Quantitation of Pluronics by High Performance Liquid Chromatography and Corona Charged Aerosol Detection

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Overview

Purpose: To develop HPLC methods for the quantitation of Pluronic® polymers, as both diluted and as a component in a fluoride mouth wash sample, using an HPLC system with a charged aerosol detector.

Methods: Two methods using the Thermo Scientific™ Acclaim™ Surfactant Plus HPLC column and the charged aerosol detector are outlined. One method was used for neat poloxamers, and a second method with greater selectivity was used to determine the amount of poloxamer in a commercial product containing multiple matrix components.

Results: Near-single peaks were analyzed for a number of poloxamers, and the amount of poloxamer 407 was accurately determined in a fluoride mouth wash sample. Sensitivity was determined at 156 ng o.c. Spike recoveries were within 15% of target.

Introduction

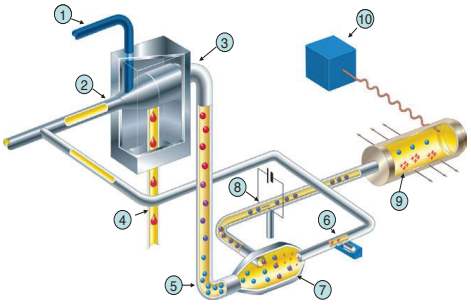
Surfactants are generally used to control or affect the consistency of mixtures, to alter surface tension, and as an aid in mixing materials that normally would not mix. These surface-active agents exist in four different categories, amphoteric (zwitterionic), cationic, anionic, and non-ionic, each with a specific form of activity and use. Within each category hundreds of different compounds exist providing nearly any range of property needed for a specific application or use. Pluronic polymers, or poloxamers, are a special class of non-ionic surfactants, consisting of a triblock copolymer of one polypropylene oxide molecule connected to two polyethylene blocks. They are used in many detergents, consumer, and pharmaceutical products.

Like other surfactants, poloxamers pose several analytical challenges: they are nonvolatile, which eliminates the use of highly sensitive gas chromatography; they also lack a good chromophore for fluorescence or ultraviolet detection following their separation by HPLC. Chemically, they are nonionic surfactants and do not typically chromatograph well due to their polymeric nature. This makes reversed-phase chromatography difficult, usually resulting in broad peaks. Since poloxamers do not respond to light absorption detectors, a universal detector such as the Thermo Scientific™ Dionex™ Corona™ ultra RS™ charged aerosol detector, is ideal. It enables low nanogram detection and quantitation of many surfactant classes.

The charged aerosol detector is a sensitive, mass-based detector, especially well-suited for the determination of any nonvolatile analyte independent of chemical characteristics. 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. The charge is then measured by a highly sensitive electrometer, providing reproducible, nanogram-level sensitivity. This technology has greater sensitivity and precision than ELSD and refractive index (RI), is gradient compatible and is simpler to operate than a mass spectrometer (MS). Compounds do not have to possess a chromophore (unlike UV detection) or be ionized (as with MS).

This sensitivity, combined with the specificity of the new Acclaim Surfactant Plus column, provides a unique and complete analytical solution for sensitive, reproducible, and routine analysis of surfactant-containing samples. Several examples of poloxamer HPLC separations are detailed.

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

Standard and Sample Preparations
Standards (neat Pluronic solutions) were dissolved in isopropanol/water (1:1) solvent at a concentration of 10 mg/mL. Further dilutions were in isopropanol/water (1:1).

The sample, a fluoride mouth wash, was placed directly into the HPLC vial without dilution.

Liquid Chromatography – Neat Poloxamers

HPLC System:	Thermo Scientific™ Dionex™ UltiMate™ 3000 RS system: DGP-3600RS pump, WPS-3000 RS autosampler, and TCC-3000RS column oven
HPLC Column:	Acclaim™ Surfactant Plus 3 μm, 3.0 150 mm
Column Temperature:	40 C
Mobile Phase A:	100 mM ammonium formate, pH 4
Mobile Phase B:	n-propanol
Flow Rate:	0.6 mL/min
Injection Volume:	40 μL
Sample:	Pluronic F127 in isopropyl alcohol/water (1:1)
Detector:	Corona ultra RS
Nebulizer Temperature:	10 C
Filter:	5
Data Rate:	10 Hz
Power Function:	1.75
Flow Gradient:	

Time (min)	%A	%B	Curve
-5	95	5	5
0	95	5	5
1	95	5	5
7	50	50	2
10	10	90	5
10	10	90	5

Liquid Chromatography – in Sample Matrix

HPLC System:	UltiMate 3000 RS system, as above
HPLC Columns:	Acclaim Surfactant Plus, 3 μm, 3.0 150 mm and Acclaim Phenyl-1, 3 μm, 2.1 150 mm
Column Temperature:	50 C
Mobile Phase A:	100 mM Ammonium Formate, pH 4
Mobile Phase B:	*n*-Propanol/Acetone (1:1)
Flow Rate:	0.4 mL/min
Injection Volume:	20 μL
Sample:	Fluoride mouth wash, containing Polaxamer 407, undiluted
Detector:	Corona ultra RS
Nebulizer Temperature:	ambient
Filter:	5
Data Rate:	10 Hz
Power Function:	1.75
Flow Gradient for Characterization:	

Time (min)	%A	%B	Curve
-5	95	5	5
0	95	5	5
1	95	5	5
7	50	50	2
10	10	90	5
10	10	90	5
19	10	90	5
19	95	5	5

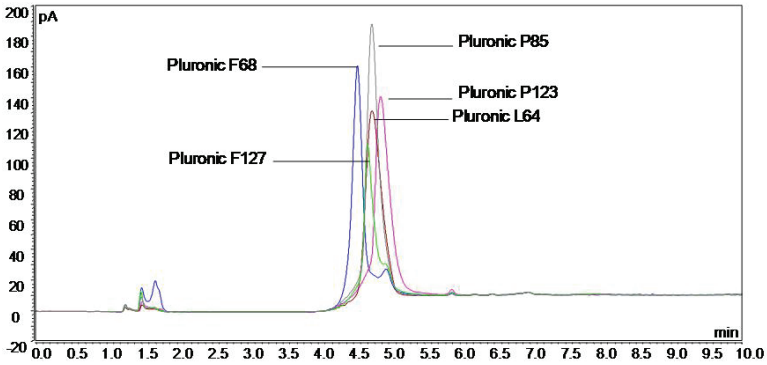
Data Analysis
All HPLC chromatograms were obtained and compiled using Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software, 7.1 SR 1.

Results

Sample Analysis

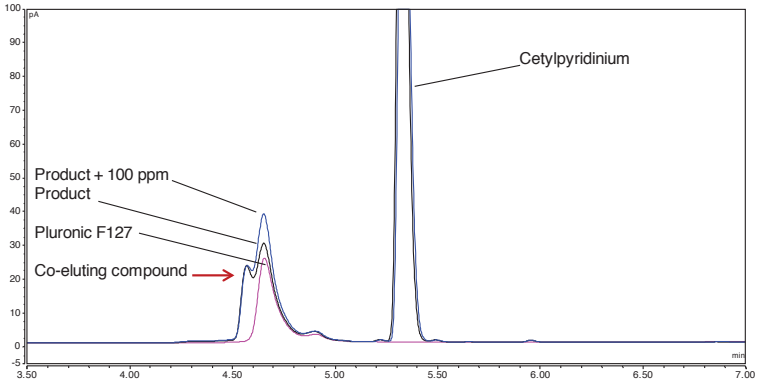
The Acclaim Surfactant Plus column can be used for the HPLC analysis of a wide variety of surfactants (cationic, anionic, non-ionic, and amphoteric).[1] An advantage of this column is that it can elute complex analyte mixtures as a near-single peak (e.g., Triton X-100™), which is helpful in quantitation of polymeric or complex surfactant mixtures. Using a buffered mobile phase and a fast organic gradient, five different pluronics were analyzed, including L64, F68, F127, P85, and P123, as shown in Figure 2. The pluronics eluted within a retention time window of approximately one minute, with the gradient adjusted to provide best resolution and peak shape for these compounds while maintaining a single peak for best sensitivity for quantitation.

FIGURE 2. Overlays of five different Pluronic compounds (10 mg/mL in isopropanol/water (1:1))



When this method was used with a consumer product, a fluoride mouth wash, a small peak was found to elute as a shoulder on the Pluronic analyte peak, as shown in Figure 3. Gradient adjustments failed to resolve this compound so a second column, an Acclaim Phenyl-1 column, was added in series after the Surfactant Plus column to provide some selectivity based on π-π interactions. Unfortunately, this approach broadened the Pluronic peak greatly. To address this issue, acetone was added to the n-propanol mobile phase (50% by volume). After some gradient adjustments, the second method was developed that resolved the cetylpyridinium surfactant, the Pluronic, and the co-eluting component, as shown in Figure 4.

FIGURE 3. Overlays of the fluoride mouth rinse product spiked with Pluronic F127 (blue), the product itself (black), and the Pluronic F127 standard (250 ppm, pink)



With the analyte resolved from the sample matrix using this two-column method, a calibration curve was created using amounts of Pluronic F127 from 156 to 20,000 ng o.c. Each solution concentration was injected three times, resulting in the chromatogram overlays in Figure 5. Replicate peak areas (n=3) varied from 0.28 to 2.09 %RSD. The calibration curve is shown in Figure 6, and the LOQ value was determined at a value of 156 ng o.c., based on a signal-to-noise ratio of 10. Solvent blanks were used to remove baseline artifacts.

The charged aerosol detector is a non-linear detector, but through the use of the Power Function parameter, linear calibration curves are now possible. For Pluronic F127, a Power Function value of 1.75 was found to be optimal for the fitting of a linear calibration curve. A high correlation coefficient (r^2 = 0.9993) and precision (calibration %RSD= 2.30) was determined.

The fluoride mouth wash was then analyzed without dilution. Three other samples were prepared for spike recovery determinations: a sample comprised of 980 µL of fluoride mouth wash with 20 µL each of water, a 1 mg/mL solution (+200 ng o.c., 10 ppm) of Pluronic F127, and a 0.5 mg/mL (+400 ng o.c., 20 ppm) solution of Pluronic F127. The overlaid chromatograms are shown in Figure 4.

Replicate injection peak areas (n=3) varied from 0.28 to 2.09 %RSD. The calibration curve is shown in Figure 5, and the LOQ value was determined at a value of 156 ng o.c., based on a signal-to-noise ratio of 10.

The results of the analysis of the fluoride mouth wash are presented in Table 1. Spike recovery values were calculated by dividing the found-spiked amount by the theoretical-spiked amount, and these values were within 15% of theoretical values. This demonstrates good sensitivity and accuracy, considering that the spike levels were less than 7% of the product's poloxamer concentration.

FIGURE 4. HPLC chromatogram overlay of over-the-counter fluoride mouth wash product, spiked and unspiked, and the Pluronic F127 standard at 10,000 ng o.c.

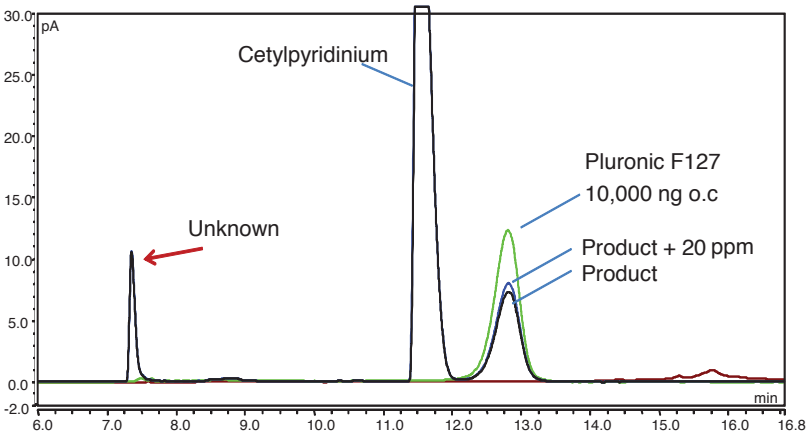


FIGURE 5. HPLC chromatogram overlays of Pluronic F127 standard solutions, from 156 to 20,000 ng o.c. with triplicate analyses

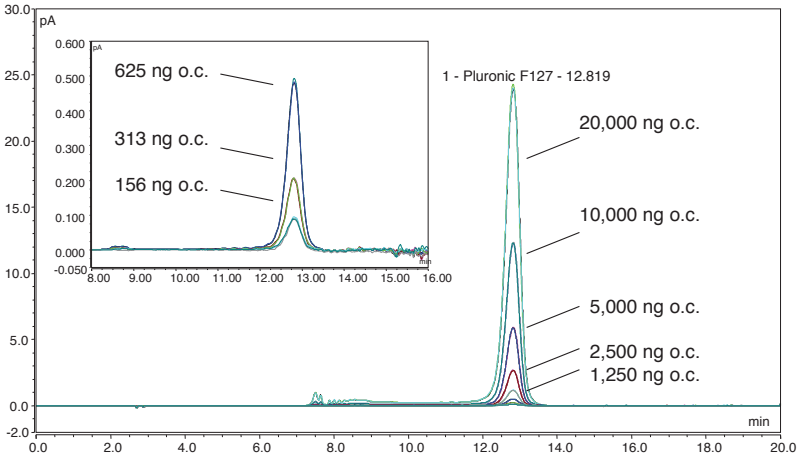


FIGURE 6. Linear Pluronic F127 calibration curve, from 156 to 20,000 o.c., each amount analyzed in triplicate

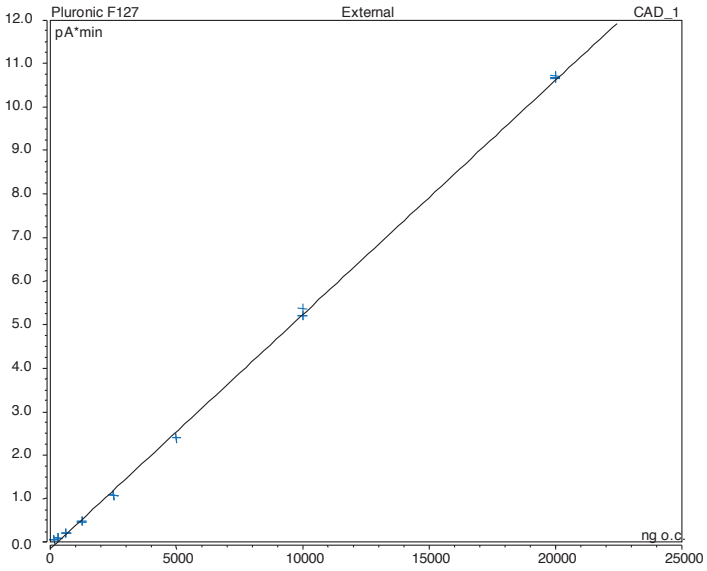


TABLE 1. Spike recovery results for fluoride mouth wash at two spike values

Sample	Theoretical Amount (ng o.c., ppm)	Experimental Amount Found (ng o.c., ppm)	Spike Recovery (%)
Fluoride mouth wash	--	5894, 294.7	--
Spiked + 10 ppm	6094, 304.7	6123, 306.2	114.5
Spiked + 20 ppm	6294, 314.7	6342, 317.1	112.0

Conclusions

A basic method was developed for quantitation of poloxamers using the Acclaim Surfactant Plus column and the Corona ultra RS detector. The method showed a linear response to analyte concentration with a sensitivity to 100 ng o.c. The analysis of fluoride mouth wash showed that there was poor resolution between a matrix component and the pluronic analyte. This was overcome through the use of an Acclaim Phenyl-1 column placed in series, after the Acclaim Surfactant Plus column and with acetone added to the organic eluent to sharpen the Pluronic F127 peak. Spike recovery measurements were within 15% of the targeted spike amounts.

References

1. http://www.dionex.com/en-us/webdocs/113473-Pittcon12_830-13_MPlante_PN70055_Surfactantsr1.pdf (last accessed 04 Feb 2013)

Acknowledgement

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