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Surfactants in cell culture media: Impact on HEK and CHO cells in cultivation and transfection

In this work, the impact of surface active agents (surfactants) on cultivation and transfection of HEK and CHO cells is presented. Surfactants are commonly used cell culture medium components for reducing shear stress in non-static suspension culture. Despite the preferred application of poloxamer 188, there is an ongoing discussion about surfactant-related process deviations. Furthermore, surfactants have shown to interact with polyplexes as well as polymer nanoparticles within various applications (e.g. transfection, encapsulation). Better understanding the related mechanisms of action will facilitate finding alternative components for progressive cell culture media.

RESULTS

A. Impact of surfactant on growth, shear stress and transfection

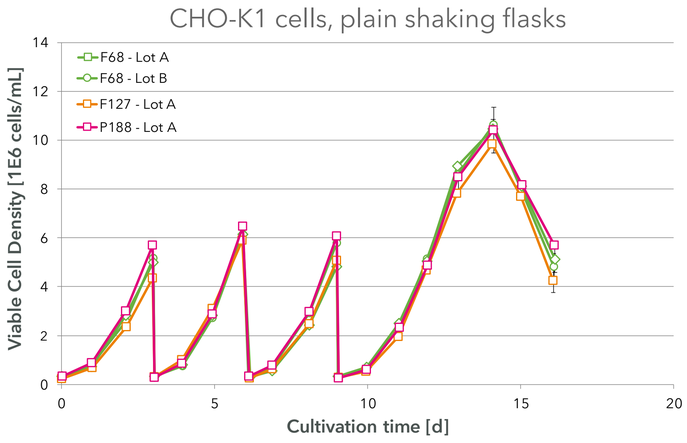


FIG. 1: Growth performance of suspension CHO-K1 cells in CHO TF medium containing poloxamers 188 (Pluronic® F-68 or Kolliphor® P188) and 407 (Pluronic® F-127) in 125 mL plain shaking flasks with 40 mL culture volume (n=3 replicates in batch phase). Under these conditions, similar viable cell densities were detected for the tested surfactants and different lots thereof.

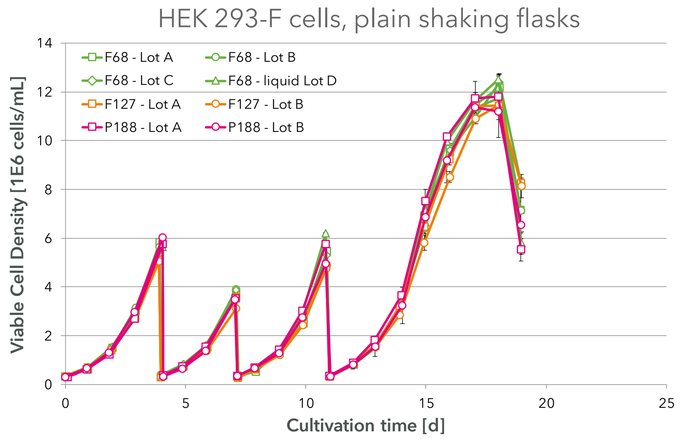


FIG. 2: Growth performance of HEK 293-F cells in HEK TF medium containing poloxamers 188 (Pluronic® F-68 or Kolliphor® P188) and 407 (Pluronic® F-127) in 125 mL plain shaking flasks with 40 mL culture volume (n=3 replicates in batch phase). Under these conditions, the tested surfactants and different lots thereof (Lot D: Pluronic® F-68 solution #P5556, Sigma-Aldrich) showed similar viable cell densities.

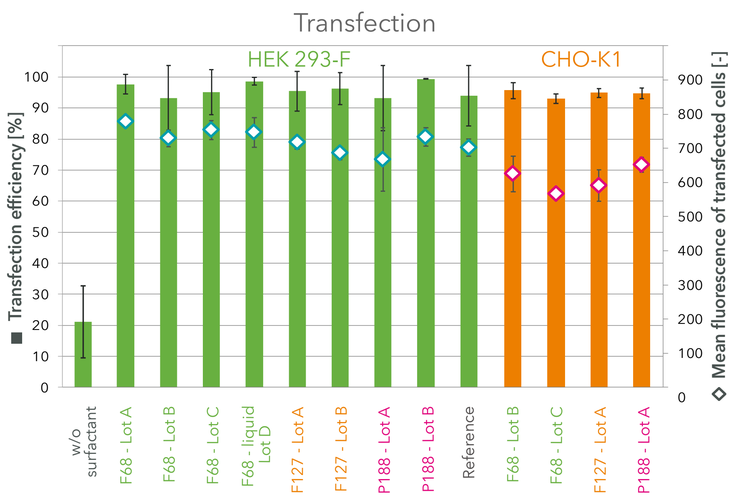


FIG. 3: Analysis of poloxamers Pluronic® F-68, F-127 and Kolliphor® P188 as well as different lots thereof regarding transfection efficiency of HEK 293-F (green) and CHO-K1 (orange) cells based on GFP expression 48 h post transfection (n=3). Additionally, the mean fluorescence of transfected cells was compared (◊). A commercially available medium suitable for transfection was used as reference. Based on these experiments no differences were detectable.

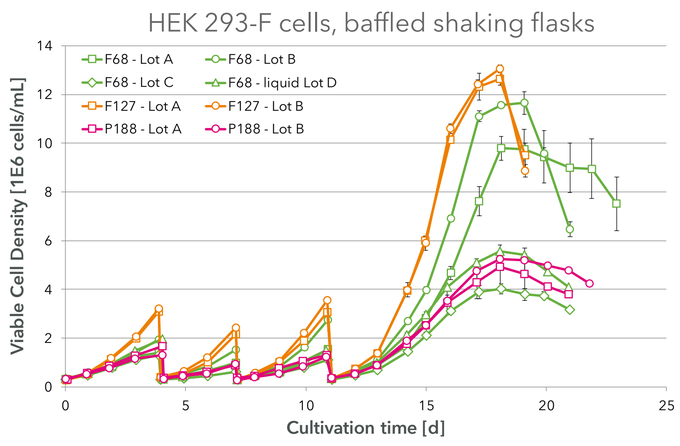


FIG. 4: Growth performance of HEK 293-F cells in 125 mL baffled shaking flasks with 40 mL culture volume (n=3 replicates in batch phase). Using these turbulent conditions, distinct differences between tested surfactants and also different lots were observed. Especially Kolliphor® P188 showed inferior performance in all examined batches. For Pluronic® F-68, a high lot-depending variation was detected.

B. Surfactant uptake and structural analysis of lot-to-lot divergence

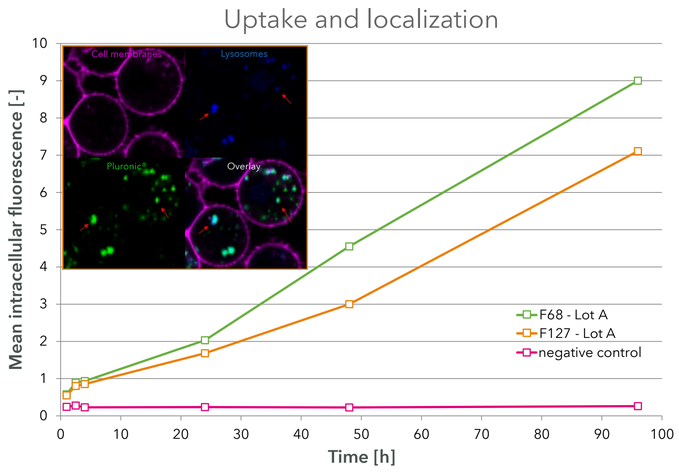
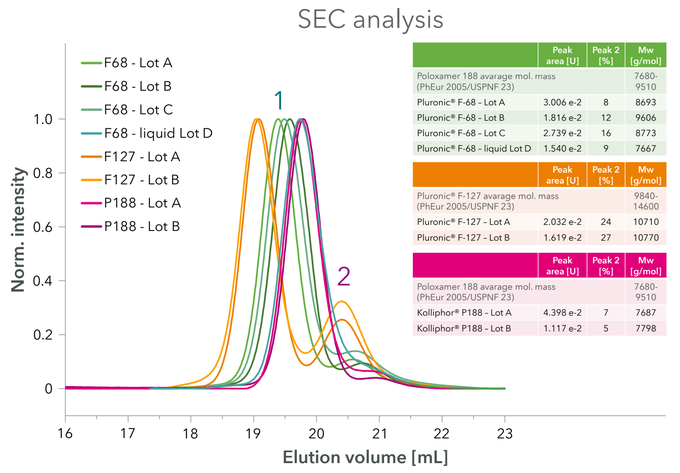


FIG. 5: Flow cytometry of fluoresceine-labeled Pluronic® into HEK 293-F cells showed a time-dependent cellular uptake of F-68 and F-127. Confocal fluorescence microscopy images revealed a co-localization of labeled Pluronic® (green) and cellular lysosomes (blue).



	Peak area [U]	Peak 2 [%]	Mw [g/mol]
Poloxamer 188 avarage mol. mass (PhEur 2005/USPNF 23)			7680-9510
Pluronic® F-68 - Lot A	3.006 e-2	8	8693
Pluronic® F-68 - Lot B	1.816 e-2	12	9606
Pluronic® F-68 - Lot C	2.739 e-2	16	8773
Pluronic® F-68 – liquid Lot D	1.540 e-2	9	7667

	Peak area [U]	Peak 2 [%]	Mw [g/mol]
Pluronic® F-127 avarage mol. mass (PhEur 2005/USPNF 23)			9840-14600
Pluronic® F-127 - Lot A	2.032 e-2	24	10710
Pluronic® F-127 - Lot B	1.619 e-2	27	10770

	Peak area [U]	Peak 2 [%]	Mw [g/mol]
Poloxamer 188 avarage mol. mass (PhEur 2005/USPNF 23)			7680-9510
Kolliphor® P188 - Lot A	4.398 e-2	7	7687
Kolliphor® P188 - Lot B	1.117 e-2	5	7798

FIG. 6: Overlay of size exclusion chromatography (SEC) chromatograms of the different surfactant lots for Pluronic® F-68 (green), Pluronic® F-127 (orange) and Kolliphor® P188 (pink). While lots of F-127 and P-188 showed specific and consistent profiles, a higher inter-lot variation in molecular weight was detected for F-68. Subsequent to a preparative SEC (data not shown), fractions from peak 1 and 2 were subjected to NMR.

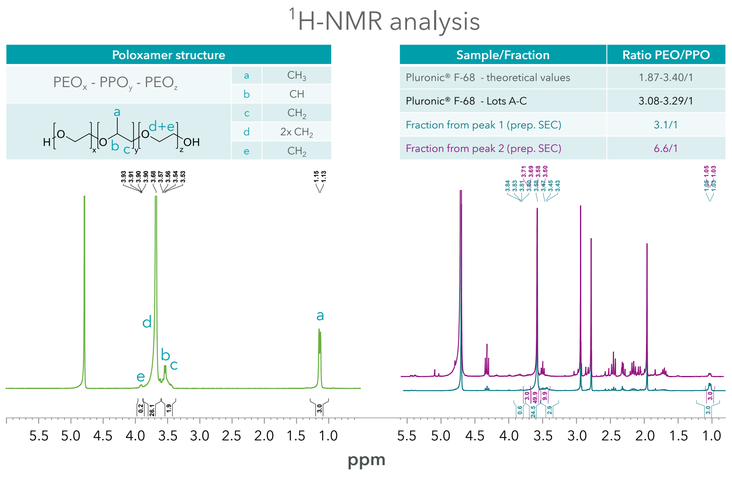


FIG. 7: ^{1}H-NMR spectra of the surfactants (exemplary data and poloxamer structure shown on left side) as well as two fractions generated from a preparative SEC (right side), which allowed calculation of ratios of PEO and PPO blocks. Results revealed that the fraction from peak 2 contained a high portion of PEO, which is likely a remnant from synthesis.

CONCLUSIONS

- Growth performance during precultures and batch curves in plain shaking flasks did not show any difference among tested surfactants or lots thereof and cell densities reached 10-12·10^6 cells/mL.
- Experiments with HEK cells at elevated power input in baffled shaking flasks revealed significant differences between Pluronic® F-68, F-127 and Kolliphor® P188, with F-127 showing the best performance and viable cell densities comparable to plain shaking flasks.
- Similar transient transfection efficiency and mean fluorescence of transfected cells independent from applied sufactant and lot indicated no major impact of respective poloxamer.
- Interestingly, experiments using fluorescein-labeled Pluronic® showed a time-dependent uptake into HEK cells.
- Co-localization of Pluronic® F-68 and lysosomes indicate an endocytic uptake of poloxamers from cell membrane.
- It remains unclear if the different effect of Pluronic® F-68, F-127 and Kolliphor® P188 on growth under high shear stress is only due to membrane protection or also due to effects occuring during and after cellular uptake.
- In this context, SEC of surfactants showed differences in low molecular weight fraction, especially for the tested lots of Pluronic® F-68. This fraction represents mostly the PEO block (revealed by NMR), a remnant from synthesis.
- Further experiments will focus on investigation of specific fractions from SEC.

METHODS

Cultivation HEK 293-F and CHO-K1 suspension cell lines were cultivated in shaking flasks or tube spin bioreactors using standard conditions (37 °C, 80 % humidity on shaking platform at 185 rpm, 5 cm orbit) in either chemically defined Xell media (HEK TF or CHO TF) or relevant reference media.

Analytics Viable cell density and viability were measured using a Cedex automated cell counter. Transfection efficiency was quantified via GFP expression and flow cytometry. Fluorescence confocal microscopy was based on fluoresceine (Pluronic®), LysoTracker® Blue DND-22 (lysosomes) and CellMaskTM Deep Red (cell membranes). SEC and NMR measurements were performed at JCSM, Jena.

Transfection Cells were transfected at densities of 3·10^6 cells/mL in Xell media with PEI-MAX (40.000 MW) and 0.67 pg pCMV-GFP DNA/cell. Culture volumes during transfections ranged from 4 mL to 10 mL in plain shake flasks and tube spin bioreactors shaken at 185 rpm.

PARTNERS & ACKNOWLEDGEMENTS

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