Direct Analysis of Surfactants using HPLC with Charged Aerosol Detection

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Overview

Purpose: Examples of HPLC methods for the determination of surfactants using the universal Thermo Scientific Dionex Corona charged aerosol detector with the Thermo Scientific Acclaim Surfactant Plus column were evaluated.

Methods: HPLC methods, using buffered mobile phases and different elution programs are outlined.

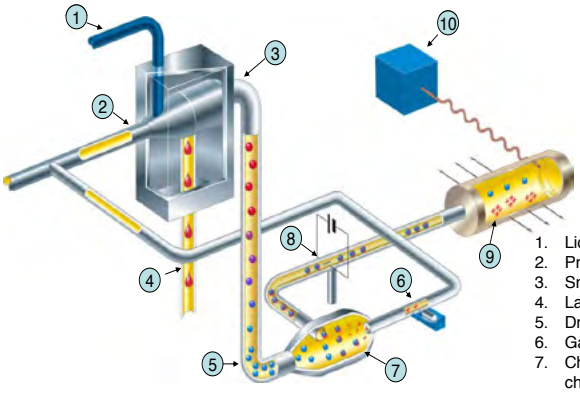
Results: The method was used to generate chromatograms of a mixture of anionic, cationic, and non-ionic surfactants, and samples of Span™ (80, 83, and 85), TWEEN® 80 and 85, Pluronic™ F68, and a laundry detergent.

Introduction

Surfactants are a diverse group of chemicals whose structures vary widely but typically contain an oil-soluble hydrocarbon chain and a water-soluble ionic group. Surfactants can be categorized based upon their structure and include nonionic, anionic, and cationic classes. They have widespread use as detergents in shampoos and cleaning products, ion pairing agents used in chromatography, and complex dispersants used to treat oil spills. Many of these commercial surfactants are mixtures of members of a homologous series, and such mixtures can be defined using LC. Chromatographic approaches can separate the molecules on the basis of carbon chain length, chain branching or positional isomer distribution. Surfactants typically do not contain a UV-chromophore so are usually measured using RP-HPLC with non-suppressed or suppressed mode conductivity or indirectly using photometric detection. Charged aerosol detection can measure any non-volatile, and many semi-volatile compounds, typically to low ng sensitivity. Furthermore, as response is similar for all compounds and independent of chemical structure, charged aerosol detection is ideal for measurement of surfactant species. Generally, the reproducibility for methods using charged aerosol detection is better than 2% RSD. Sensitive methods are described herein for the analysis of various surfactant classes including anionic alkyl sulfonates (lauryl sulfate), cationic quaternary amines (laurylmethylbenzylamine), non-ionic block copolymer (Pluronic F-68), and complex mixtures of oil dispersants (Span 80).

The Corona™ ultra RS™ charged aerosol detector (CAD™) is a sensitive, mass-based detector, especially well-suited for the determination of non-volatile and many semi-volatile analytes. As shown in Figure 1, the detector uses nebulization to create aerosol droplets. The mobile phase evaporates in the drying tube, leaving analyte particles, which become charged in the mixing chamber. This technology has greater sensitivity and precision than evaporative light scattering detectors (ELSD), and it is simpler to operate than a mass spectrometer (MS). Typical characteristics of chromatography with charged aerosol detection include: low-nanogram on-column (o.c.) sensitivity, over four orders of magnitude of dynamic range, and high precision results, typically less than two percent of peak area RSD. Analyte response is also largely independent of chemical structure, providing clear relationships among different analytes in a sample

FIGURE 1. Schematic and functioning of charged aerosol detection.



1. Liquid eluent enters from HPLC system
2. Pneumatic nebulization occurs
3. Small droplets enter drying tube
4. Large droplets exit to drain
5. Dried particles enter mixing chamber
6. Gas stream passes over corona needle
7. Charged gas collides with particles and charge is transferred
8. High mobility species are removed
9. Charge is measured by a highly sensitive electrometer
10. Signal transferred to chromatographic software

Methods

Sample Preparation

Samples were dissolved in a isopropanol, isopropanol/water (1:1), or acetonitrile/water (1:1) to a concentration of 10 or 20 mg/mL.

Liquid Chromatography

HPLC System:	Thermo Scientific Dionex UltiMate 3000 x2 Dual RSLC system
HPLC Column:	Acclaim™ Surfactant Plus, 4.6 × 250 mm
Column Temperature:	30 °C (Gradient, Inverse Gradient, Detergent) 40 °C (Pluronic F68)
Mobile Phase A:	100 mM Ammonium acetate, pH 5.4
Mobile Phase A1:	50 mM Ammonium acetate, pH 5 in water/acetonitrile (9:1)
Mobile Phase B:	n-Propyl alcohol
Mobile Phase C:	Acetonitrile
Mobile Phase C1:	50 mM Ammonium acetate, pH 5 in acetonitrile/methanol/water (4:5:1)
Detector:	Corona ultra RS Nebulizer Temperature: ambient Filter Setting: 0
Sample Temperature:	Ambient
Injection Volume:	5.0 µL

Gradients:

Gradient Elution

Time (min)	Flow Rate (mL/min)	%A	%C
-5	1.0	98	2
0	1.0	98	2
15	1.0	5	95
20	1.0	5	95

Inverse Gradient

Time (min)	Flow Rate (mL/min)	%A	%C
-4	1.0	0	100
0	1.0	0	100
1	1.0	0	100
16	1.0	93	7
21	1.0	93	7

Pluronic F68

Time (min)	Flow Rate (mL/min)	%A	%B
-5	0.6	95	5
0	0.6	95	5
5	0.6	30	70
10	0.6	5	95

Detergent

Time (min)	Flow Rate (mL/min)	%A1	%C1
-5	0.6	0	20
0	0.6	0	20
2	0.6	0	20
12	0.6	10	90
17	0.6	10	90

Data Analysis

All HPLC chromatograms were obtained and compiled using Thermo Scientific Dionex Chromeleon 7.1 SR 1. The inverse gradient was calculated using the Inverse Gradient Calculator using the parameters of void volume difference and setting for maximum acetonitrile content, which increases analyte response.

Results

Sample Analysis

Using the conditions above, a mixture of eight surfactants, consisting of five anionic, two non-ionic, and one cationic surfactant were analyzed using the single-pump gradient elution program and the inverse gradient program (both pumps, as programmed with the gradient elution and the inverse gradient conditions). As shown in Figure 2, the column clearly separates the different surfactant classes, including separation of components within more complex surfactants. The use of the inverse gradient provides two benefits: it flattens the baseline and it eliminates increases in relative response factors that are associated with increases in nebulization efficiency resulting from increased organic content of the mobile phase. This yields more aesthetic chromatograms which are less error-prone towards peak integration and, more importantly, relative response factors across the gradient are more consistent which allows for improved results on mass-percent values in the sample.

Two surfactants, TWEEN 80* (polyoxyethylene-20-sorbitan monooleate) and TWEEN 85* (polyoxyethylene-20-sorbitan trioleate), were dissolved in isopropanol and analyzed using the gradient elution conditions. The two chromatograms are overlaid, as shown in Figure 3. Note that not only are the subcomponents of each TWEEN distinguished, but also TWEEN 85 elutes later than the TWEEN 80 due to the greater amount of oleate moieties contained within the polymer.

*used in COREXIT® 9500[1]

FIGURE 2. HPLC with charged aerosol detection chromatogram of a surfactant mix in water/acetonitrile (1:1), containing cationic, anionic, and neutral surfactants. Single-pump eluent gradient conditions in black, and dual-pump inverse gradient conditions in blue.

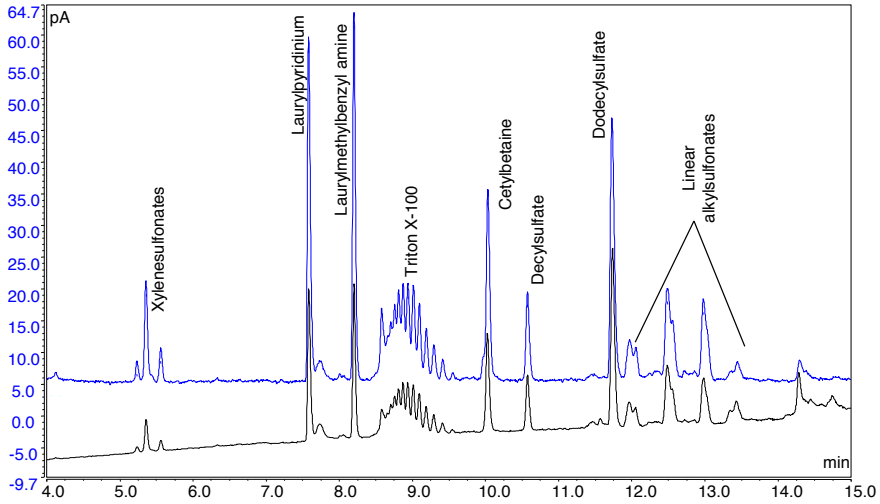
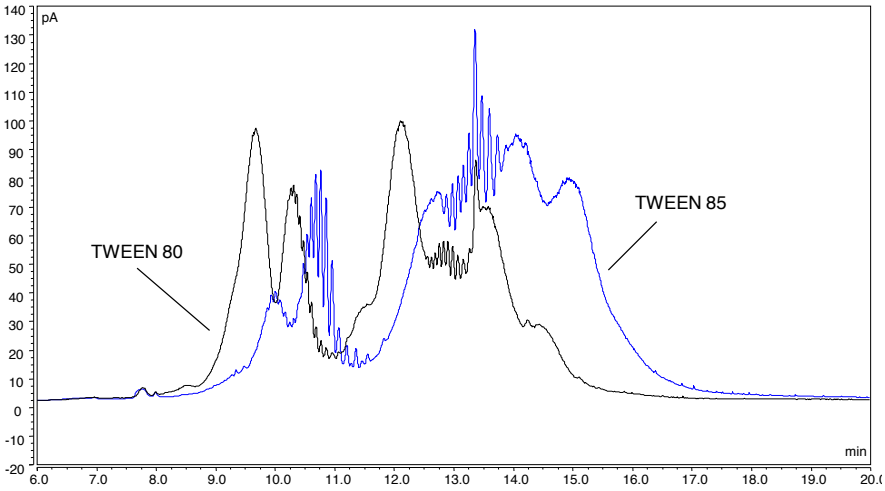


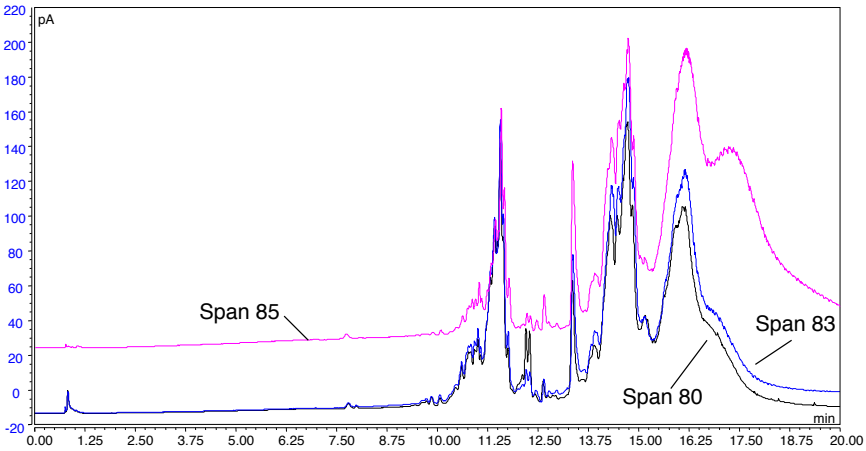
FIGURE 3. Overlaid chromatograms of TWEEN 80 (black) and TWEEN 85 (blue), 20 mg/mL in isopropanol using single gradient conditions.



Six Span surfactants were analyzed, using the single-pump gradient elution parameters shown above. Span-80* (sorbitan monooleate), -83 (sorbitan sesquioleate), and -85 (sorbitan trioleate) were dissolved in isopropanol at a concentration of 20 mg/mL. The similarity between the Span 80 and 83 chromatograms reflects the similarity in composition: Span 83 is similar to Span 80, except that it contains 50% more oleate than Span 80. This may be reflected in the slight increase of the later eluting portions of the Span 83 chromatogram. Taking this difference further, the triolein form of Span 80, called Span 85, contains the greatest amount of later-eluting, hydrophobic material than the other two, which is clearly seen in the chromatogram overlays in Figure 4.

A common surfactant in pharmaceutical/biotechnology products is Pluronic F68 (polyoxyethylene-polyoxypropylene block copolymer). Like other surfactants, Pluronic lacks a chromophore, and its polymeric nature makes reversed-phase chromatography difficult, usually resulting in peaks with broad tailing. One recent paper uses a restricted access media column with a step gradient and ELSD.[2] The use of step gradients typically causes baseline disruptions which can interfere with analytical results, especially at low levels.

FIGURE 4. HPLC-CAD chromatogram overlays of Span 80, 83, and 85 at 20 mg/mL in isopropanol.



Use of the Acclaim Surfactant Plus column also generated acceptable chromatography for Pluronic F68, using the Pluronic F68 conditions described in Methods. Triplicate injections of Pluronic F68 at concentrations of 20 mg/mL diluted sequentially to 0.31 mg/mL (or 1.6 µg o.c.) in isopropanol/water (1:1), is shown in Figure 5. Precision was good, with peak area percent RSD values of 0.61 (20 mg/mL) to 6.5 (0.31 mg/mL). A calibration plot, fitted to an inverted second-order polynomial for concentrations, between 0.31 and 10 mg/mL, is shown in Figure 6. The correlation coefficient, r^2, was 0.9995. The signal to noise ratio (S/N) at 1.6 µg o.c. was 139, for an limit of quantitation (LOQ) value of 115 ng o.c., based on a S/N ratio of 10.

FIGURE 5. Chromatogram overlays of Pluronic F68, in triplicate, from 20 to 0.31 mg/mL in isopropanol/water (1:1)

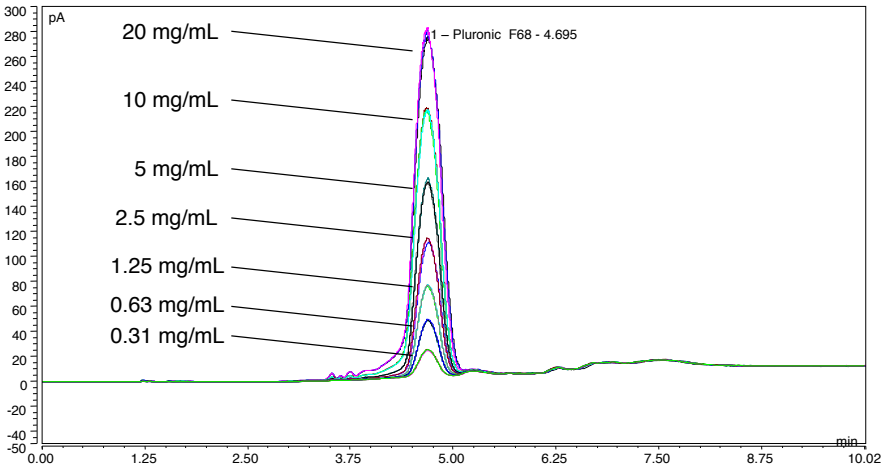
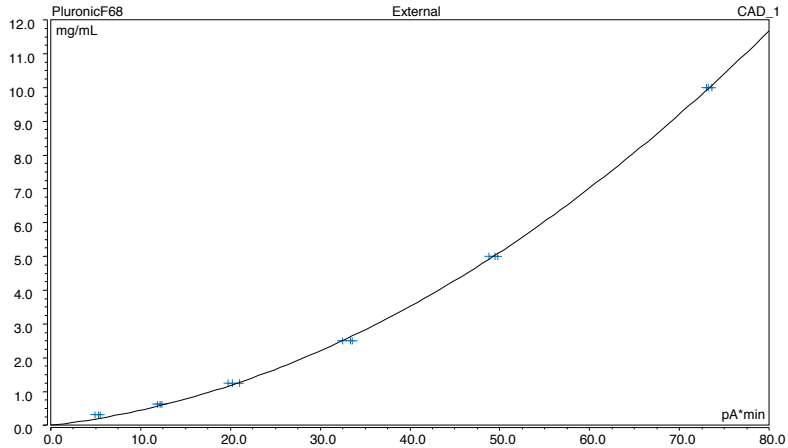
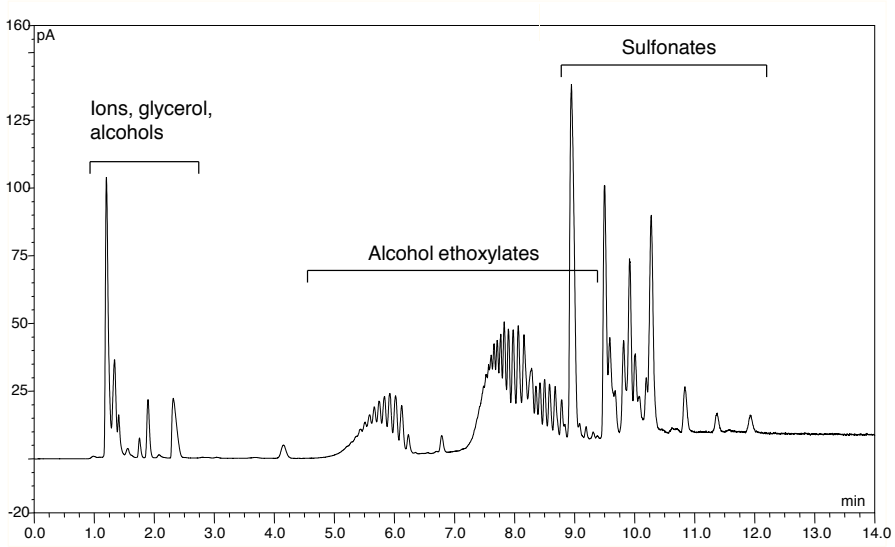


FIGURE 6. Calibration plot and inverted polynomial fit for Pluronic F68 from 0.31 to 10 mg/mL.



An off-the-shelf laundry detergent product was diluted in water at a concentration of 50 mg/mL; 5 µL was analyzed using the detergent conditions described in Methods. As can be seen in Figure 7, this detergent appears to contain alcohol ethoxylates, two main varieties of sulfonates, and a variety of more hydrophilic materials that elute before 3 minutes.

FIGURE 7. Chromatogram of a laundry detergent



Conclusion

Combining the use of the Surfactant Plus column with the universality, reproducibility, and sensitivity of the Corona charged aerosol detector enables a simplified approach to chromatography method development.

- Methods used gradient elution for fast, quantitative results, while providing resolution for sample characterization.
- The methods shown are capable of separating and quantifying many of the typical classes of surfactants, from the simple surfactant to the complex, polymeric surfactants and mixtures.
- The use of the inverse gradient enabled more consistent response throughout the gradient.
- Analysis times were less than 21 minutes.
- Similar surfactants were differentiated consistent with their composition.
- Other surfactants have been analyzed, including Aerosol OT (docusate sodium) as a single peak, as well as Span 20 and 60.

References

1. Nalco North America Home Page. http://www.nalco.com/news-and-events/4297.htm (accessed February 29, 2012)
2. Chung H.H.; Zhou C.; Khor H.K.; Qiu J. *J. Chromatogr. A.* **2011**, 1218(15), pp. 2106-13.

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