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MULTIMODAL CHROMATOGRAPHY COMBINING STERIC EXCLUSION AND CATION EXCHANGE AS AN INTERMEDIATE DOWNSTREAM STEP TO PURIFY YELLOW FEVER VIRUS-LIKE PARTICLES

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Yellow fever (YF) is an hemorrhagic viral disease transmitted by infected mosquitoes, which is endemic in many African and Central/South American countries. The severe symptoms and the high mortality rate of the disease can have devastating effects in case an outbreak occurs in an area where the population is non-vaccinated. Before the current YF vaccine became available, outbreaks in cities like Barcelona (Spain) and Philadelphia (USA) led to the death of approximately 10% of the population. Recent outbreaks have shown that YF continues to be a major public health threat due to production capability issues and shortage of vaccine stockpiles, which even led to the use of an emergency fractional (1/5) dose in Africa in 2016 and in Brazil in 2018. Yellow fever virus-like particles (VLPs) represent an interesting alternative to develop a new YF vaccine.

With the aim of developing an efficient and affordable process to purifiy yellow fever VLPs, in this work we developed a multimodal strategy combining cation exchange (CEX) and steric exclusion chromatography (SXC) under conditions where the product of interest does not bind to the CEX adsorber, whereas many contaminants do. In this way, the product of interest is retained just due to steric exclusion by the polyethylene glycol (PEG) added to the mobile phase. Product desorption can be achieved by decreasing PEG concentration, while contaminants remain bound to the adsorber and are eluted in the regeneration step. To the best of our knowledge, the application of such a multimodal strategy has not been published before.

Cell culture supernatant containing yellow fever virus-like particles was produced by stable HEK293 cell lines constitutively expressing the VLPs. Bioreactor was operated in perfusion mode with an ATF2 as cell retention device, eliminating the need for a clarification step. Prior to the multimodal chromatography step, a capture step using a 3-mL anion-exchange (AEX) membrane adsorber (ReadyToProcess™ Adsorber Q Nano membrane, GE Healthcare, Sweden) was performed in bind-and-elute mode to concentrate the product and remove part of the contaminants. The eluate from the AEX capture step was then purified by the proposed multimodal technique, using a 75-cm^2 cation-exchange membrane adsorber (Sartobind S75, Sartorius, Germany) and 8% (m/v) of PEG 6,000 in the equilibration buffer and feed. In order to compare to the multimodal strategy, simple SXC was carried out under identical conditions, except for the stationary phase, which was a stack of disks of chromatographic paper (3mm Chr Whatman™, GE Healthcare, Sweden) packed to a total area of 76.34 cm^2 in a filter holder designed in house to provide low dead volume. The processes were evaluated for VLP recovery, purification capability, and removal of host-cell DNA and proteins. VLP was quantified by means of an ELISA assay developed in-house, total protein was analyzed using Pierce™ BCA Protein assay kit, and DNA was determined by means of the Quant-iT™ PicoGreen™ assay kit (ThermoFisher Scientific, USA).

Recovery of VLPs in both the multimodal chromatography step and the simple SXC step were above 95%. The multimodal technique was able to remove more DNA (82%) than conventional SXC (71%), whereas total protein removal was similar for the multimodal strategy (92.9%) and the simple SXC (92.5%). SDS-PAGE analysis indicated a higher degree of purity for the multimodal strategy. The overall 2-step process (Q capture followed by multimodal chromatography) provided an overall yield above 80%, with removal of 99.9% of DNA and 99.9% of total proteins. This process could serve as platform for the purification of other viral particles (whole viruses or VLPs).