POLYMER GRAFTED CHROMATOGRAPHY MEDIA FOR DIRECT CAPTURE AND HIGH-RESOLUTION PURIFICATION OF ENVELOPED VIRUS-LIKE PARTICLES

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Usually, the downstream processing of viruses and virus-like particles (VLP) does not include conventional chromatography media (beads) in the capture and/or purification steps. For large biomolecules, the binding capacity of conventional resins is limited to the outer surface of the beads. We developed a purification process based on polymer-grafted media, which allows a swift purification of HIV-1 VLP from CHO cell culture supernatant. The dynamic binding capacity is one order of magnitude lower than convective media but still in the range of 5-7x10^{11} particles/ml packed bed, which is unexpectedly high. For that reason, the binding mechanism was studied in detail. As expected, transmission electron micrographs showed that the VLPs only adsorb at the outer surface of the beads. This was corroborated by confocal microscopy using florescence labelled VLPs by incorporating cell membrane label. In batch update experiments, we observed a biphasic behavior with a fast uptake within minutes followed by a slow adsorption within hours. Desorption was also occurring very fast within minutes. Modeling linear gradient elutions with different gradient slopes showed that the number of effective charges involved in the adsorption is in the range of 30 and the adsorption is not really affected by salt. This explains why VLPs can be directly loaded from culture supernatant without further preconditioning. Scalability is not an issue, because these polymer grafted media can be packed in any scale from less than 0.5 ml to several hundred liters and in any column geometry.