CONTINUOUS VACCINE PURIFICATION UTILIZING MULTI-STAGE AQUEOUS TWO-PHASE EXTRACTION

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The COVID-19 pandemic accentuated the value of time- and cost-effective vaccine manufacturing in an effort to quickly distribute doses to patients. Continuous manufacturing could reduce the manufacturing time and footprint necessary to produce vaccines. Aqueous two-phase extraction (ATPE) provides a purification method more cost-effective and more compatible with continuous processing than traditional liquid chromatography purification. ATPE is here composed of semi-miscible polyethylene glycol (PEG) polymer and citrate salt solutions. By leveraging hydrophobic and electrostatic interactions between the viral product and the ATPE components, the partitioning behavior of the viral product can be adjusted. By changing pH, component concentrations, and volume ratios, a non-enveloped vaccine model porcine parvovirus (PPV) was partitioned initially to the polymer phase in stage one and secondly to the salt phase in stage two to facilitate purification, recovery, and processability.

During the first stage of ATPE, PPV must be partitioned to the PEG phase in order to separate it from cell culture impurities. At a neutral pH, PPV is negatively charged due to its low isoelectric point. The citrate salt solution also contains a net negative charge at this pH and exerts a repulsive force on the PPV. Meanwhile, PPV's amphiphilic nature interacts favorably with the amphiphilic PEG molecules, coaxing an average of 60% of the PPV particles into the PEG phase with removal of 95% of host cell DNA and 89% of host cell proteins. However, the PEG solution is highly viscous. PPV must be back-extracted into the salt solution to allow for further processing. This can be achieved by reversing the aforementioned conditions. By harvesting the PPV-laden PEG and introducing a more dilute citrate, the repulsive and amphiphilic forces fade to permit an average of 99% of PPV particles into the citrate phase. This process yields an average 49% recovery of PPV particles. PPV recovery may be improved by supplementing the first stage with osmolytes to further displace PPV into the PEG phase.

When adapted to process continuously, the PPV recoveries have corresponded to batch experiments. This continuous purification framework presents a strategy for more efficient vaccine manufacturing at a lower cost compared to traditional methods, which could reduce cost and increase access to patients in need of therapies.

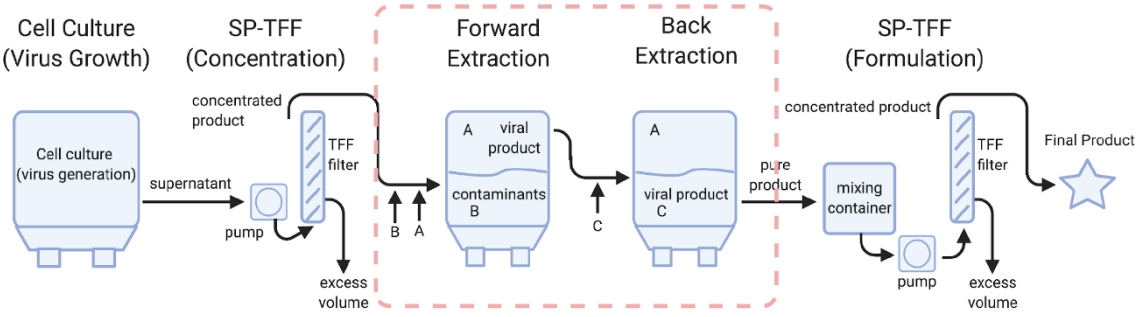

Figure 1: A continuously-processing purification system which uses multi-stage aqueous two-phase extraction to purify porcine parvovirus from cell culture impurities. Tangential flow filtration is used to concentrate the virus solution before purification and to formulate the solution after purification