The Use of Orthogonal Techniques to Enhance Confirmation of Analyte Identification in Inorganic Analysis

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- At Waters there are times that we will perform analyses of customer samples in order to show the feasibility of the analysis. Many times these samples contain additional constituents beyond the intended analytes. This talk will focus on the analysis of anionic species that were present in an aqueous extract of an immiscible organic monomer and the use of orthogonal techniques to gain information on one of these constituents.

Overview of Analytical Techniques Employed

- ► Ion Chromatography with Direct Conductivity Detection (no chemical suppression)
- ► Ion Chromatography with Direct Conductivity Detection after Chemical Suppression
- ► Ion Chromatography with Direct Conductivity and Photodiode Array Detection after Chemical Suppression
- ► Capillary Ion Analysis (CIA) with Indirect UV Detection

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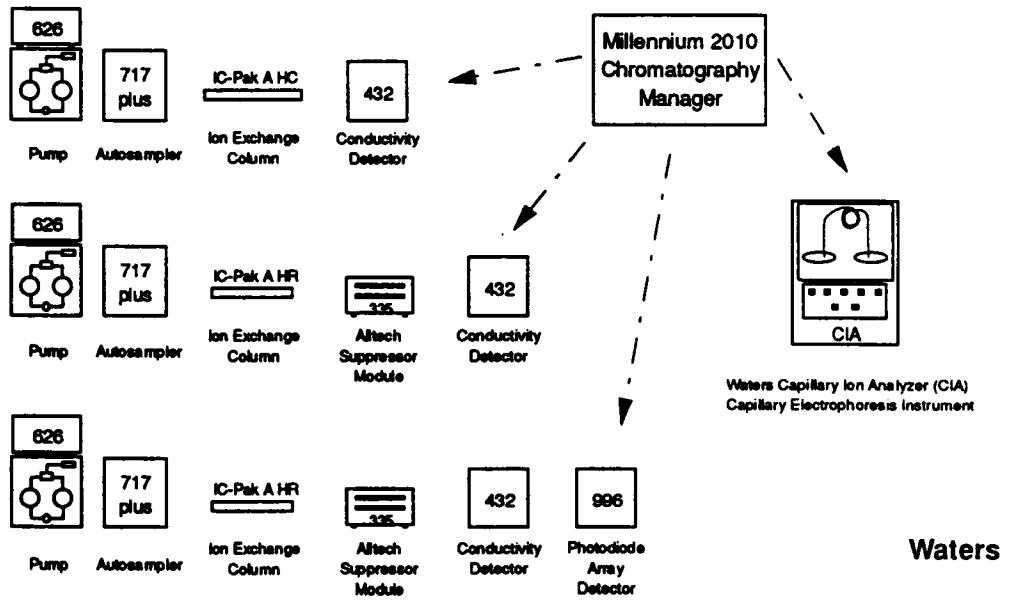
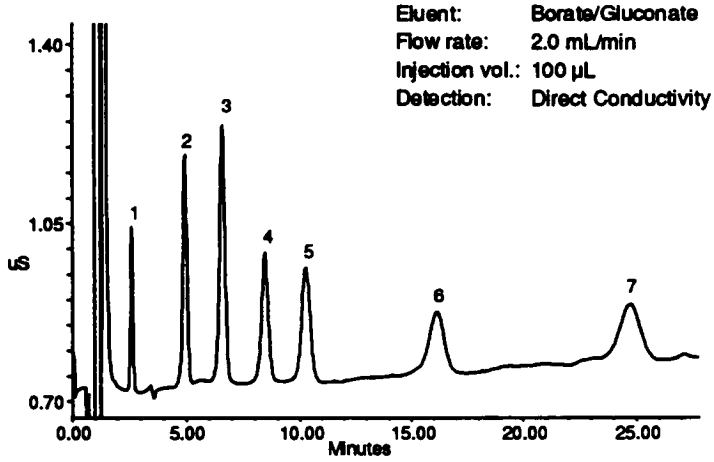
- A number of instrumental components were used to analyze these samples. The samples were first analyzed by Ion Chromatography (IC) with direct conductivity detection. An Alltech packed-bed suppressor was then added to the system, the ion exchange column and eluent changed and the samples re-run using conductivity detection after chemical suppression. The addition of Waters Photodiode Array (PDA) detector gave the capability of performing spectral analysis of UV-absorbing species. This will be discussed later. The orthogonal technique of Capillary Ion Analysis (CIA) provides a separation technology with different selectivity that aids in further confirmation of analyte identity.

Analytical Equipment Used



- This is a diagrammatic representation of the equipment used. All equipment was from Waters with the exception of the Alltech Model 335 Suppressor Module. A personal computer with Waters Millennium 2010 Chromatography Manager software was used for all data collection as well as control of the Waters CIA instrument and the PDA detector.

Seven Anion Standard
Borate Gluconate Eluent



1. Fluoride 1 ppm
2. Chloride 2 ppm
3. Nitrite 4 ppm
4. Bromide 4 ppm
5. Nitrate 4 ppm
6. Phosphate 6 ppm
7. Sulfate 4 ppm

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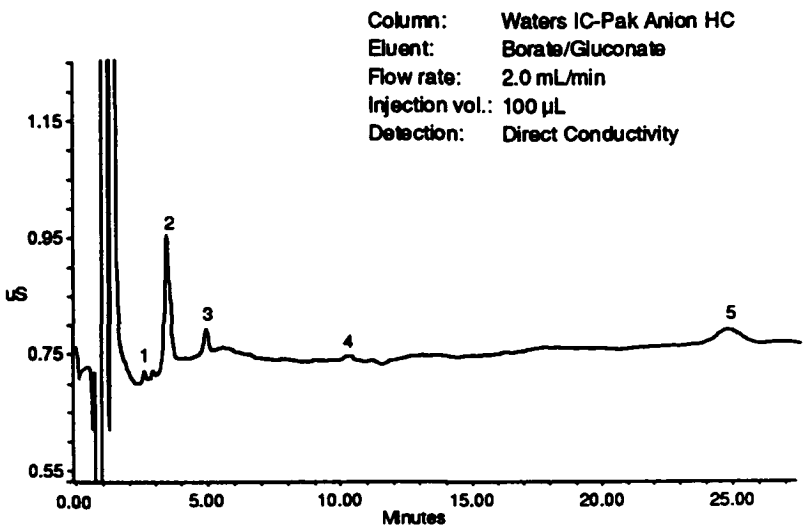
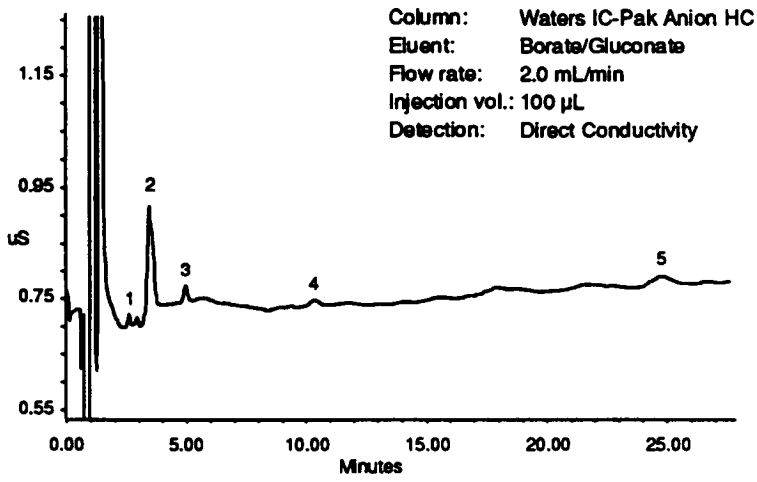
- This is a chromatogram of seven inorganic anions typically analyzed using IC. The levels, as seen in the slide, are in the low ppm (mg/L) range. This is IC without the use of chemical suppression and the eluent used is borate/gluconate.

Conductivity Detection of Aqueous Extract of Monomer



- There were a number of samples analyzed, but I will focus on only two, sample #1 and sample #5. In all cases, the monomer, which was immiscible with water, was extracted with and aliquot of 18 Megohm water by shaking. The lower layer was removed, filtered (0.45 um) and then injected. Low levels of fluoride, chloride, nitrate and sulfate are seen, but this work will focus on peak #2, which under these conditions was originally thought to be carbonate due to the adsorption of atmospheric carbon dioxide.

Direct IC Analysis of Aqueous Extract of Immiscible Monomer



Sample #5

1. Fluoride 67 ppb
2. ----------
3. Chloride 123 ppb
4. Nitrate 143 ppb
5. Sulfate 569 ppb

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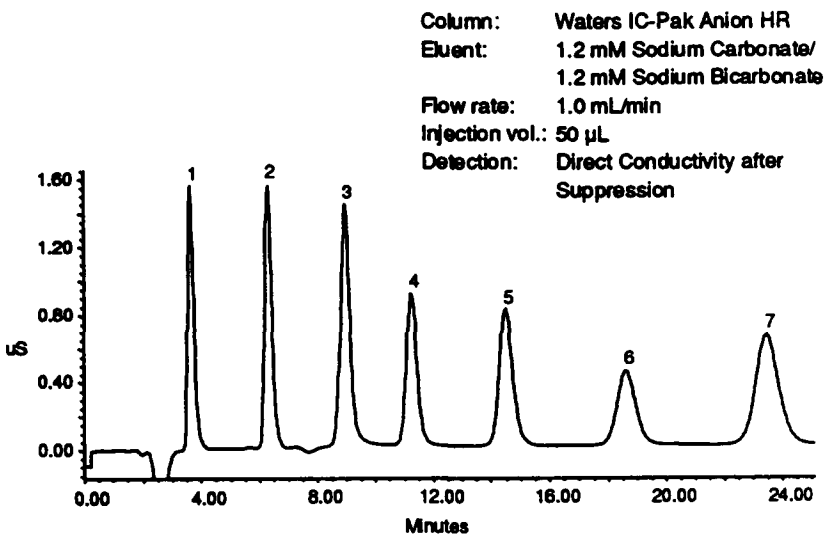
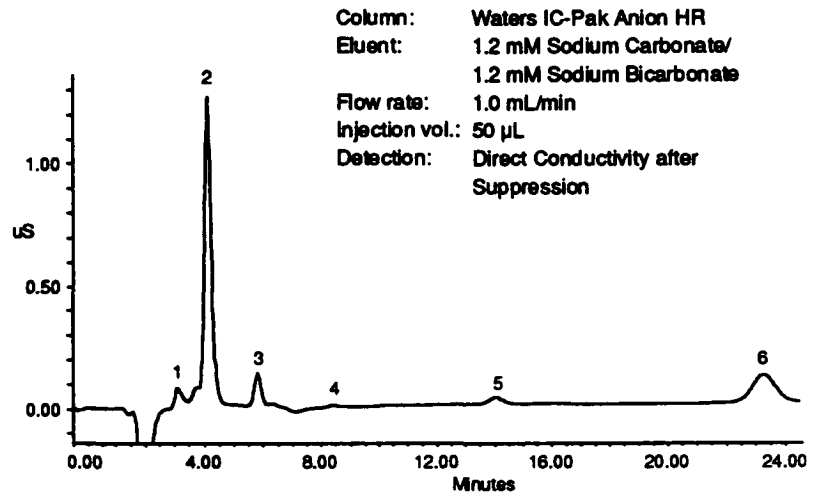
- Sample #5 shows a very similar profile to that of sample #1, with low levels of fluoride, chloride, nitrate and sulfate. Again peak #2 is prevalent. The region between peaks #1 and #3 is an area in which carbonate and short-chain organic acids such as acetate and formate elute. Complex organic acids, such as humic and fulvic acids which are found in some environmental samples, also may appear in this area. The small unlabeled peak between #1 and #2 is suspected to be acetate. Although this was not confirmed by spiking the sample with acetate, acetate was seen in the same sample analyzed using CIA.

Seven Anion Standard Analyzed Using Chemical Suppression



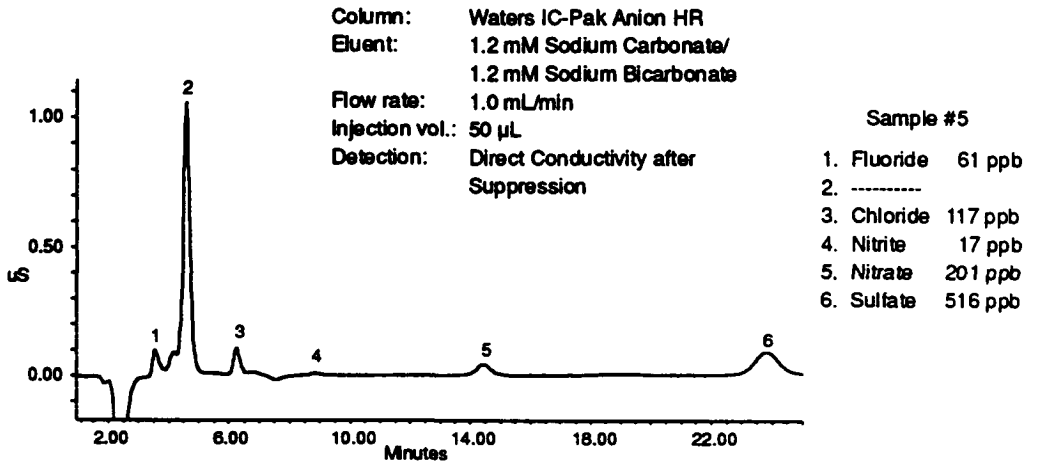
- The instrumental configuration used to produce this chromatogram was the second configuration shown earlier. The analytical column was changed to an IC-Pak A HR (High Resolution) and the eluent changed to 1.2 mM sodium carbonate/1.2 mM sodium bicarbonate. This eluent, upon passage through the packed-bed suppressor becomes converted to carbonic acid, which significantly reduces the background conductivity. Note also that the resolution between the peaks is different with this column/eluent combination than that seen earlier.

Monomer Extract - Conductivity Detection after Chemical Suppression



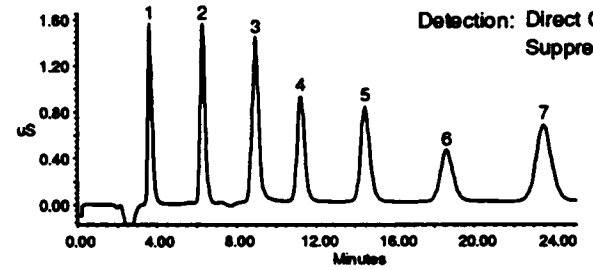
- This is the chromatogram obtained for sample #1 using suppressed IC and conductivity detection. Note that peak #2 is still present. Originally this peak was thought to be carbonate, but it is not because it too would be suppressed if it were, and not be conductive. Peak #2 is still very prevalent which suggests that it is not a weak acid as very weak acids are suppressed themselves under these conditions, to some degree. The profile seen here is similar to that seen using borate/gluconate with the exception that a small amount of nitrite is seen. This is due the combination of the HR column and the use of suppression.

Monomer Extract - Conductivity Detection after Chemical Suppression



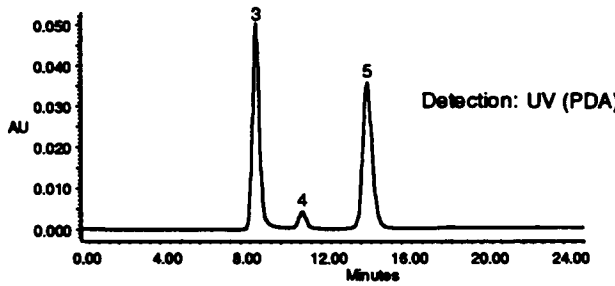
- The chromatogram for sample #5 analyzed by suppressed IC is also similar to that seen earlier. A small amount of nitrite is present, as was seen for sample #1 in the previous slide. One of the disadvantages of using a suppressor of any type is the possibility of the formation of a "system peak". It is characteristic of an eluent/suppressor combination and here appears close to the nitrite peak. This can lead to variability in the quantitation of the nitrite peak. Peak #2 is still very apparent. The non-resolved peak on the leading edge of peak #2 is probably acetate. Recall that this was completely resolved with the High Capacity (HC) column and borate/gluconate eluent.

Conductivity and UV Detection in Series



Detection: Direct Conductivity after Suppression

Column: Waters IC-Pak Anion HR
Eluent: 1.2 mM Sodium Carbonate/ 1.2 mM Sodium Bicarbonate
Flow rate: 1.0 mL/min
Injection vol.: 50 µL



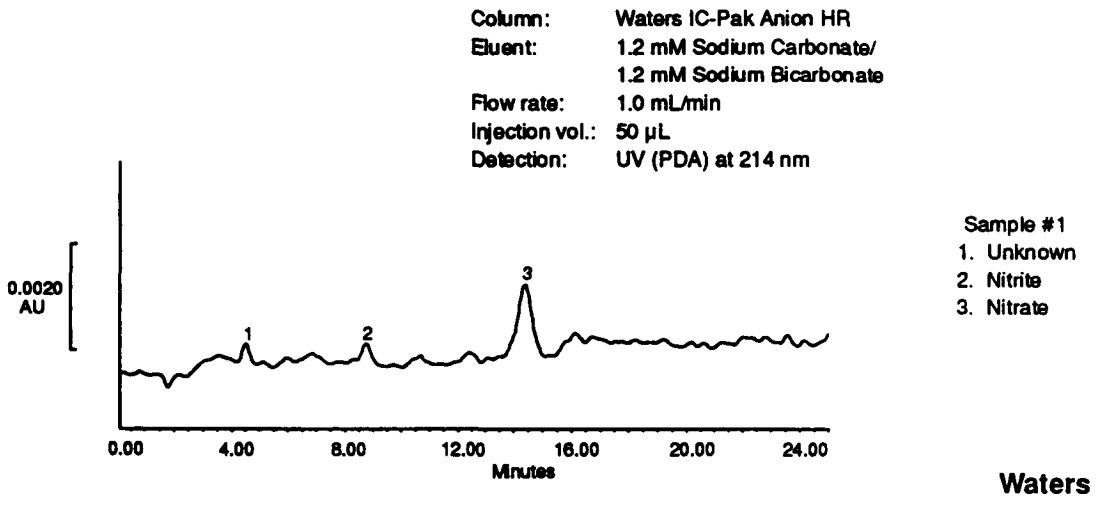
Detection: UV (PDA) at 214 nm

1. Fluoride 1 ppm
2. Chloride 2 ppm
3. Nitrite 4 ppm
4. Bromide 4 ppm
5. Nitrate 4 ppm
6. Phosphate 6 ppm
7. Sulfate 4 ppm

Waters

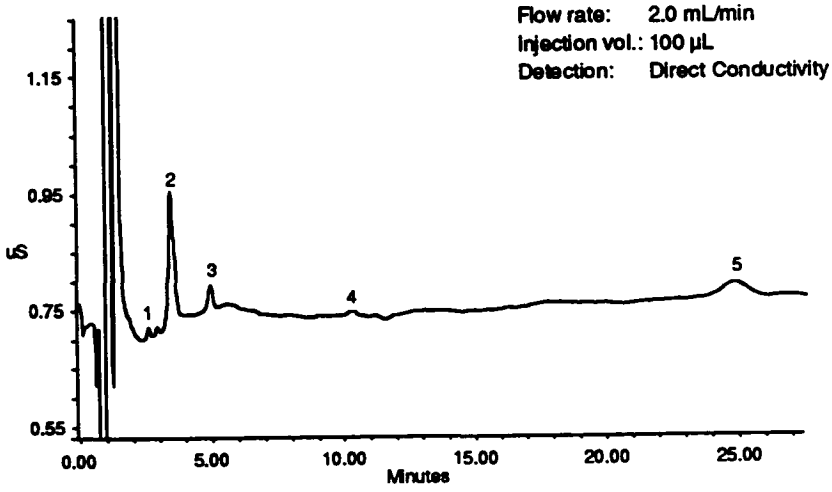
- The most common detector used in IC is conductivity. The use of a UV/Vis detector is not usually considered however, there are a variety of inorganic species which absorb in addition to those seen here. Also, a number of short-chain organic species absorb in the low-UV range. The use of a PDA detector for the analysis of these species gives useful additional information such as the spectral characteristics of a separated species. Spectral matching with user-generated libraries affords an additional degree of confirmation. In this work the range of 200 - 300 nm was collected. What is seen as the lower chromatogram is an extraction of the 214 nm wavelength from the collected range.

Monomer Extract #1 - Photodiode Array 214 nm Extracted Wavelength



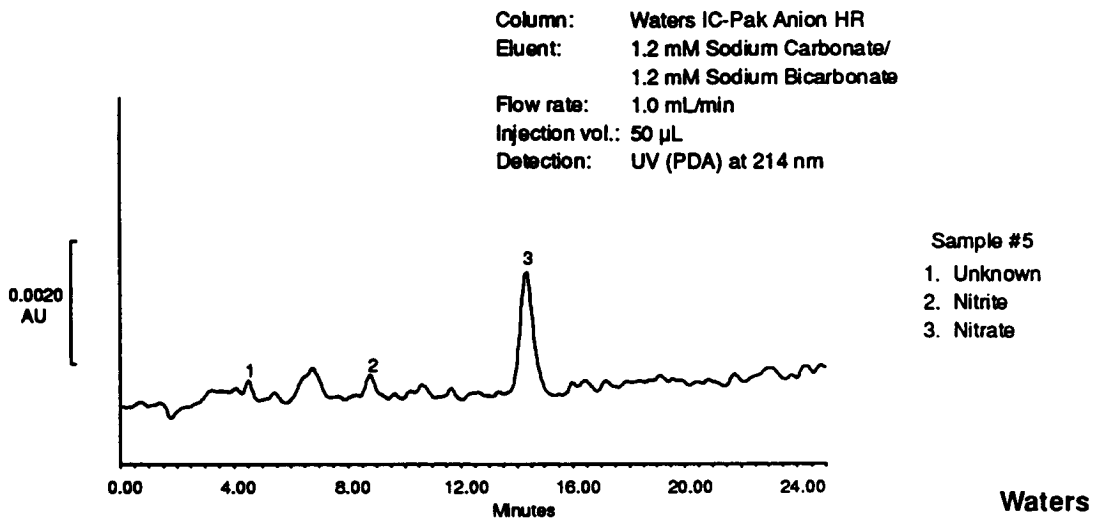
- Here is an extracted wavelength (214 nm) for sample #1. Peaks #2 and #3 correspond to the chromatographic peaks seen with suppressed conductivity. Peak #1 however, doesn't correspond to either acetate or formate which elute in this region as was mentioned previously. The relatively large amount of noise in the baseline is most likely due to organic co-extractants from this or previous injections that are slowly "bleeding" off the polymeric backbone of this column, since there is no organic constituent in the mobile phase. Such an organic would tend to prevent non-specific adsorption.

Monomer Extract #1 - Conductivity



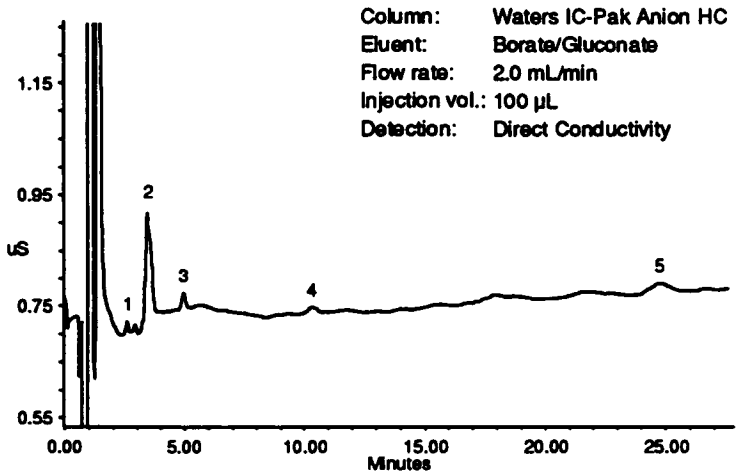
- Here again is the chromatogram using Waters IC-Pak A HC column and conductivity detection. The "unknown" seen as peak #1 in the 214 nm trace, corresponds to peak #2 here. The very small peak between #1 and #2 is acetate. It did not appear in the UV trace due to the very low levels present as well as the relatively high UV background.

Monomer Extract #5 - Photodiode Array 214 nm Extracted Wavelength



- A similar extracted UV chromatogram is seen for sample #5. Peak #1 corresponds to.....

Monomer Extract #5 - Conductivity



Sample #5

1. Fluoride 67 ppb
2. ----------
3. Chloride 123 ppb
4. Nitrate 143 ppb
5. Sulfate 569 ppb

Waters

- .....peak #2 in this chromatogram.

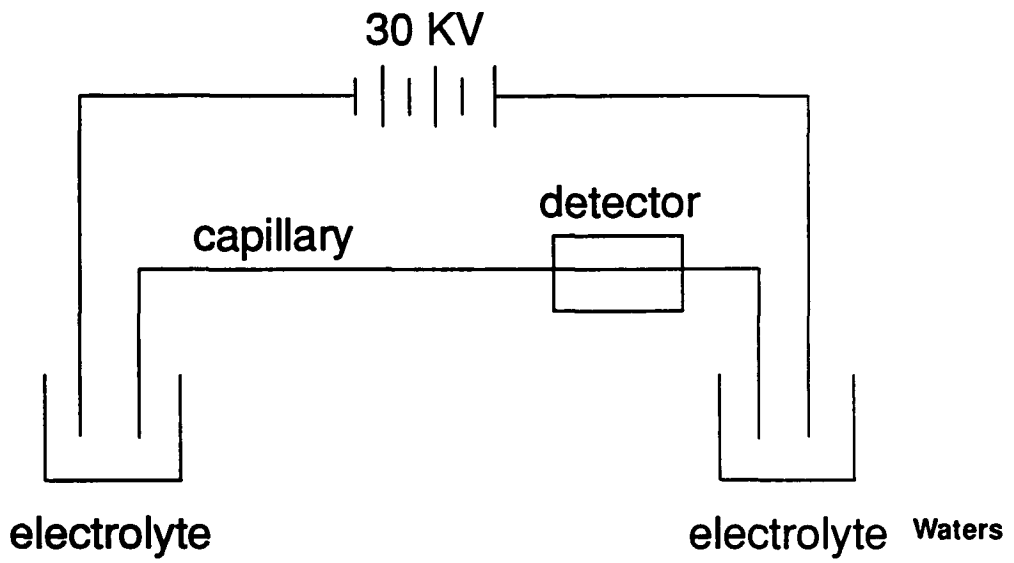
Capillary Ion Analysis

- CIA is a CE technique optimized for the rapid analysis of highly mobile small molecular weight ions
  - Selectivity is based on the mobility of the analyte in the electrolyte
  - Speed of the analysis is optimized by ensuring the EOF direction is always towards the detector
  - Sensitive detection is based predominately on the indirect UV principal

Waters

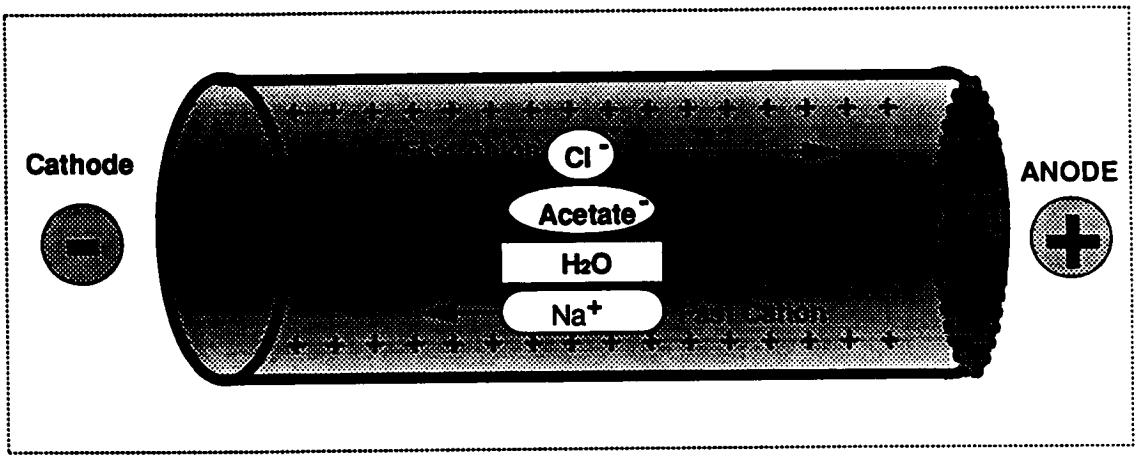
- The samples were also analyzed by the orthogonal technique of Capillary Ion Analysis (CIA).
- Read slide
- Electro-osmotic flow or Endo-osmotic flow = EOF This is the bulk movement of electrolyte that occurs when voltage is applied.

Capillary Electrophoresis System Schematic



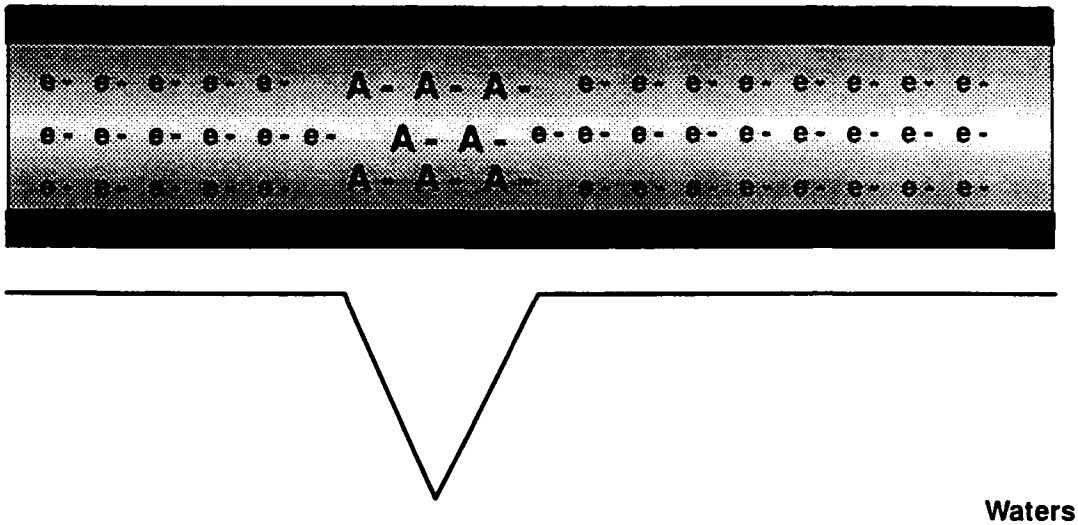
- This is a general schematic of a capillary electrophoresis system. The Waters CIA instrument uses a negative power supply for the analysis of anions which is capable of generating voltages up to 30,000 volts.

Capillary Ion Analysis



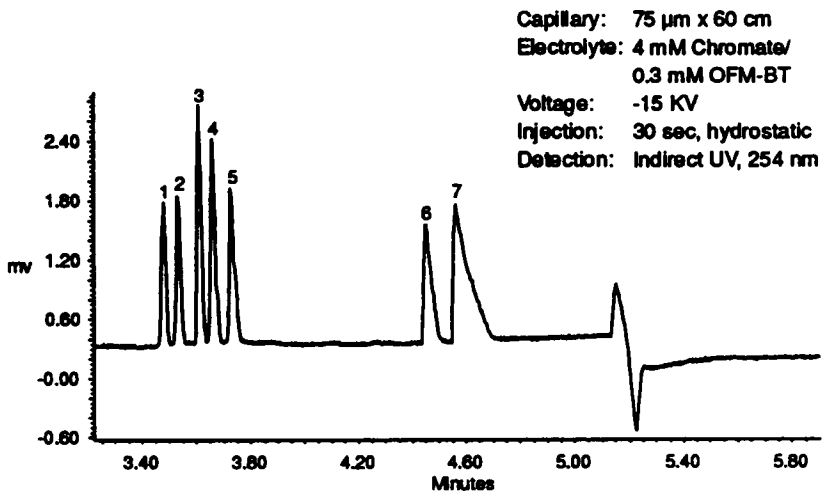
- An Osmotic Flow Modifier (OFM) is used in the electrolyte to ensure anodic osmotic flow. Under these conditions, anions migrate when voltage is applied toward the anode and move ahead of (faster than) the EOF. Neutral compounds migrate at the same rate and direction as the EOF while positively charged compounds move opposite to the EOF direction and toward the cathode.

Capillary Ion Analysis



- A UV-absorbing anionic species, chromate, is in the electrolyte. The principle of indirect (or vacancy) UV detection is used because the majority of inorganic species are not UV-absorbers. An analyte anion displaces the chromate anion which results in a "lack" of UV-absorber which appears as a negative peak. The polarity of the signal is reversed to give a positive peak as an output.

Capillary Ion Analysis (CIA) of 7 Anion Standard



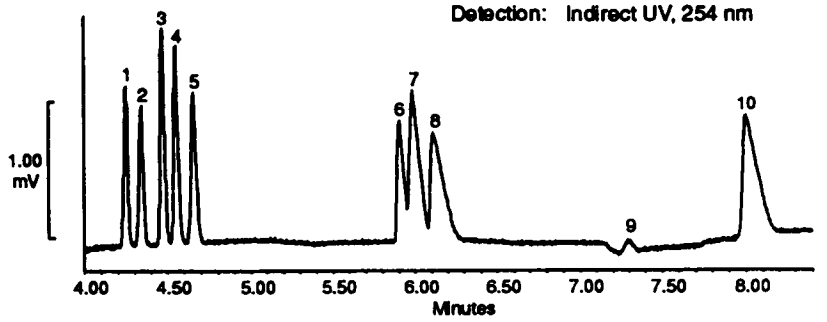
1. Bromide 4 ppm
2. Chloride 2 ppm
3. Sulfate 4 ppm
4. Nitrite 4 ppm
5. Nitrate 4 ppm
6. Fluoride 1 ppm
7. Phosphate 6 ppm

Waters

- The seven anion standard shown earlier by non-suppressed and suppressed IC is shown here using Waters CIA. Note that the selectivity is quite different than that seen by IC. The elution order for IC shown earlier is fluoride, chloride, nitrate, bromide, nitrate, phosphate and sulfate.

7 Anion Standard + Formate and Acetate

Capillary: 75 µm x 60 cm
Electrolyte: 4 mM Chromate/
0.3 mM OFM-BT
Voltage: -15 KV
Injection: 30 sec, hydrostatic
Detection: Indirect UV, 254 nm



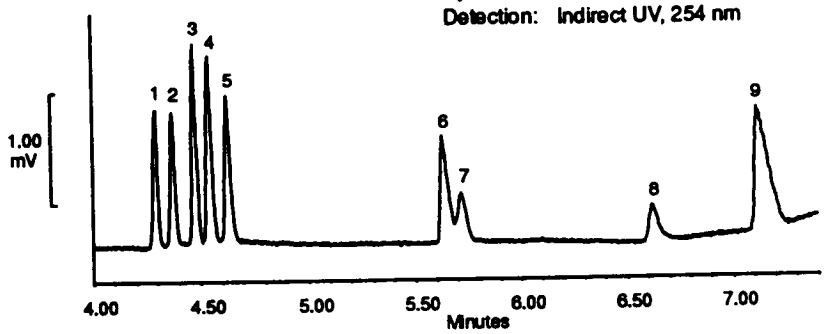
1.	Bromide	5 ppm
2.	Chloride	2 ppm
3.	Sulfate	4 ppm
4.	Nitrite	4 ppm
5.	Nitrate	4 ppm
6.	Fluoride	1 ppm
7.	Formate	4 ppm
8.	Phosphate	4 ppm
9.	Carbonate	--------
10.	Acetate	4 ppm

Waters

- The two common short-chain organic acids, formate and acetate, were combined with the other seven anions to show where they appear in the pherogram. Formate migrates between fluoride and phosphate while acetate migrates later, after carbonate. The carbonate was not added to the standard but was present due to the adsorption of atmospheric carbon dioxide.

Anion Standard (no Phosphate) + Formate and Acetate

Capillary: 75 µm x 60 cm
Electrolyte: 4 mM Chromate/
0.3 mM OFM-BT
Voltage: -15 KV
Injection: 30 sec, hydrostatic
Detection: Indirect UV, 254 nm

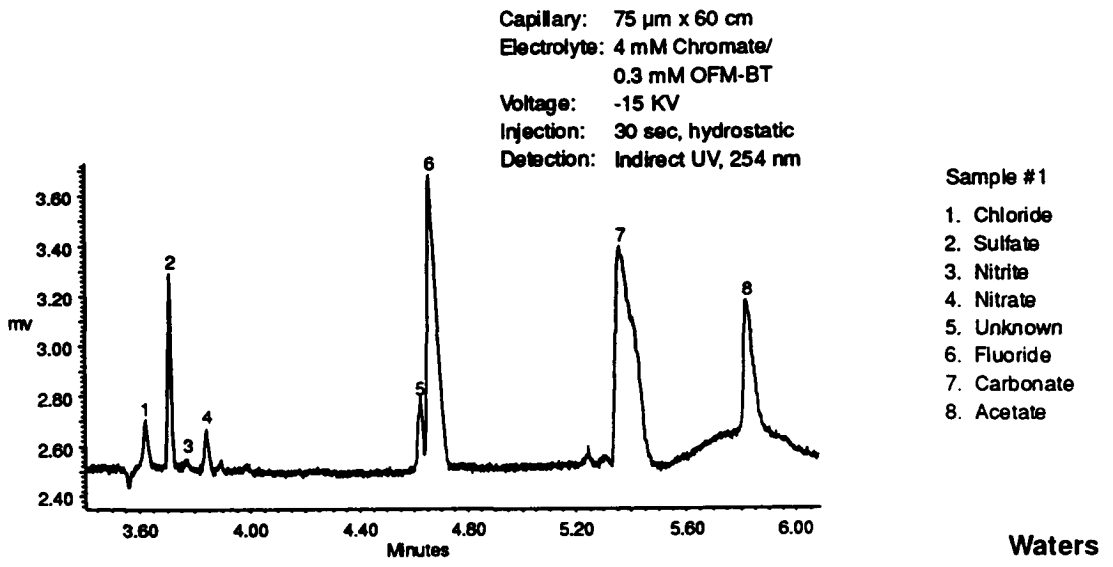


1. Bromide 5 ppm
2. Chloride 2 ppm
3. Sulfate 4 ppm
4. Nitrite 4 ppm
5. Nitrate 4 ppm
6. Fluoride 1 ppm
7. Formate 1 ppm
8. Carbonate --------
9. Acetate 4 ppm

Waters

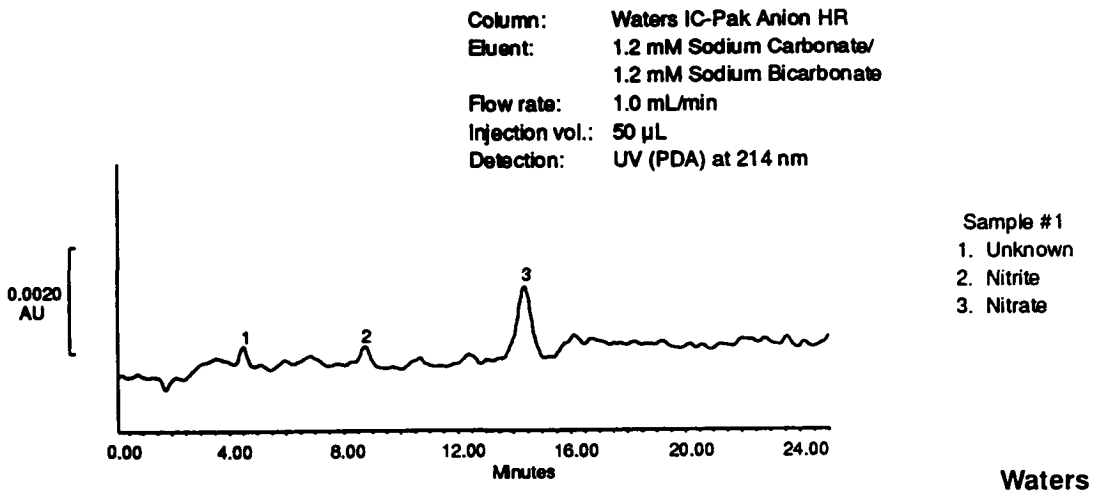
- Since by IC neither sample #1 nor sample #5 contained phosphate, another standard including the organic acids but without phosphate was prepared. The resulting separation is shown here.

CIA of Monomer Extract #1

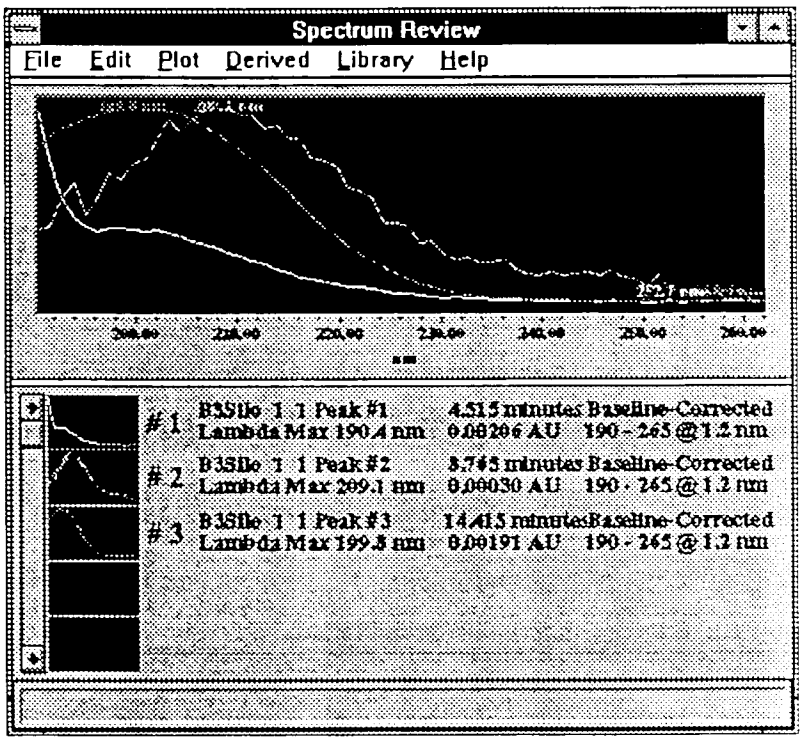


- When sample #1 was analyzed there was good correlation between CIA and IC for the compounds present. Acetate was seen as a small peak in the borate/gluconate IC separation as well as here in the CIA pherogram. A small amount of nitrite is seen here as was seen in the suppressed separation.
- Note however, peak #5 that was seen in both suppressed and non-suppressed IC chromatograms. By CIA it migrates before fluoride, not after as does formate. In this region it is common that multi-valent, relatively larger organic acids such as citrate or tartrate appear.

Monomer Extract #1 - Photodiode Array 214 nm Extracted Wavelength



- This is the extracted wavelength from PDA that was seen earlier. "Unknown" peak #5 seen by CIA in the last slide corresponds to peak #1 here. Peak #2 is nitrite and #3 is nitrate. We will use the power of the PDA software to help confirm the identities of each of these peaks.

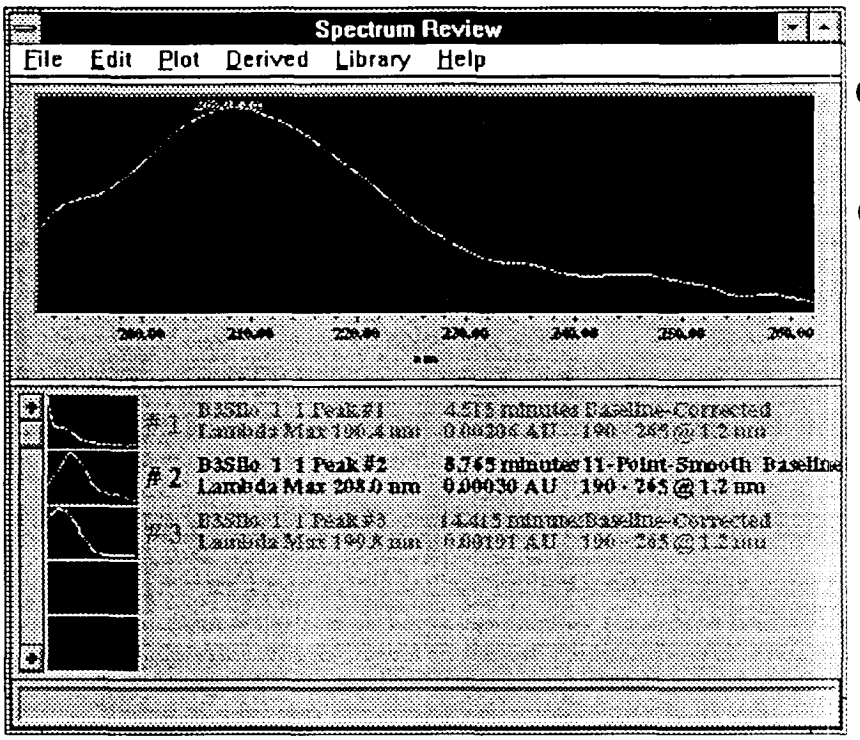


Spectrum Review of Monomer Extract

Sample #1

Waters

- Waters Millennium 2010 Chromatography Manager software controls the functions of the 996 PDA detector as well as the collection and analysis of the data from the optics bench. Shown here is a screen from the software which displays the entire spectrum of each of the chromatographic peaks seen on the previous slide. Recall from that slide that the nitrite peak (peak #2), was quite small. The corresponding spectrum (green) is quite noisy due to the low level present and also in part to the noisy background.

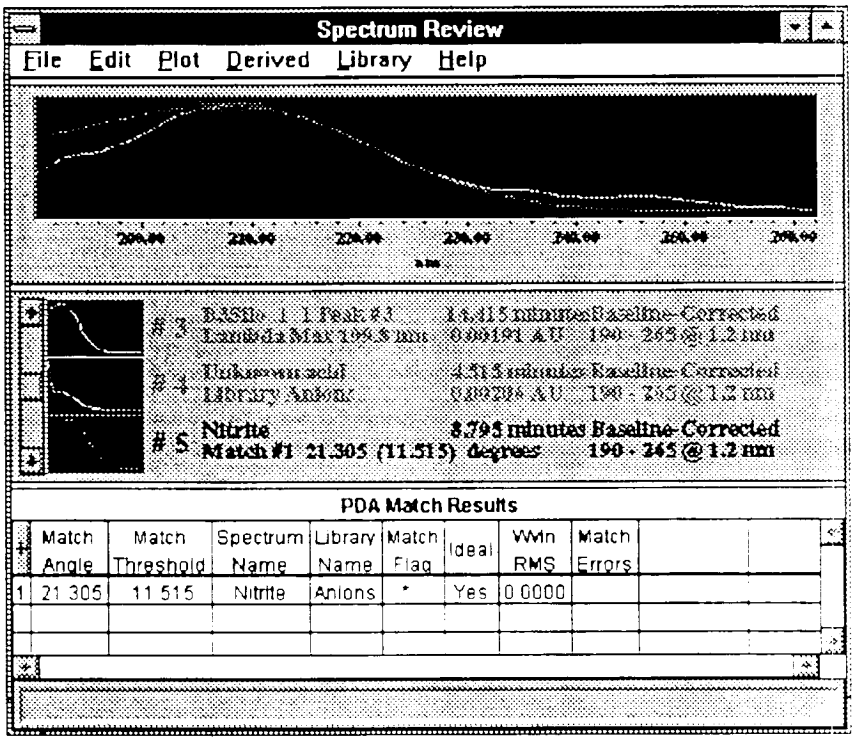


Smoothing of Spectrum (Peak #2) of Monomer Extract

Sample #1

Waters

- From the "Derived" pull-down menu, the spectrum was smoothed to make visual comparisons easier.

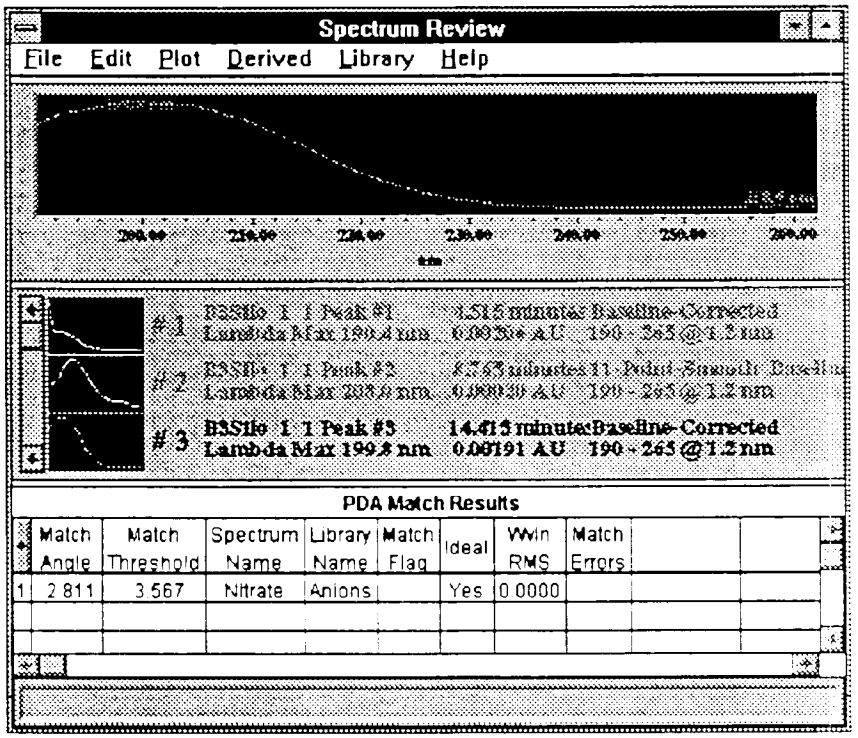


Library Matching Results (Peak #2) of Monomer Extract

Sample #1

Waters

- The software can be used to find the best match between an analyte spectrum and a spectrum that has previously been collected and stored in a library. When this procedure was performed, the best match was with the library spectrum of nitrite which agrees with the conductivity data also. In this case, the match is not a very good one in terms of match angle but is good in terms of identity.

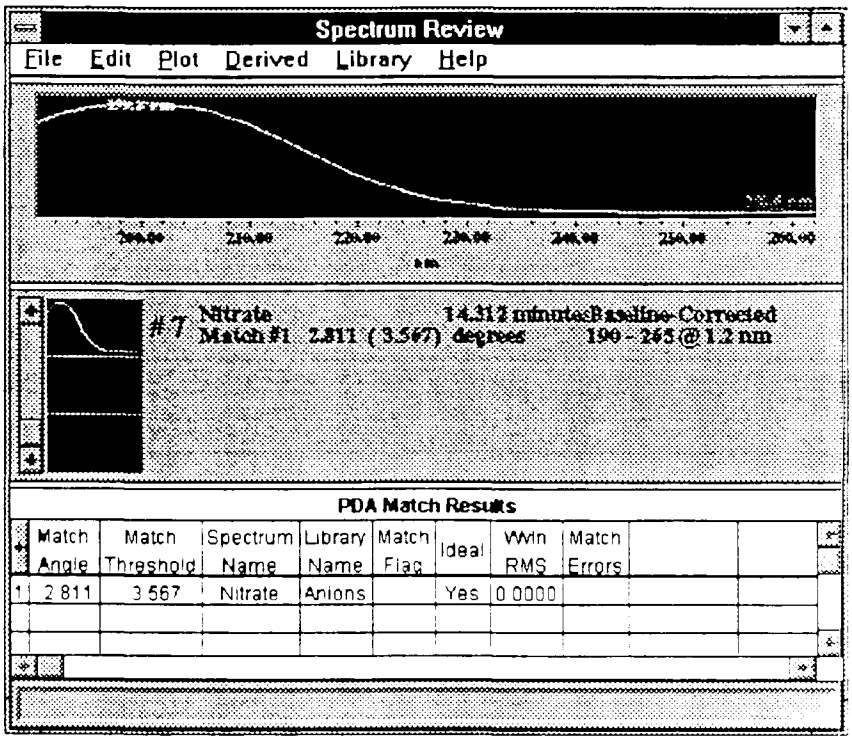


Spectrum Review of Monomer Extract (Peak #3)

Sample #1

Waters

- This is the spectrum review of peak #3.

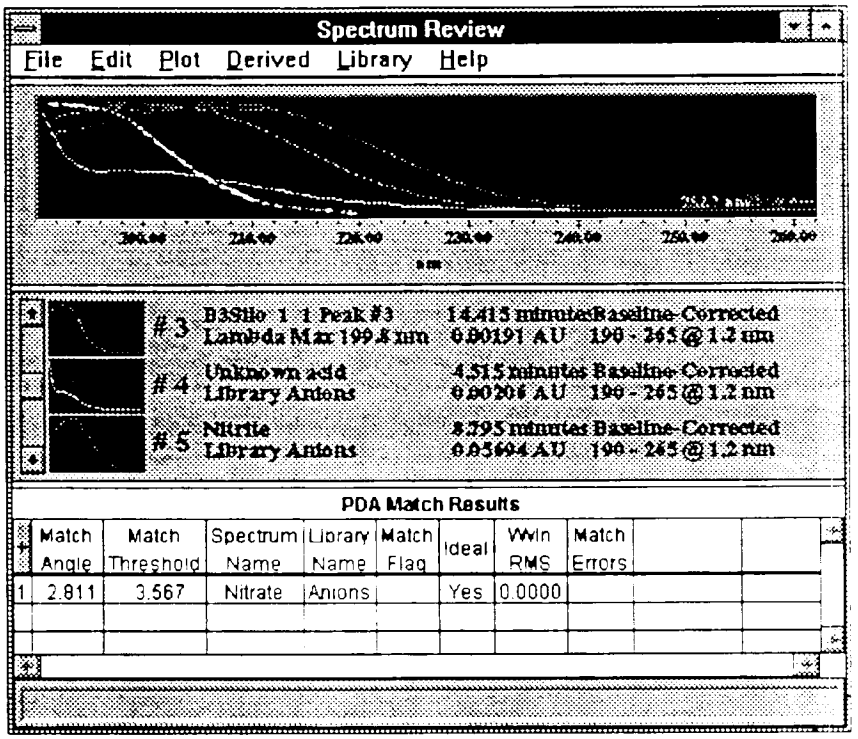


Library Matching Results (Peak #3) of Monomer Extract

Sample #1

Waters

- The library match for peak #3 was found to be nitrate. The spectra of peak #3 and that of nitrate from the library are much more similar than seen in the library match for peak #2. Therefore, there is a better match angle as evidenced by it being lower than the match threshold. It is also apparent visually as there is almost complete overlap of the sample and library spectra.



Spectrum Review of Monomer Extract (Peak #3)

Sample #1

Key

Library Name	Color
Unknown acid	Light blue
Nitrite	Red
Bromide	Dark blue

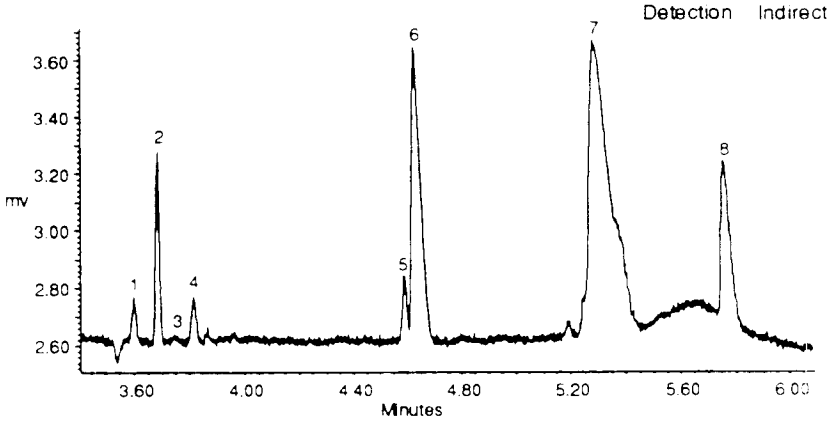
Peak #3 - Nitrate - Purple

Waters

- An overlay of the spectrum of peak #3 and the library spectra of the "unknown acid", nitrite and bromide show the dissimilarities between these and peak #3 which was correctly identified as nitrate.
- (Note)
- There are four spectra shown; (top to bottom they are:)
- nitrite (red),
- peak #3, nitrate, (purple),
- bromide (dark blue) [This is barely visible in a photocopy]
- "unknown acid" light blue

CIA of Monomer Extract #5

Capillary: 75 µm x 60 cm
Electrolyte: 4 mM Chromate
0.3 mM OFM-BT
Voltage: -15 KV
Injection: 30 sec. hydrostatic
Detection: Indirect U.V. 254 nm

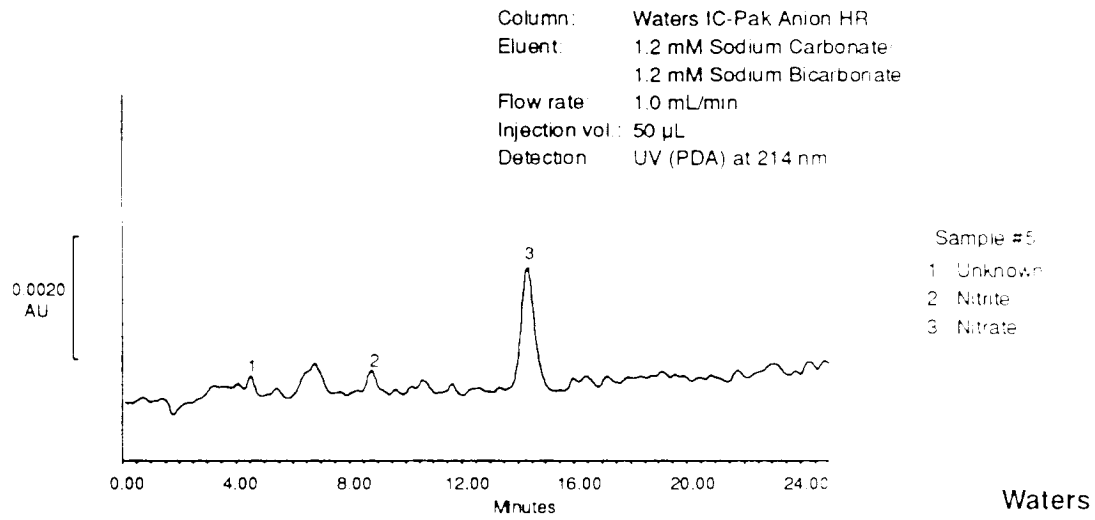


Sample #5
1 Chloride
2 Sulfate
3 Nitrite
4 Nitrate
5 Unknown
6 Fluoride
7 Carbonate
8 Acetate

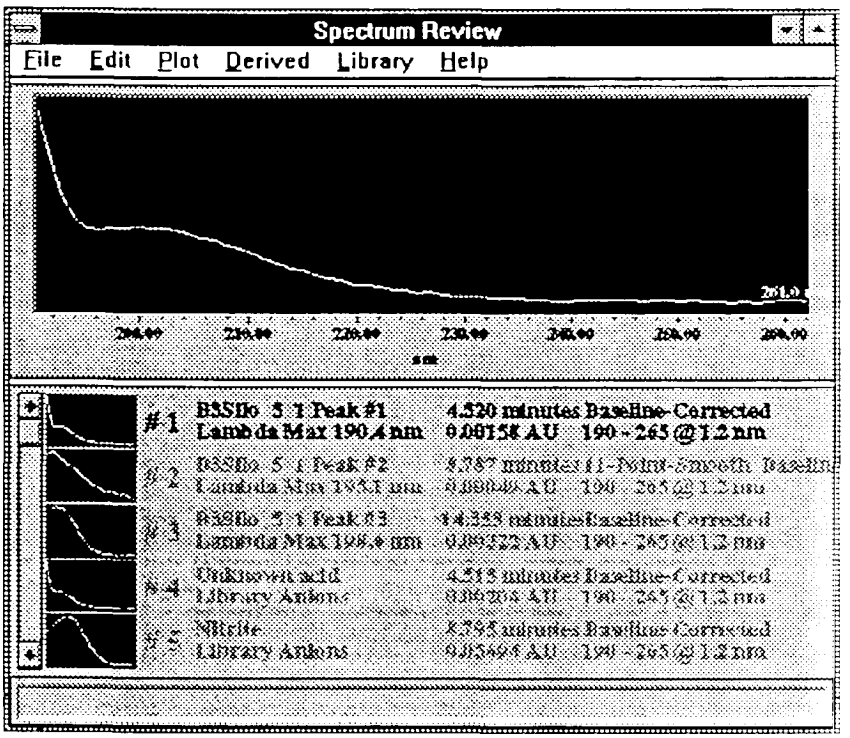
Waters

- Sample #5 showed a similar appearance to sample #1. Acetate and nitrite are present and the "unknown'" (peak #5) is also present in the CIA pherogram.

Monomer Extract #5 - Photodiode Array 214 nm Extracted Wavelength



- Peak #5 from the previous slide corresponds to peak #1 here.

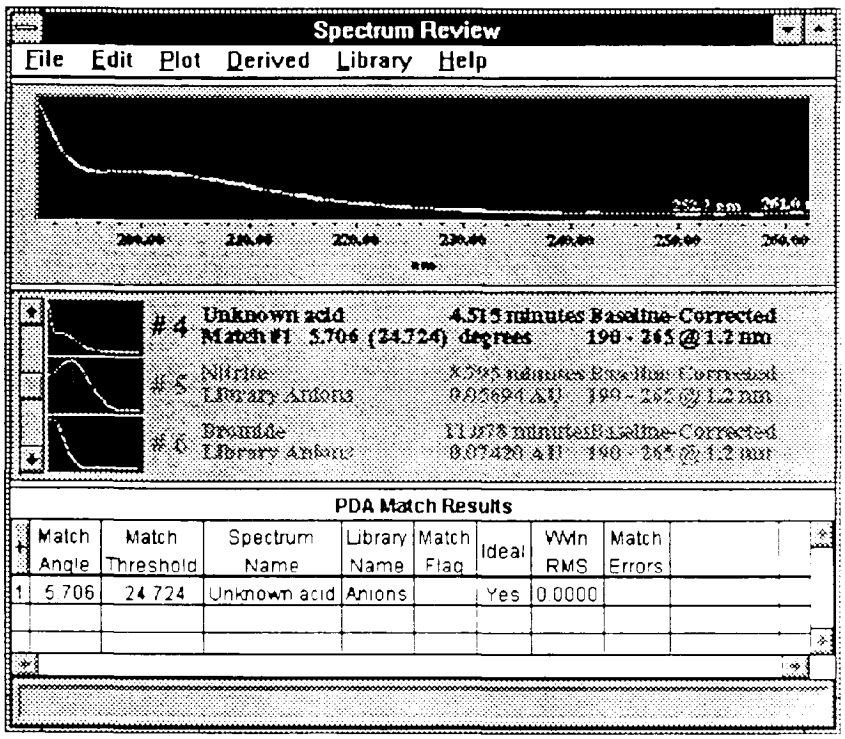


Spectrum Review of Monomer Extract (Peak #1)

Sample #5

Waters

- This is the spectrum review of peak #1 of sample #5. It did not match the library-stored spectra of nitrite, nitrate, bromide, formate or acetate.
- It however did appear similar in shape to the spectrum obtained for peak #1 of sample #1.
- The spectrum of peak #1 of sample #1 was added to the library and when the library was again searched for a match to peak #1 of sample #5.....

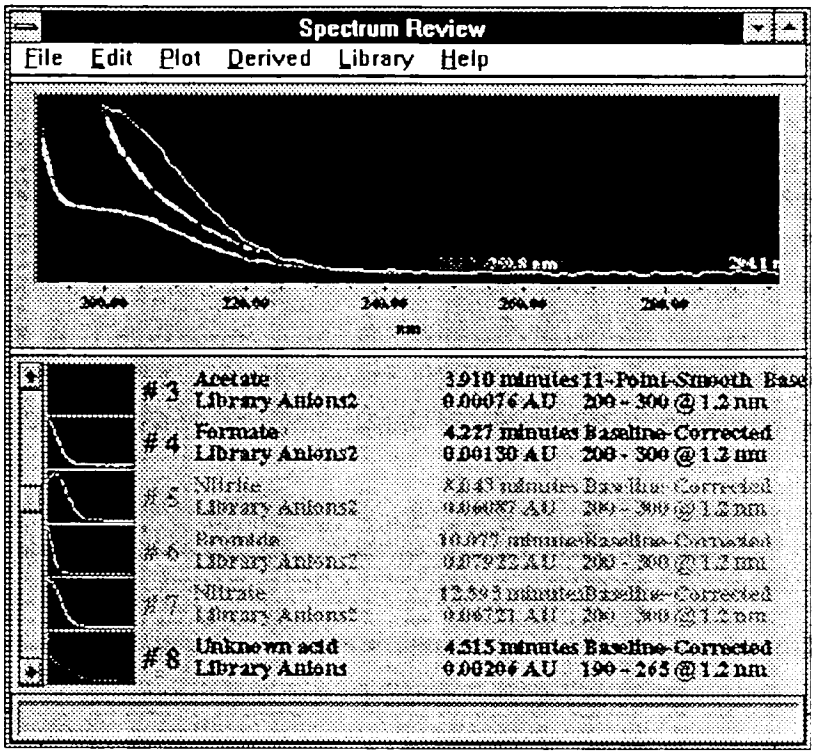


Library Matching Results (Peak #1) of Monomer Extract

Sample #5

Waters

- ..... the best match was found for the "unknown acid" (peak #1 of sample #1). This confirms that this peak is not only the same in terms of retention time by both suppressed and non-suppressed IC. as well as by CIA, but also the same spectrally.



Spectrum Review (Peak #1) of Monomer Extract

Sample #5

Key

Library Name	Color
Formate	Yellow
Acetate	Dark blue
Unknown acid	Red

Waters

- As mentioned, it is not spectrally similar to either formate or acetate as can be seen here visually.
- (Note)
- There are three spectra shown; (top to bottom they are:)
- formate (yellow),
- acetate (dark blue) [This is barely visible in a photocopy]
- "unknown acid" (red)

Conclusions

- ► Use of both chemically-suppressed and non-suppressed conductivity yields more information than either alone
- ► UV detection (PDA) for inorganic species
  - ► enhances analyte identification
  - ► increases inter-sample information
- ► The technique of CIA offers a unique and predictable selectivity as compared to ion chromatography

Waters

- Non-suppressed- able to see acetate as a resolved peak
- Suppressed- able to see nitrite and confirm that the 'unknown' peak was not carbonate.
-
- PDA helped confirm that nitrite and nitrate were present and that the "unknown acid" was not formate or acetate.
- The best match for the peak #1 from sample #1 was the same peak in sample #5. This strongly suggests that they are the same compound.
-
- CIA confirmed that: acetate was present in the samples: the "unknown peak" in both samples had similar migration times: and formate was not present in the samples.