Characterization and Lot-to-Lot Variability of Complex Surfactants by High Performance Liquid Chromatography and Charged Aerosol Detection

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Overview

Purpose: To develop a semi-quantitative method for determining lot-to-lot variability of complex surfactants using HPLC and the Thermo Scientific™ Dionex™ Corona™ Veo charged aerosol detector.

Methods: A reversed phase gradient HPLC method was used to create a chromatographic profile for complex surfactants such as polysorbates 20 and 80, Triton™ X-100, Brij™ 35, and Pluronic™ F127 (poloxamer 407). Peak sections were integrated and compared against a reference.

Results: The general method can be used to profile surfactants for lot-to-lot studies. The method can be adapted, with mobile phase adjustments, to obtain profiles for a variety of polymeric surfactants. A chromatogram for poloxamer 407 and a more complete analysis of polysorbate, with lot-to-lot variation, are described.

Introduction

Surfactants are present in biopharmaceutical products, pharmaceuticals, and over-the counter products. Polymeric surfactants, such a polysorbate 20, polysorbate 80, Triton X-100, Brij 35, and Pluronic F127 are used to promote the solubility of APIs, and for controlling the solubility and stability of proteins. Raw material testing and qualification of these surfactants are an important criterion of quality control measures, and the ICH Q6B Guidelines recommends that "liquid chromatographic patterns for identity, homogeneity, and purity."[1]

Some methods provide narrower groups of peaks for surfactants, where the elution is more appropriate for quantitation.[2] A published method comparing charged aerosol detection (CAD) and evaporative light scattering detection (ELSD) showed that CAD was 10x more sensitive than ELSD.[3] This increase in sensitivity can provide a more consistent measurement of surfactant profiles, and can be also used in low level quantitation applications.

CAD is a mass sensitive technique used for determining levels of any non-volatile and many semi-volatile analytes after separation by HPLC. HPLC methods using Corona Veo CAD have limits of detection typically between high-picogram to low nanogram amounts on column and have a wide dynamic range from nanogram to microgram levels, with high reproducibility. A schematic of the Corona Veo charged aerosol detector is shown in Figure 1.

A non-aqueous reversed phase HPLC method, based on an HPLC-ELSD and mass spectrometry (MS) method by R. Zhang,[4] was used to separate these complex, polymeric surfactants on a solid-core C18 column into their respective subsets. Poloxamer 407 was analyzed and two different lots of Tween 80 were compared against a reference lot, using relative peak areas.[4] This provides a suitable method for lot-to-lot product characterization for quality control purposes.
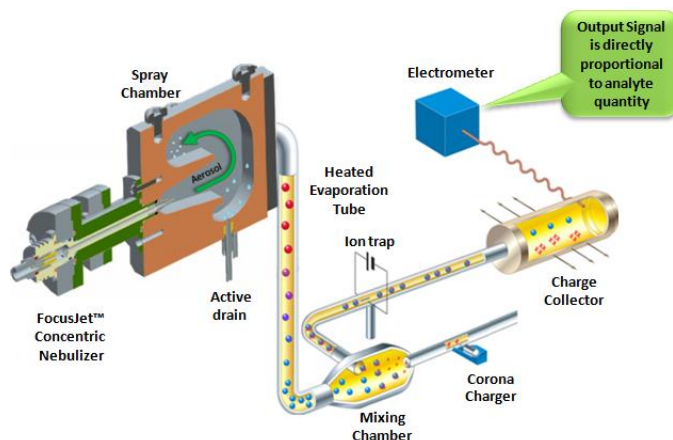

FIGURE 1. Schematic of the Corona Veo Charged Aerosol Detector.

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Methods

Sample Preparation

Solutions were made at a concentration of 20 mg/mL of the surfactant in water. Samples were mixed in a vortex mixer for 5 minutes to dissolve completely.

Liquid Chromatography

HPLC System: Thermo Scientific™ Dionex™ UltiMate™ 3000 with LPG-3400 SD pump (normal phase), WPS-3000 autosampler, TCC-3000RS column oven, Corona Veo SD charged aerosol detector

Mobile Phase A: Acetonitrile / Water (1:1)
Mobile Phase B: Tetrahydrofuran (unstabilized)
Mobile Phase C: Water
Column: Thermo Scientific™ Accucore™ C18 2.6 µm, 2.1 x 150 mm
Column Temperature: 50 °C
Flow Rate: 0.4 mL/min
Detector: Corona Veo SD
Evaporation Temperature: High
Data Rate: 10 Hz
Filter: 5 s
Power Function Value: 1
Gradient: Poloxamer 407

Time (min)	%A	%B	%C	Curve
-6	50	0	50	--
0	50	0	50	5
3	50	0	50	5
25	25	50	25	4
33	5	90	5	5
34	50	0	50	5

Gradient: Polysorbate 80

Time (min)	%A	%B	%C	Curve
-6	100	0	0	--
0	100	0	0	5
1	100	0	0	5
25	30	70	0	4
33	10	90	0	5
34	100	0	0	5

Data Analysis

All HPLC chromatograms were obtained and compiled using Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data Station, 7.2 SR 2 software.

Results

Poloxamer 407

Poloxamer is a tri-block polymer consisting of a polyoxypropylene with hydrophilic polyoxyethylene chains on both sides, as shown in Figure 2. A poloxamer 407 solution was analyzed using the poloxamer gradient method, and the resulting chromatogram is shown in Figure 3.
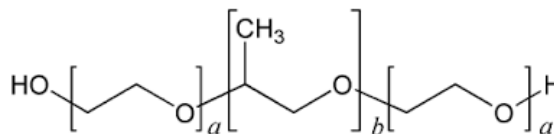

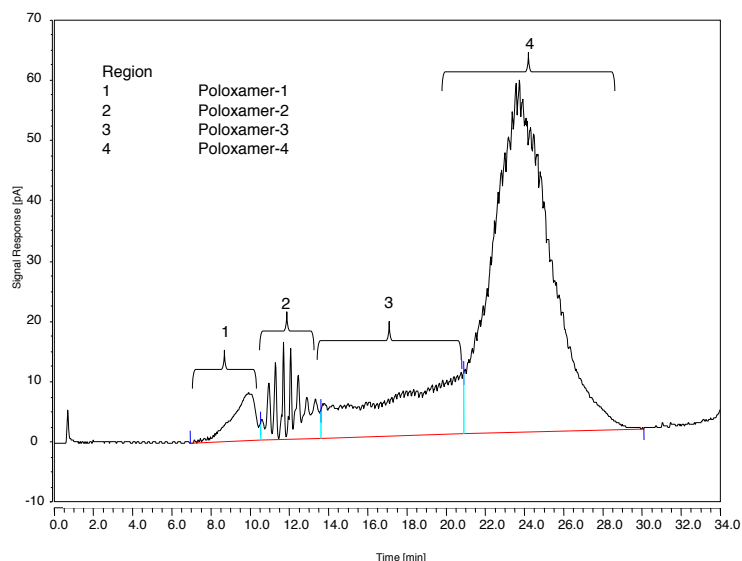FIGURE 2. Structure of poloxamer 407, where a = 2 – 130, b = 15 – 67.

FIGURE 3. HPLC-CAD chromatogram of 20 mg/mL of poloxamer 407, showing four possible retention time window assignments that can be used for comparison to other lots

These four regions of the chromatograms in Figure 3 can be used to compare one batch of Poloxamer with another, as is demonstrated with polysorbate 80, below.

Polysorbate 80/Tween 80

Polysorbates are a nonionic surfactant, based on a sorbitan with three hydrophilic polyethoxylate chains and an oleic acid, as shown in Figure 4.

The polysorbate solution was analyzed using the polysorbate gradient, which contains less water than the poloxamer gradient as part of the mobile phase. The chromatogram overlays of a reference lot of Tween 80 (MFR1_Batch1) used to compare two lots from a different manufacturer (MFR2_Batch1 and MFR2_Batch2) is shown in Figure 5, showing the peak region assignments used for further calculations.
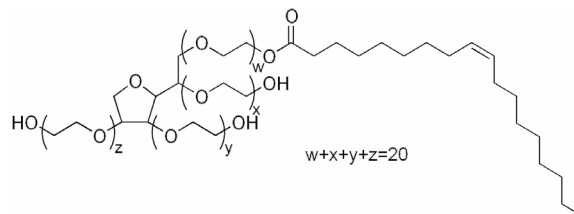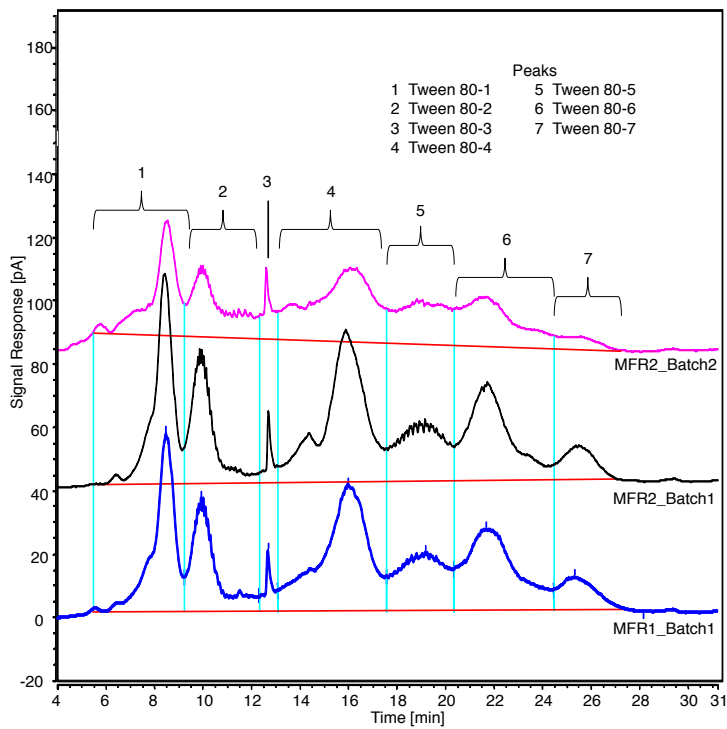

FIGURE 4. HPLC-CAD chromatogram of 20 mg/mL of polysorbate 80

FIGURE 5. HPLC-CAD chromatogram overlays of three lots of Tween 80 at 20 mg/mL, showing retention time window assignments for comparison

Table 1. Lot-to-lot comparison using sum of absolute differences between a two samples and a reference lot of Tween 80.

Peak Number	MFR_1 Peak Area - %	MFR_2_Batch1		MFR_2_Batch2	
		Peak Area - %	|Difference| Peak Area - %	Peak Area - %	|Difference| Peak Area - %
Tween 80-1	17.43	17.86	0.43	17.19	0.24
Tween 80-2	12.86	13.34	0.48	14.72	1.86
Tween 80-3	1.85	1.75	0.10	2.97	1.12
Tween 80-4	27.83	28.54	0.71	30.66	2.83
Tween 80-5	13.29	13.08	0.21	13.43	0.14
Tween 80-6	20.48	19.52	0.96	17.68	2.80
Tween 80-7	6.27	5.91	0.36	3.34	2.93
Sum of |Difference|			**3.25**		**11.92**

Conclusions

- A flexible and fast reversed phase gradient HPLC method was developed for the analysis of different polymeric surfactants for characterization using the charged aerosol detector, which provides greater sensitivity and reproducibility than ELSD and is simpler to operate in a quality control environment than MS.

- The method can be adapted for different surfactants, such as polysorbate 20, Tween 20, Brij-25, and Triton X-100, (see appslab.thermoscientific.com for further details).

- The sum of absolute differences, using relative peak area, can be used as a semi-quantitative measure for lot-to-lot variation: the smaller the value, the more similar the sample is to reference.

- This method allows for important actionable decisions concerning product quality that can be made sooner in the manufacturing process.

References

1. International Conference on Harmonisation, "Q6B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products,"(1999), Pages 3, 9, 19.

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3. Lobback, C.; Backensfeld, T.; Funke, A.; Weitschies, W. *Pharmaceutical Technology* (May 2010): 48–54.

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Similarity Calculation

To determine how similar two batches of a surfactant are, relative peak areas of the integrated regions are used to compare different lots of material. The use of peak areas themselves opens the possibility of variation from concentration differences. To determine possible lot differences percent peak areas from a reference lot is first calculated. Then percent peak area values are subtracted from a test material and added together (as absolute values) to calculate a sum of absolute differences, as indicated by the following equation.

$$\text{Sum of absolute differences} = \sum_{i=1}^{\#peaks} \left| RPA_{original,\,i} - RPA_{test,\,i} \right|$$

where RPA is the relative peak area of each component peak.

Using this equation, the resulting value will determine similarity of peak regions between different samples, and those with values closer to zero will be of increasing similarity. For the two Tween samples compared against the reference standard (blue trace in Figure 5), the sum of absolute differences were 3.25 (MFR2_Batch1) and 11.92 (MFR2_Batch2). The first batch was obtained from a different vendor and produced a small sum of absolute differences. The small difference calculated may be considered similar enough for equivalent usage. However, larger differences were found in the second sample (MFR2 Batch2), which was an older sample of Tween, indicating that this lot of material may have possibly degraded.

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