Endotoxin separation and detection by SEC

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Endotoxins (ET) are an integral part of the outer membrane of gram-negative bacteria and thus may appear as a contaminant in biotechnological production streams. They are pyrogenic and can cause a septic shock with a few ng introduced into the human blood stream. Thus, products for parenteral administration must be controlled for as low as < 5 endotoxin units (EU)/dose and kg body weight.[1]

ETs form aggregates of different shapes and structures in a molecular weight (MW) range of < 10 to > 6000 kDa depending on solvent, salts, detergents, chelating agents and temperature. Furthermore, the aggregation depends on the type of ET monomer, which is highly variable with an average MW of 2 kDa for rough ET mutants and up to 42’000 kDa for smooth ETs. The biological response depends on ET aggregation and can therefore be different for the same concentration of ET in different formulations.[2]

When measuring ETs with the widely used biological Limulus Amebocyte Lysate (LAL) assay, samples are usually diluted in ET free water. This is believed to ensure equal aggregation and thus comparable ET measurements. However, the LER (Low Endotoxin Recovery) effect has recently received much attention because some formulations with spiked ET standard could not be recovered with LAL.[3]

ET aggregation in different formulations is of interest for ET testing and for potential purification strategies. *E.g.* separation of ET from a target molecule by size can be assessed. Thus, new simple and fast analytical tools for this purpose are needed. Accordingly, we analyzed ETs in common buffers such as PBS with SEC-UV/CAD, techniques available in most analytical laboratories. Polymer and silica based SEC columns in the MW range from <1000 Da to 10^6 Da were used. ET aggregation was reduced from large aggregates towards intermediate MW and monomeric forms using salts, detergents, elevated temperature, sonication and different aqueous/organic mobile phases. The effects were different for ET from different bacterial strains. Time dependent aggregation could also be observed. Apparent MW estimation is difficult due to a lack of suitable MW-standards. Thus, we aimed for the development of MS based MW determination of ETs after SEC separation. SEC eluents were adapted accordingly for SEC-qTOF analysis. Furthermore, the monomer MW of ETs was determined with MALDI-TOF-MS, using different matrices and additives.

[1] https://www.fda.gov/iceci/inspections/inspectionguides/inspectiontechnicalguides/ucm072918.htm (Accessed: 10.02.2017)
[2] D. Su, R.I. Roth, M. Yoshida, J. Levin, *Infect. Immun.*, **1997**, 65, 1258-1266.
[3] https://www.outsourcedpharma.com/doc/the-great-ler-debate-0001 (Accessed: 20.01.2017)