,5	9,6	101	9,1	9,9	109	4,1	4,0	98
10,6	10,6	100	10,1	10,1	100	4,6	4,6	100
Average recovery		102			112			99
Standard deviation		2,3			12,3			3,7
RSD		2,2			10,9			3,7

Suppressed system:

A carbonate / bicarbonate eluent was used with suppression (background conductivity of approximately 17 µS/cm) to evaluate linearity, repeatability and recovery.

- The system was found to be non-linear when used across a wide range of concentrations. This non-linearity of the calibration plot in suppressed IC work is a well documented effect (Doury-Berthod *et al.,* 1985). As the sample concentration decreases, the slope approaches a limiting value (h_{lim}), whilst at higher concentrations of solute, the slope approaches a different limiting value (h'_{lim}). This is shown in Figure 1 for the chloride ion over the concentration range of 0,01 ppm to 20 ppm. Two regression lines are shown calculated from 0,01 to 5 ppm and 5 to 20 ppm chloride. It has been ascribed to the effect of hydrogen ions in the sample band affecting ionisation and resulting in non-gaussian peak shapes, especially at low concentrations. Care must therefore be taken when analysing samples at concentrations not brac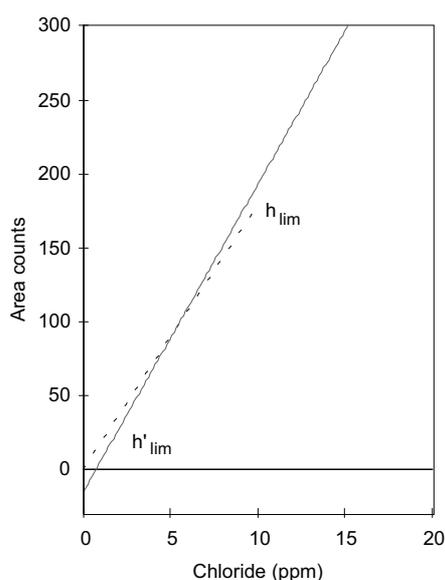keted by a range of standards. The calibration range was limited to 0,5 to 5 ppm for phosphate and 5 to 20 ppm for chloride and sulphate to overcome this effect (Table 6). If the entire range was to be used a polynomial regression would be required to fit the data.

- The precision of the method was checked by repeatedly injecting a suitably diluted molasses sample (Table 7) and found to be acceptable.

- Recovery experiments were employed to quantitatively evaluate the method. Known amounts of chloride, phosphate and sulphate were added to a sucrose solution and the recovery of the added ions was calculated (Table 8). The average recovery was found acceptable provided recoveries were calculated in the calibration range. The non-linear effect of the calibration can be seen in the recovery at low concentrations (110%).

- A minimum detection limit was determined for the method for chloride, phosphate and sulphate. For a 20 µl injection, it was found to be less than 0,05 ppm for chloride and phosphate and less than 0,08 ppm for sulphate in solution. It can be seen that the suppressed system has lower detection limits than the non-suppressed system, especially for phosphate - an important anion in factory streams. However, the system does not afford the same flexibility of eluent pH adjustment to monitor ions of particular interest.

FIGURE 1. Example of a non-linear calibration plot in suppressed IC for chloride. h_{lim} shows the linear portion of the curve from 0,01 to 5 ppm whilst h'_{lim} shows the linear portion for 5 to 20 ppm chloride ion.

Table 6. Linearity regression data for the suppressed analysis of selected anions (20 µl injection volume; n=7).

Ion	Range (ppm)	Slope	R^2	Standard Error of Estimation
Chloride	5 - 20	21,4 ± 0,347	0,999	4,13
Phosphate	0,5 - 5	4,32 ± 0,038	0,999	0,16
Sulphate	5 - 20	5,87 ± 0,032	0,999	0,42

Table 7. Repeatability for chloride, phosphate and sulphate in molasses by suppressed ion analysis (n=10).

	Anion (ppm)		
	Chloride	Phosphate	Sulphate
Mean	13838	1008	13984
Standard error of mean	24	47	18
95% confidence limits	54	90	47
Standard deviation	76	47	58
RSD (%)	0,5	4,7	0,5

Table 8. Recovery of chloride, phosphate and sulphate added to diluted 1st boiling sugar and analysed by suppressed ion chromatography.

Added ion (ppm)	Chloride		Phosphate		Sulphate	
	Found (ppm)	Recovery (%)	Found (ppm)	Recovery (%)	Found (ppm)	Recovery (%)
0,5	0,7	130	0,5	100	0,7	144
1,0	1,4	137	1,0	99	1,2	117
2,0	2,2	112	2,0	99	2,2	109
5,0	4,8	98	5,0	100	5,1	102
8,0	7,9	99	8,0	100	8,1	101
10,0	10,2	102	10,2	102	10,1	101
12,0	11,9	99	12,1	101	12,0	100
15,0	15,3	102	15,5	103	15,1	101
20,0	20,6	102	21,0	105	20,35	101
Average recovery [1]		100,6		99,6		100,9
Standard deviation		1,9		0,7		0,6
RSD (%)		1,8		0,7		0,6

Effect of eluent pH

Polyvalent anions such as phosphate, aconitic and oxalic acid dissociate in multiple steps. The fraction of the ionic species for any particular polyvalent anion present in solution as a function of pH can be calculated (see Appendix 2) and shown graphically (Figure 2). At pH 4.3, all of the phosphate present in a juice or molasses sample has a negative one charge. It will therefore elute early in the chromatogram and cannot be easily analysed (Figure 3a). At pH 8,5 or greater, all the phosphate present has a charge of negative two and will be well retained on the ion exchange column, eluting after the singly charged ions (see Figure 3b). By contrast, approximately 65% of the aconitic acid in solution has a negative two charge at pH 4,3 and all of the acid present a negative three charge at pH 8,5. Thus it is well retained at pH 4,3 (Figure 3a) whilst taking an inordinate length of time to elute from the column at pH 8. As the peak will be very broad, it is not seen eluting from the column. A similar reasoning applies to oxalic acid. It can be seen after the sulphate at a pH greater than 6 as the divalent ion (Figure 3b) but not at pH 4 as the monovalent ion (Figure 3a). Eluent pH therefore has a profound effect on retention of poly-

(a) (a - H_3PO_4; b- $H_2PO_4^-$; c- HPO_4^{2-}; d - PO_4^{3-})

(b) (a - H_3Ac; b - H_2Ac^-; c - HAc^{2-}; d - Ac^{3-})

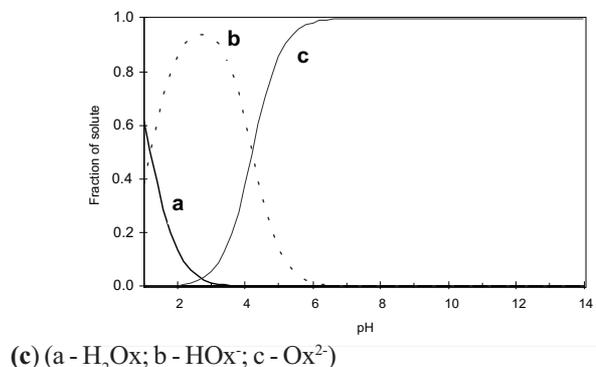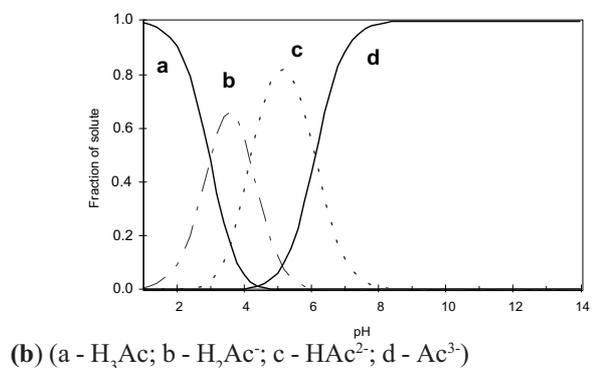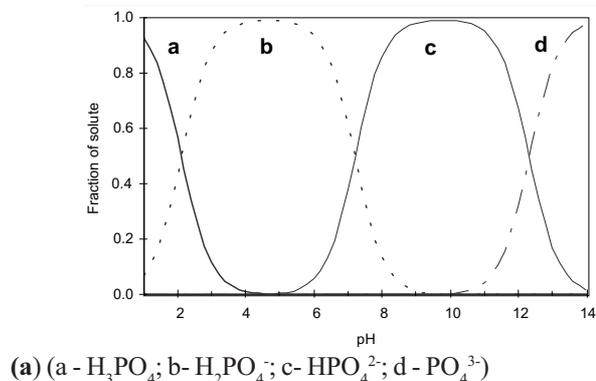**(c)** (a - H_2Ox; b - HOx^-; c - Ox^{2-})

FIGURE 2. Calculated fraction of the ionic species present in solution as a function of pH for (a) phosphate, (b) aconitic acid and (c) oxalic acid. (Ionic species present indicated under the graph).
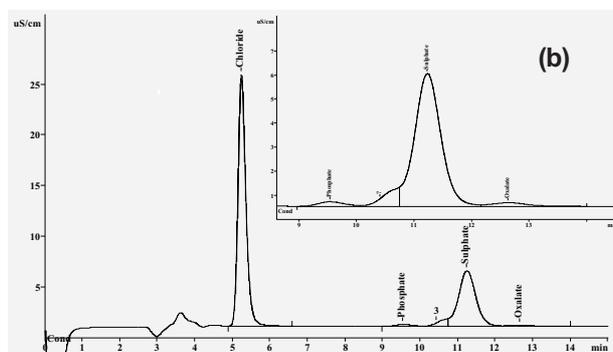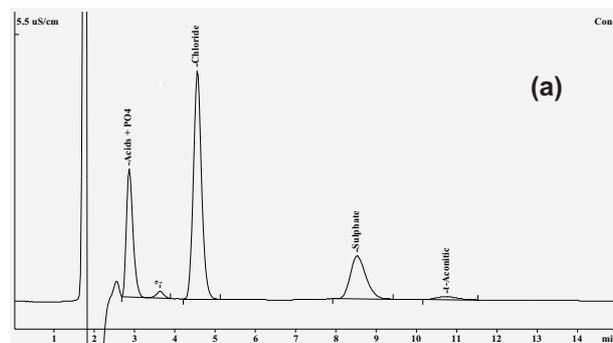

FIGURE 3. Chromatogram of molasses (a) nonsuppressed at pH 4,3; (b) suppressed. Insert shows minor components phosphate, oxalate and an unknown.

valent ions and can be used to increase the separation and selectivity of the technique. Generally phosphate and oxalate will be separated from chloride and sulphate using a high pH eluent, whilst a lower pH eluent is used to analyse for aconitic acid in the presence of chloride and sulphate.

Comparison of IC technique with other chemical techniques

Ion chromatography is accepted worldwide as an officially approved technique for specific ion analysis (see Table 1). Comparison with traditional techniques was considered in all these studies and found acceptable. Therefore only a small comparative study was made for chloride and phosphate. Comparison was made on a series of raw sugar samples for chloride using suppressed chromatography and a potentiometric argentimetric titration. Suppressed chromatography was chosen as it was more sensitive than non-suppressed. Phosphate was measured by Atomic Emission Inductively Coupled Plasma (AE ICP) and suppressed chromatography on a series of juices from a varietal trial. The results obtained are shown in Table 9 and 10. There is a very good correlation with statistical analysis of the data showing no significant difference between the techniques.

Table 9. **Comparison of chloride concentrations in raw sugar by potentiometric titration and suppressed ion chromatography (IC).**

Sample	Chloride (ppm)	
	Potentiometric	IC
A	263	265
B	132	131
C	103	102
D	130	131
E	83	87
F	249	250
G	148	155
H	102	103
I	124	117
J	226	225
K	119	123
L	264	261
Average	162	163
Minimum	83	87
Maximum	264	265
Standard deviation	68	68

Table 10. **Comparison of phosphate concentrations in mixed juice by inductively coupled plasma atomic emission spectroscopy (ICP AE) and suppressed ion chromatography (IC).**

Sample	Phosphate as PO_4 (ppm)	
	ICP AE	IC
A	77	80
B	46	50
C	79	85
D	85	89
E	57	58
F	49	54
G	44	51
H	90	79
I	76	69
J	49	50
Average	65	67
Minimum	44	50
Maximum	90	89
Standard deviation	18	16

Factory samples:

The IC method has successfully been used for the analysis of anions in mixed juice (MJ), clear juice (CJ), syrup, A-sugar, C-molasses. An example of use of the method was in part of a study of the clarification process, a project of the SMRI 2000 Research Programme. The efficiency of phosphate removal and effect of the inclusion of tops and trash on the ash values was monitored. A random selection of the on-sample values obtained are shown in Table 11. Samples 1 to 4 are clean, fresh cane. It can be seen that the introduction of tops and trash introduces more soluble ash into the mixed juice (samples 5 to 9). These include chloride and sulphate (approximately twice as much) as well as aconitic (approximately three times more) and oxalic acids. Increased levels of both aconitic and oxalic acids have a practical impact on the clarification process. More lime is required to neutralise this acid leading to increased ash in the clear juice and subsequent lower sucrose recovery. Increased levels of aconitic acid with the inclusion of tops and trash is a consequence of the inclusion of growing regions of the cane. Aconitic acid is directly associated with cane growth and metabolism (Celestine-Myrtil-Marlin, 1990). Phosphate removal can be seen to occur across the clarification stage. These techniques show why the rate of lime usage will increase with increasing proportions of tops and trash.

A second example of the use of this technique of analysis has been the monitoring of the effect of heating and subsequent decomposition of final molasses. Samples of molasses stored at elevated temperatures for extended periods of time were analysed by both non-suppressed (chloride, sulphate and aconitic acid) and suppressed (phosphate) techniques. The results are shown in Table 12. The chloride and sulphate remain constant whilst the aconitic acid decomposes producing CO_2. Decarboxylation is seen to occur at the higher temperatures as it is a thermal decomposition. The gassing produced under these conditions has been found to be a decarboxylation and not the "Maillard" type reaction as is often surmised (Morel du Boil, *et al.,* 2000). A good correlation between aconitic acid decomposition and CO_2 gas production was found (Figure 4). Decarboxylation of aconitic acid is not the only source of CO_2 gas production. Other organic acids present in the molasses can undergo a similar reaction which may account for the difference in the measured and predicted gas evolution. At 85°C oxalic acid appears to also be produced. This agrees with the findings that aconitic acid can ultimately form oxalic acid when decomposed (Walford and Walthew, 1996).

Conclusions

Ion chromatography has been shown to be a simple, reliable technique for the simultaneous analysis of both inorganic and organic ions found in sugar streams. The results obtained for phosphate and chloride are equivalent to the traditional wet-chemical techniques. Suppressed techniques are more sensitive than non-suppressed but do not have the wide linear range or eluent versatility of the latter. Both suppressed and non-suppressed ion chromatographic techniques have value in the analysis of ions found in sugar streams.

Table 11. Levels of anions in MJ / CJ clarification trials.

Sample		Anion (ppm on sample)				
Test	Juice	Chloride	Phosphate	Sulphate	Aconitic	Oxalate
1	MJ	523	152	415	273	14
	CJ	566	40	452	269	18
2	MJ	507	195	424	154	17
	CJ	528	45	440	272	19
3	MJ	529	173	421	323	16
	CJ	560	55	448	324	14
4	MJ	507	187	428	281	15
	CJ	536	62	451	289	16
5	MJ	1011	270	626	No	49
	CJ	1061	108	664	sample	41
6	MJ	1009	351	644	913	50
	CJ	1074	94	681	924	53
7	MJ	1114	334	847	1010	31
	CJ	1176	77	898	973	45
8	MJ	1244	510	890	1148	46
	CJ	1301	63	915	1167	37
9	MJ	1017	255	677	901	36
	CJ	1071	48	704	880	25

Table 12. Levels of anions during heating trials on final molasses.

Temperature (°C)	Heating time (hr)	Anion (%)				
		Chloride	Phosphate	Sulphate	Aconitic	Oxalic (ppm)
Starting molasses	0	2,03	0,3	2,22	2,36	781
65	24	2,04	0,3	2,26	2,17	738
	48	2,03	0,3	2,30	2,21	514
	75	2,03	0,3	2,34	2,27	478
75	24	2,02	0,3	2,38	2,20	759
	50	2,02	0,3	2,35	1,99	774
	95	2,04	0,3	2,33	1,48	753
85	24	2,03	0,3	2,37	1,61	484
	48	2,04	0,3	2,21	1,08	1803
	96	2,05	0,3	2,22	0,58	1006

REFERENCES

Celestine-Myrtil-Marlin, AD (1990). Influence of cane age on sugars and organic acid distribution in sugarcane stalks. *Sugar Y Azucar*. 85: August pp 17-24.

De Bruijn, JM and Heringa, R (1992). Determination of anions and cations in sugar factory samples by ion chromatography. *Proc. Sugar Processing Research Conf*. pp 69-83.

Doury-Berthod, M, Giampaoli, P, Pitsch, H, Sella, C and Poitrenaud, C (1985). Theoretical approach of dual-column ion chromatography. *Anal. Chem.* 57 : 2257-2263.

Haddad, PR and Jackson, PE (1990a). Applications of ion chromatography. *Ion Chromatography. Principles and Applications.* Elsevier, Amsterdam. Chapters 16 - 22 : 487-733

Haddad, PR and Jackson, PE (1990b). Eluents for ion exchange separation. *Ion Chromatography. Principles and Applications.* Elsevier, Amsterdam. p 85.

Haddad, PR and Jackson, PE (1990c). Conductivity detection. *Ion Chromatography. Principles and Applications.* Elsevier, Amsterdam. p 255.

James, AT, Martin, AJP and Randall, SS (1951). Automatic fraction collectors and conductivity recorders. *Bichem J.* 49 : 293-299.

Morel du Boil, PG, Walford SN and Wienese S (2000). Thermal degradation of molasses. *Sugar Milling Research Institute, February 2000, Monthly Report.* p 3.

Puke, H, Thielecke, K and Hallmann, J. (1996). Quantitative ion-chromatographic anion determination for the assessment of the technical value of sugar beet. *ZuckerIndustrie* 121 : 408-413.

Rearick, DE and Little, M. (1990). Applications of ion chromatography to sugarbeet process problems. *Proc. Sugar Processing Research Conf.* pp 432-443.

Small, H, Stevens, TS and Bauman, WC (1975). Novel ion exchange chromatographic method using conductrimetric detection. *Anal. Chem.* 47 : 1801-1809.

Van der Poel, PW, de Bruijn, JM, de Visser, NHM and Konings, J. (1990). Balance of cations and anions in sugar beet processing. *ZuckerIndustrie* 115 : 943-949.

Walford, SN and Walthew, DC (1996). Preliminary model for oxalate formation in evaporator scale. *Proc S Afr Sug Technol Ass* 70 : 231-235.

Preparation of eluents

4 mM pHBA / 2,5% methanol.

Para-hydroxybenzoic acid (pHBA) (1,1 g) was dissolved in methanol (50 ml) and added to 1900 ml water. The pH was adjusted to 8,5 with 0,05 M sodium hydroxide with stirring. The solution was made up to 2,0 *l* with water and filtered (0,45 μm)

8 mM phthalic acid / 2% acetonitrile

Phthalic acid (2,66 g) was dissolved in acetonitrile (40 ml) and added to 1900 ml water. The pH was adjusted to 4,3 with tris(hydroxymethyl)-aminomethane (TRIS) with stirring. The solution was made up to 2,0 *l* with water and filtered (0,45 μm).

Carbonate / hydrogen carbonate eluent

Anhydrous sodium carbonate (0,53 g) and sodium hydrogen carbonate (0,40 g) were dissolved in water and made up to 2,0 *l*. The solution was filtered (0,45 μm) before use. A CO_2 absorber tube was attached to the 2 *l* eluent bottle. This was filled with indicating soda-lime pellets to minimise CO_2 adsorption by the eluent.

Suppressor eluents

Suppressor regeneration eluent consisted of 20 mmol/l H_2SO_4 whilst suppressor rinsing solution consisted of deionised water. Both suppressor eluents were filtered (0,45 μm) before use.

APPENDIX 2

Composition of polyfunctional acids as a function of pH

The example shown here is for aconitic acid, the most complex of the polyfunctional acids considered. Phosphoric and oxalic acid can be considered by ignoring the relevant equations. Aconitic acid is a tricarboxylic acid with three protons available for dissociation. Given

H_3A represents the un-ionised acid

H_2A^- represents the mono-ionised acid

HA^{2-} represents the di-ionised acid

A^{3-} represents the totally ionised acid

and

$$K_1 = \frac{[H^+][H_2A^-]}{[H_3A]} = 1.02 \times 10^{-3}$$
$$K_2 = \frac{[H^+][HA^{2-}]}{[H_2A^-]} = 6.55 \times 10^{-5}$$
$$K_3 = \frac{[H^+][A^{3-}]}{[HA^{2-}]} = 7.80 \times 10^{-7}$$

Where K_i = dissociation constant.

The fractions of solute for each form are given by :

$$F_{H_3A} = \frac{[H_3A]}{\delta_t}$$
$$F_{H_2A^-} = \frac{[H_2A^-]}{\delta_t}$$
$$F_{HA^{2-}} = \frac{[HA^{2-}]}{\delta_t}$$
$$F_{HA^{3-}} = \frac{[HA^{3-}]}{\delta_t}$$

Where δt, the total molar concentration of substrate, is

$$[H_3A] + [H_2A^-] + [HA^{2-}] + [A^{3-}]$$

Combining these equations leads to expressions for the fractions of solute as a function of the dissociation constants and hydronium ion concentration :

$$F_{H_3A} = \frac{[H^+]^3}{[H^+]^3 + K_1[H^+]^2 + K_2[H^+] + K_1K_2K_3}$$

$$F_{H_2A^-} = \frac{K_1[H^+]^2}{[H^+]^3 + K_1[H^+]^2 + K_2[H^+] + K_1K_2K_3}$$

$$F_{HA^{2-}} = \frac{K_2[H^+]}{[H^+]^3 + K_1[H^+]^2 + K_2[H^+] + K_1K_2K_3}$$

$$F_{A^{3-}} = \frac{K_1K_2K_3}{[H^+]^3 + K_1[H^+]^2 + K_2[H^+] + K_1K_2K_3}$$

Figure 2b shows, F_{H_3A}, $F_{H_2A^-}$, $F_{HA^{2-}}$ and $F_{A^{3-}}$, and plotted as a function of pH.