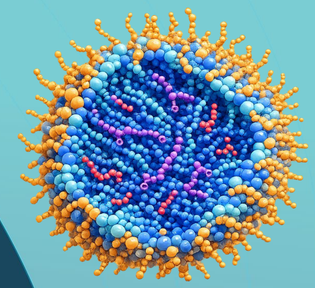
Analytical strategy to monitor surfactant integrity by LC-based methods and orthogonal particle characterization

Amber Raines[1], Georg Schuster[2], Simon Eisele[2], Daniel Demminger[2], Gökce Cagatay[2], Daniela Stadler[2], Tim Menzen[2], Andrea Hawe[2]

[1] Coriolis Pharma US Inc., 8 Davis Drive Suite 324, Durham NC 27713, U.S.A.
[2] Coriolis Pharma Research GmbH, Fraunhoferstraße 18b, 82152 Martinsried, Germany

Coriolis Pharma

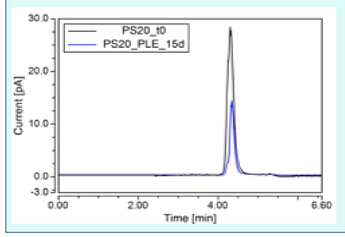


Surfactants (PS20, PS80, P188) protect protein therapeutics from interfacial stress, but their hydrolytic and oxidative degradation can lead to loss of protection, free fatty acid release, and particle formation.
Comprehensive monitoring is essential for formulation stability and root-cause investigations.

Methods: We developed versatile, tiered liquid chromatography-charged aerosol detection (LC-CAD) platform methods for monitoring surfactants which are complemented by orthogonal Micro-Flow Imaging particle characterization using:

Tier 1 (Routine): A rapid, single-peak LC-CAD method for total surfactant quantification in R&D and GMP environments.

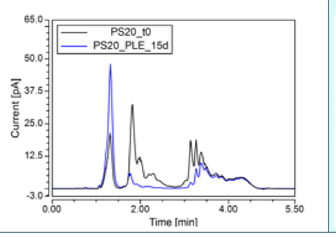
- Use tight content criteria (e.g., ≤±10%).
- No information on degradation pathway



Tier 2 (Mechanistic): Gradient LC-CAD with off-line protein removal to resolve individual surfactant species and compositional shifts during degradation.

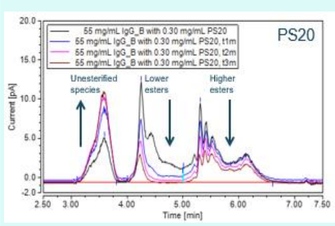
Track peak pattern with parameter sensitive to degradative changes:

- relative peak area of the unesterified species (or early eluters for P188)
- peak-to-valley ratio of monoesters (or main species for P188)



Tier 3 (High-Throughput): An automated, multi-dimensional (2D-) LC-CAD method featuring online matrix clean-up for direct, hands-free quantification in high-concentration formulations

- High throughput, quantitative, and qualitative analysis of all three surfactants (PS20, PS80 and P188) in mAb formulations with no additional sample preparation
- Fast analysis of only 8 min for PS20/PS80 and 12 min for P188
- Significantly aid formulation development and stability assessment of mAb formulations containing common non-ionic surfactants by tracking potential degradation pathways



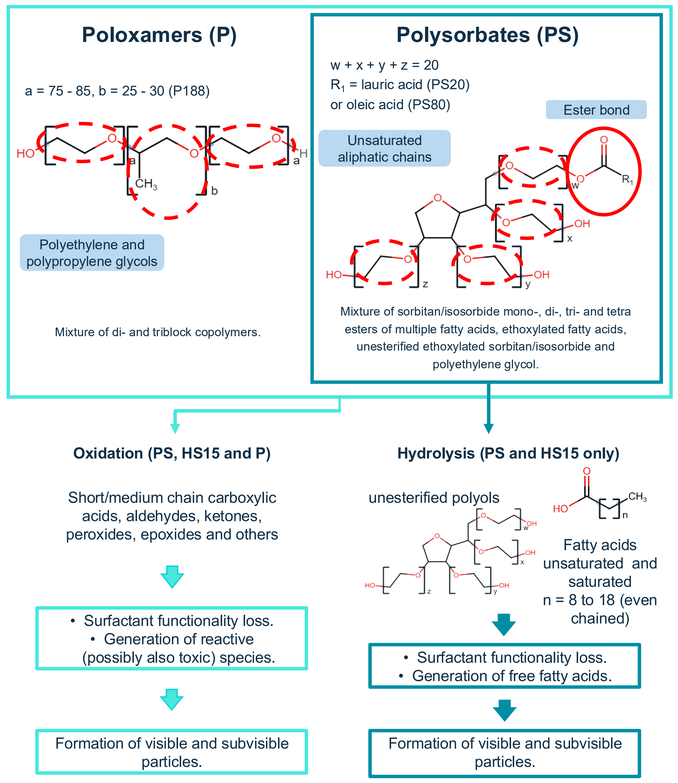
Analytical priority

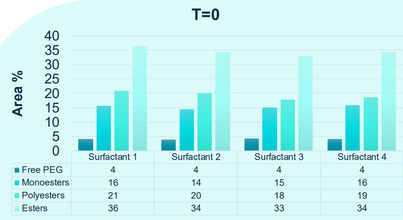
- **Simplicity** → Single peak method
- **Specificity** → LC-CAD gradient
- **Efficiency** → 2D-LC-CAD gradient

Applications

- Release testing (GMP)
- In-process controls (R&D)
- Stability studies (R&D, GMP)
- Degradation investigations (R&D)
- Formulation screenings (R&D)
- In-process controls (R&D)

Method selection is a trade-off between speed, specificity, and efficiency aligned with your application.





	Surfactant 1	Surfactant 2	Surfactant 3	Surfactant 4
Free PEG	4	4	4	4
Monoesters	16	14	15	16
Polyesters	21	20	18	19
Esters	36	34	33	34

Figure 1. Percent area of surfactant species at T=0, as determined by gradient RPHPL-CAD

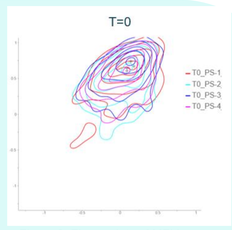
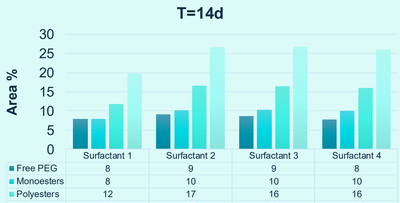


Figure 2. Fingerprints of MFI images for each surfactant at T=0

- Samples all displayed comparable profiles by both RP-HPLC-CAD and MFI + ParticleSentry^{AI} at T=0
- After enzyme degradation for 14 days, all surfactants experienced similar RP-HPLC-CAD profiles
- Only MFI + image analysis showed a distinct difference in degradation profile for Surfactant 1 compared to all other samples



	Surfactant 1	Surfactant 2	Surfactant 3	Surfactant 4
Free PEG	8	9	9	8
Monoesters	8	10	10	10
Polyesters	12	17	16	16
Esters	20	27	27	26

Figure 3. Percent area of surfactant species at T=14d, as determined by gradient RPHPLC-CAD

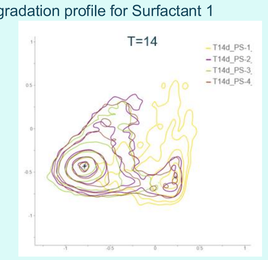
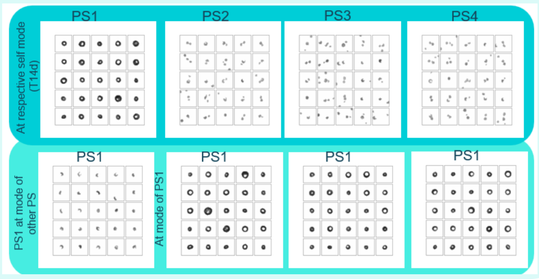
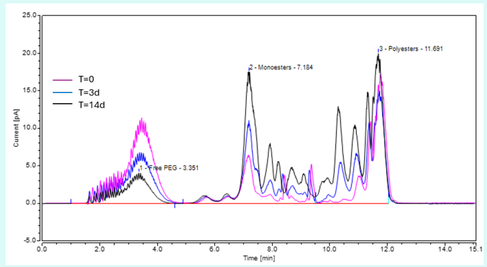


Figure 4. Fingerprints of MFI images for each surfactant at T=14d. Representative images at each surfactant mode shown below



Case Study: Four surfactants subjected to enzyme degradation for 14 days. Samples characterized by gradient RP-HPLC-CAD and MFI + ParticleSentry^{AI} (SentrySciences).



Results & Conclusion: The LC-CAD platform methods studied successfully track concentration and composition for surfactants. By correlating chromatographic profiles with subvisible and visible particle tracking, this integrated workflow establishes a robust, phase-appropriate framework. It bridges the gap between surfactant degradation and particulate risk, ultimately supporting formulation optimization, comparability studies, and lifecycle control strategies.

Martos et al. Trends on Analytical Characterization of Polysorbates and Their Degradation Products in Biopharmaceutical Formulations, J. Pharm. Sci. 2017 V106(7):1722-1735.
Zegota et al. Direct comparison of single peak and gradient chromatographic methods for routine analysis of surfactants in biopharmaceuticals, EJPS 2025 V209 107065, doi: 10.1016/j.ejps.2025.107065.

Presenter contact: amber.raines@coriolis-pharma.com

www.coriolis-pharma.com