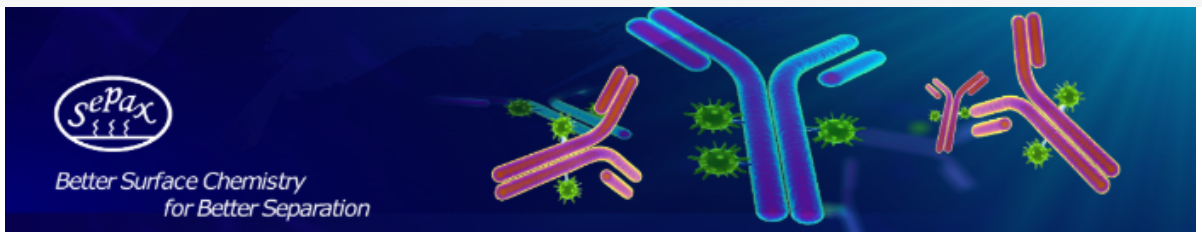

High-throughput characterization of virus-like particles by interlaced size-exclusion chromatography

with SRT SEC-1000 4.6 × 300

Interlaced SEC (iSEC) experiments were realized using the SRT SEC-1000 column at a flow rate of 0.8 mL/min. only the SRT 1000 column shows multiple peaks for the VLP samples with high peak area and recovery: The elution of B19 VP1/VP2 VLPs is split into three peak groups with two minor and one major peak, while the elution of MuPyV VLPs reveals one minor and one major peak. Peak fractionation and analysis by SDS-PAGE evidenced the presence of major viral proteins in allthree UVpeaks ofthe B19VP1/VP2 VLP sample and in the two UV peaks of MuPyV VLPs (data not shown). Moreover, the total peak areas in the SEC chromatograms generated with the SRT 1000 column are higher than in those generated with other columns. This suggests a higher recovery and less secondary interactions of VLP components with the SRT 1000 column matrix. The weaker performance of other evaluated columns was attributed to different base materials (methacrylate vs. silica) and pore sizes (450Avs. ˚ 1000A).

VLP/Virus/Vaccine on SRT SEC-1000 4.6 × 300

Virus	Family	Expression system	Recombinant protein	Diameter	References
HPV	Papillomaviridae	*S. cerevisiae*	L1 (55 kDa)	40–60 nm	[39,14]
MuPyV	Polyomaviridae	*Escherichia coli*	VP1 (42 kDa)	40–50 nm	[7]
HBV	Hepadnaviridae	*Escherichia coli*	core antigen (21 kDa)	30–34 nm	[48]
EV 71	Picornaviridae	BEVS/IC	VP1 (33 kDa), VP2 (28 kDa), VP3 (27 kDa), VP4 (8 kDa)	25–35 nm	[41,49]
Parvovirus B19	Parvoviridae	BEVS/IC	VP1 (83 kDa), VP2 (58 kDa)	25–30 nm	[50,31,32]

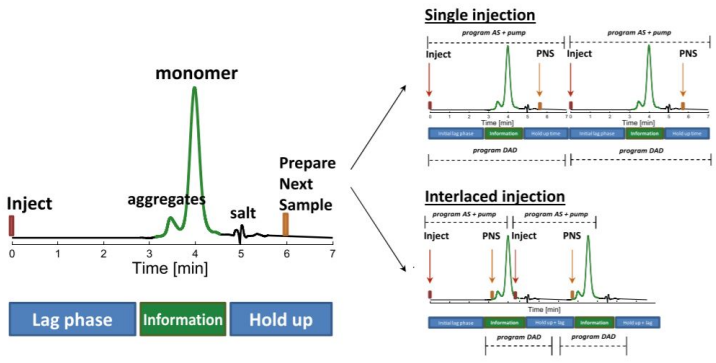

Fig. 1. Schematic illustration of SEC chromatograms for single- and interlaced-injection mode of an analyte containing aggregates and monomer. Information phases are marked by green colored bars, lag and hold-up phases by blue colored bars. Timelines for the program of the autosampler (AS) and pump and the diode array detector (DAD) are presented for two sequent injections in single- and interlaced-injection mode. (For interpretation of references to color in this figure legend, the reader is referred to the web version of the article.)

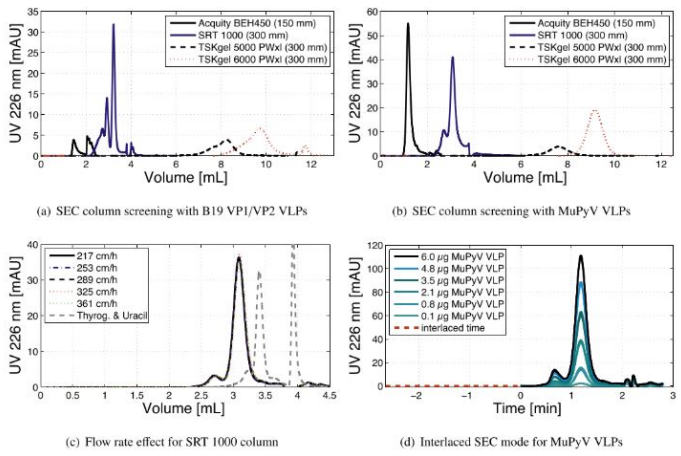

Fig. 2. Development steps of an interlaced SE-UHPLC method for VLPs. Chromatograms represent the mean of triplicate determinations. (a) Overlaid chromatograms of 8 µg human B19 parvo (B19 VP1/VP2) VLPs injected on an Acquity BEH450 (black solid line), SRT 1000 (blue solid line), TSKgel G5000 PWxl (black dotted line), and TSKgel G6000 PWxl column (red dotted line). (b) Overlaid chromatograms of 3 µg murine polyomavirus (MuPyV) VLPs injected on an Acquity BEH450 (black solid line), SRT 1000 (blue solid line), TSKgel G5000 PWxl (black dotted line) and TSKgel G6000 PWxl column (red dotted line). (c) Overlay of chromatograms of 2.1 µg MuPyV VLPs for an SRT 1000 column at varying flow rates. The elution profile of a protein standard composed of thyroglobulin and uracil is plotted as gray dotted line. (d) Chromatogram of MuPyV VLPs injected on an SRT 1000 column in interlaced-injection mode at a flow rate of 0.8 mL/min. Increasing VLP masses were loaded on the column and are plotted in triplicates. Samples were injected at −2.66 min (DAD timebase) as indicated by the red dotted line illustrating the interlaced injection time. (For interpretation of references to color in this figure legend, the reader is referred to the web version of the article.)

Column: SRT SEC-1000 4.6 × 300 (PN: 215950-4630); **Mobile Phase**: 0.2 M K2HPO4 and 0.25 M KCl pH 7 with HPV VLPs pH 7.4 for the SEC analysis of other VLPs; **Flow rate:** ; **System**: UHPLC; **Column Temperature**: ; **Detection**: ; **Sample**: protein-based VLP human B19 parvo-VLPs and murine polyoma-VLPs MuPyV, Parvovirus B19 five recombinant protein-based VLPs including human papillomavirus (HPV) VLPs, human enterovirus 71 (EV71) VLPs, and chimeric hepatitis B core antigen (HBcAg) VLPs 1) Purified HPV VLPs (HPV type 33 [29]) derived from yeast cells (Merck) at a concentration of 0.8 mg/mL in a buffer containing histidine and polysorbate 80 (pH 6.2) 2) EV71 VLPs derived from Spodoptera frugiperda Sf9 insect cells (Sentinext Therapeutics Penang, Malaysia) in a Tris buffer (pH 7.5) at a concentration of 0.1 mg/mL. 3) Chimeric HBcAg VLPs with fused tumor epitopes were expressed in E. coli and generously provided by BioNTech Protein Therapeutics Germany in a Tris buffer (pH 7.2) at a concentrationof 2.18 mg/mL. 4) B19VP1/VP2VLPs derived fromSf9 insect cells (Diarect AG Germany) at a concentration of 0.5 mg/mL in a phosphate buffer (pH 7.4) (MW: , pI: , Sample Prep:); **Injection**:

[1] Christopher Ladd Effio, Stefan A. Oelmeier, Jürgen Hubbuch, High-throughput characterization of virus-like particles by interlaced size-exclusion chromatography, Vaccine, Volume 34, Issue 10, 2016, Pages 1259-1267, ISSN 0264-410X, https://doi.org/10.1016/j.vaccine.2016.01.035.

Interlaced SEC (iSEC) experiments were realized using the SRT SEC-1000 column at a flow rate of 0.8 mL/min by shifting the ‘PrepareNextSample’ (‘PNS’) and the ‘Inject’ commands of subsequent samples to earlier points in time (Fig. 1).

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