

# Critical Evaluation of Anion Exchange Chromatography for the Separation of Water Soluble Organic Carbon.

Hui Chang, Pierre Herckes, Jeffrey L. Collett, Jr.

Colorado State University – Department of Atmospheric Science - Fort Collins, CO 80523 - USA  
<http://www.atmos.colostate.edu>

## Introduction

A number of recent, widely cited studies used anion exchange chromatography to separate water soluble organic matter into three compound classes: neutral/basic compounds, mono and dicarboxylic acids and polyacidic fractions (e.g. Decesari et al., 2000; Mayol-Bracero et al., 2002).

We applied this separation technique to fog and aerosol water soluble organic carbon (WSOC) extracts. Here we discuss some of our observations and point out possible problems with the usual compound classification by this approach.

## Analytical separation

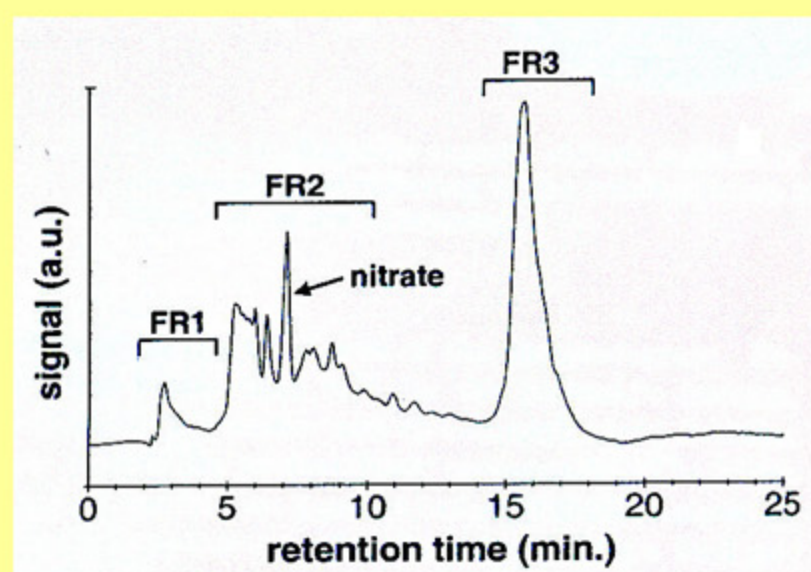
Aqueous samples were separated according to a technique first described by Decesari et al., 2000:

We used a Tosoh TSK DEAE-5PW gel column (7.5mmID, 7.5cm length). The initial mobile phase was de-ionized water. From 0.2 minutes to 2 minutes, the solvent composition was linearly increased to 0.02M NaClO<sub>4</sub>, 0.02M TRIS, 10% methanol. This eluent was kept constant until 10 minutes when the eluent was linearly changed to the final composition at 15 minutes of 0.4M NaClO<sub>4</sub>, 0.02M TRIS and 10% methanol. The pH was constant at 8. The HPLC system used was a HP 1050 system with a Diode Array Detector. The detection wavelength was set at 254nm

## Expected separation

The sample chromatogram shown below was obtained by Decesari and coworkers. The peak identification is as follows:

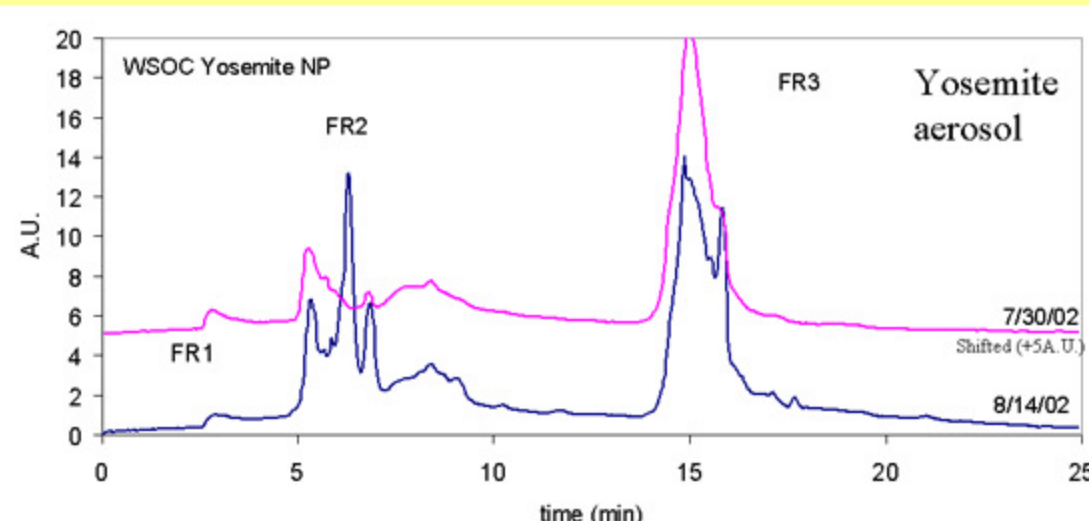
- FR1- neutral/basic compounds,
- FR2 – mono and di-carboxylic acid fraction
- FR3 – polyacidic fraction



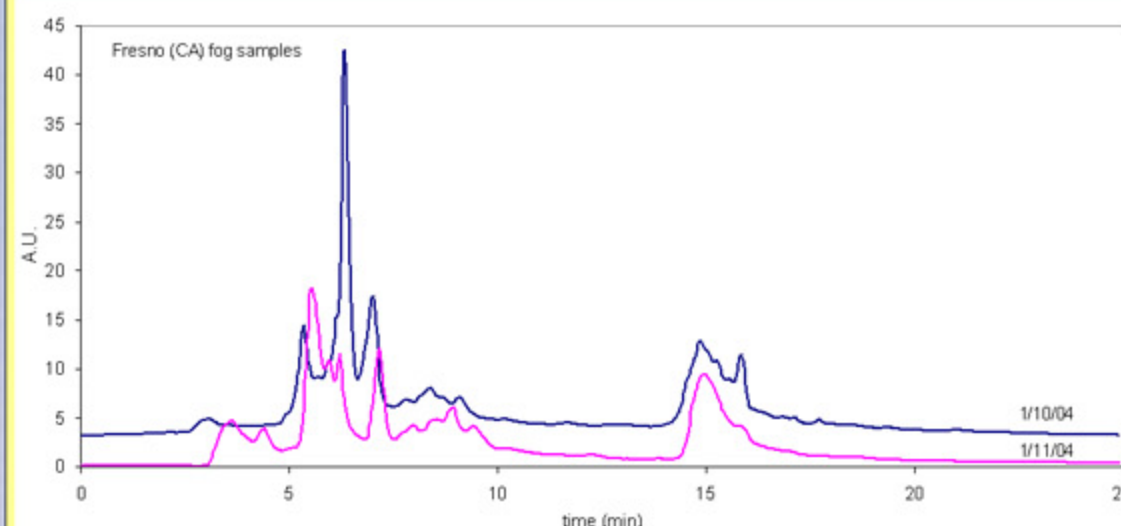
(from Decesari et al., 2000)

## Results

We obtain chromatograms very similar to the example by Decesari for WSOC extracts of aerosol samples from Yosemite National Park and for California fog samples.



Separations of WSOC samples from Yosemite National Park show 3 fractions with fraction 3 having the highest absorbance peak. Interestingly, there are some minor differences in the depicted chromatograms with the sample from August 14 presenting more distinct peaks in the second fraction than the sample from July 30. In both fractions, the neutral/basic fraction absorbance peak is much smaller than the other fractions.



The chromatograms for the California fog samples show similar features, except that the polyacidic peak (fraction 3) is smaller than the fraction 2 peak. The data show some distinct differences between different events, which will be further investigated.

## Problems ?

Because few results have been published showing retention times of individual compounds analyzed by this approach, we conducted tests for several atmospherically relevant organic compounds. Unfortunately, results of these tests often appear to contradict the usual, published classification scheme: *neutral/basic, mono/dicarboxylic acid and polyacidic fractions*.

	Retention time (min)	Expected Fraction <sup>1</sup>	Real Fraction
Methyl glyoxal	2.8, 5.4, 7.4, 14.8	1	1, 2
Acetone	2.9	1	1
Acetic acid	3.0	2	1
Formic acid	3.0	2	1
Acridine	4.5	1	1
Pyruvic acid	5.5	2	2
Nitrite	6.0	--	--
Nitrate	6.8	--	--
Syringaldehyde	7.5	1	2
Vanillin	7.8	1	2
Oxalic acid	7.8	2	2
Succinic acid	8.7	2	2
Phthalic acid	8.7	2	2
Syringol	9.0	1	2
Aniline	9.4	1	2
Guaiacol	10.6	1	2
o-Nitrophenol	11.7	1	2
Benzaldehyde	11.9	1	2
Phenol	12.9	1	2
Humic Acid (Fluka)	15.5	3	3
Tannic Acid	16.3	3	3
4-Ethylguaiacol	17.4	1	3
m-Cresol	17.9	1	3

<sup>1</sup>- based on classification in neutral/basic, mono- & di-carboxylic acid and polyacids

- Methylglyoxal (major fog component) may react with the eluent mixture.
- Phenolic compounds elute in *mono and dicarboxylic acid* fraction
  - partly dissociated at elution pH 8 (pK<sub>a</sub> ~ 7)
- Formic and acetic acid (abundant organics in fog) elute in the *neutral/basic* fraction
- It is difficult to explain why some presumably neutral compounds elute in fraction 3

## Preparative scale Quantitative Chromatography

In a second step we used a semi-preparative scale column packed with a DEAE-cellulose gel (Amersham HiPrep 16/10 DEAE). We performed a step-wise elution, identical to Decesari et al. (2000), collecting the unretained sample (fraction 0), then eluting with de-ionized water (fraction 1), a 0.05M NaHCO<sub>3</sub> buffer (FR2) and finally a 0.05M NaHCO<sub>3</sub> buffer (FR3). All fractions were analyzed for TOC by a Shimadzu TOC analyzer.

Fraction 0 and fraction 1 have been combined to FR1. The 3 fractions are considered identical to the fractions described previously. In contrast to Decesari et al., we followed elution by frequent TOC measurements instead of UV detection.

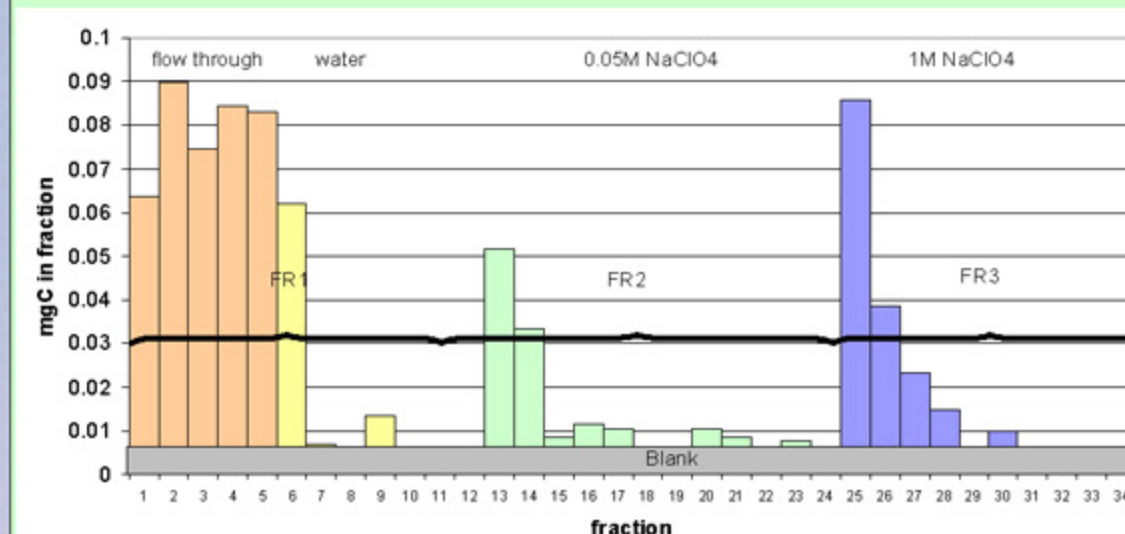
## Tests

	expected	observed
Vanillin	1	2
Levogluconan	1	1
Acetic acid	2	2
Oxalic acid	2	2
Humic acid	3	3

→ difference in separations

Prep-scale separation results are identical to analytical separation except for acetic acid. This is not surprising as FR1 on the analytical column includes eluent concentrations up to 0.02M, while in the step elution FR1 represents only water elution.

## Results



A Fresno fog sample gave the following result: 90% DOC recovery -- FR1: 54%, FR2: 16%, FR3: 14%

This contrasts with results of Decesari et al., obtained for samples from the Po Valley: 77% recovery -- FR1: 25%, FR2: 35%, FR3: 17%

Our separation result is inconsistent with high organic acid contents in these fogs (Ervens et al., 2003) and the initial analysis of our sample in particular, which showed that 20% of TOC is comprised by acetic acid. Reanalysis of the sample used for the Anion Exchange experiment showed that this aliquot, not specifically conserved for organic acid analysis, lost all formate and acetate during storage. Hence the separation might be correct but does not give a representative picture of the original sample composition.

Future work will be performed on samples treated to prevent microbial degradation of low molecular weight acids.

## Summary

- The separation appears to give similar results for different studies
- The classification of the observed fractions as *neutral/basic, mono and dicarboxylic acids and polyacids* appears inaccurate
  - Single compound tests show that compounds without carboxyl groups often elute in the second or third fractions
  - Charge-based separation might not be the only factor determining retention times as indicated by the long retention times of cresol and 4-ethylguaiacol
- UV detection to determine the fractions is problematic due to low absorption efficiencies at 254nm of many important organic compounds, including carboxylic acids. On the other hand phenolic compounds, which might elute in *wrong* fractions, present very high absorption efficiencies at 254 nm.
- Although classification problems might exist for fog samples due to the abundance of small molecular weight carbonyls and carboxylic acids, the separation technique might give a more reasonable picture for aerosol extracts.
- Large, polycharged molecules like the model humic acid compounds, do not elute completely off the column and might explain the losses in the experiment together with low molecular weight compound losses due to purging of the eluent prior to TOC analysis.

→ Further testing is warranted to evaluate the uncertainties of these techniques

## Acknowledgements

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## References

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