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CY14694-23Jun20-AN

Purification of VLP-based vaccines using a new prepacked gel filtration column

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Introduction

The use of inactivated or attenuated viruses as vaccines ("whole-agent" vaccines) carries a small but significant risk of infection. An alternative strategy is to inject a fragment of a virus lacking the nucleic acid component but still containing virus proteins and maybe also the lipid bilayer. Such structures are called virus-like particles or VLPs. VLPs are able to induce a strong immune response, but they cannot replicate. Thus, there is no risk of infecting the patient by using VLPs. Furthermore, VLPs are unlikely to need adjuvants to be highly immunogenic. This makes VLPs very promising as potential biopharmaceuticals. VLPs can be produced by recombinant technology. Two VLP-based vaccines have already been approved by the FDA.

The M_r of VLPs are often above 2 000 000 Da, which makes gel filtration with Sephacryl™ S-500 High Resolution (HR) (separation range M_r between 4×10^4 and 2×10^7) suitable for fractionation and analysis of the VLP components.

This gel filtration medium fulfills the process chromatography requirements for scalability and stability.

Purification of VLP on Sephacryl S-500 High Resolution

For this study, recombinant VLP containing three strongly immunogenic proteins from influenza virus has been used. These proteins have apparent M_r of 69 000, 54 000 and 27 000 Da. Samples containing these VLPs were fractionated on a HiPrep™ 16/60 column prepacked with Sephacryl S-500 HR. Four runs with different sample load volumes (3, 5, 7, and 9% of the total column volume) were performed to find optimal loading volumes.

The results showed that the VLPs are separated from smaller sized impurities (Fig 1). Further, SDS-PAGE analysis of the pooled fractions from the main peak showed that high purity was achieved (Fig 2).

A loading volume of approximately 5% of the total column volume was found to give a suitable fractionation between VLP proteins and the impurities. At higher loads, the amount of smaller contaminants increased above the acceptance limit.

It is shown that Sephacryl S-500 HR is very useful for fractionation and quality analysis of recombinant VLPs.

Column: HiPrep 16/60 Sephacryl S-500 HR
Sample: VLP in sodium phosphate buffer with NaCl, pH 7 (previously purified on a strong anion exchange column, Capto Q)
Sample load: 3%, 5%, 7%, and 9% of total column volume (3.6 ml, 6.0 ml, 8.4 ml, and 10.8 ml)
Buffer: 25 mM sodium phosphate, 500 mM NaCl, pH 7.2
Flow rate: 1 ml/min (30 cm/h)
System: ÄKTAexplorer™ 100

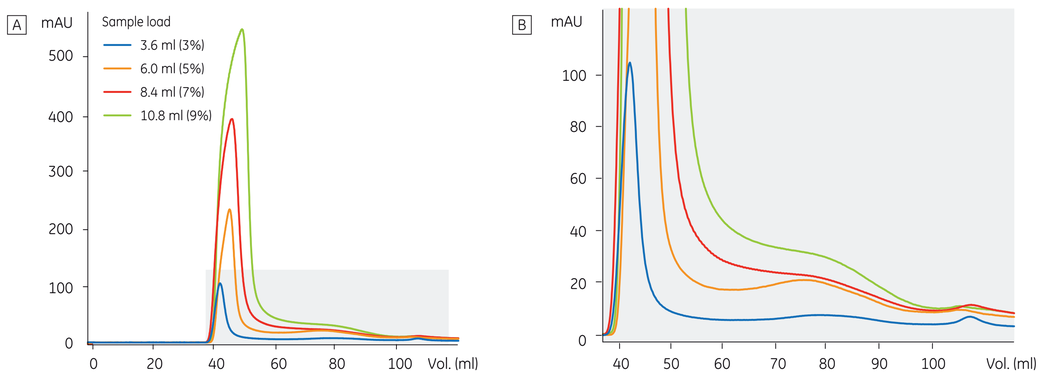


Fig 1. (A) Purification of VLP by gel filtration using HiPrep 16/60 Sephacryl S-500 HR. Various sample loads were applied to the column; (B) Enlargement of the peaks shown in (A).

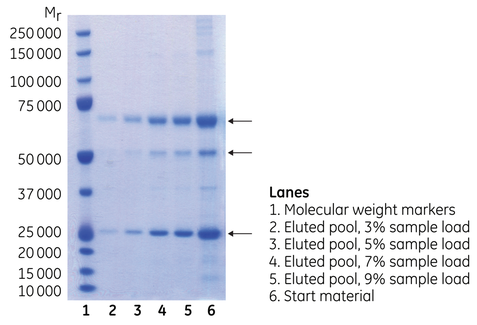


Fig 2. SDS-PAGE analysis (reducing conditions, 4-12% polyacrylamide gel, Coomassie™ stained) of pooled eluted fractions after application of VLP to a HiPrep 16/60 Sephacryl S-500 HR column. The arrows indicate the 3 proteins of the VLP at positions representing the expected molecular masses of 69 000, 54 000, and 27 000.

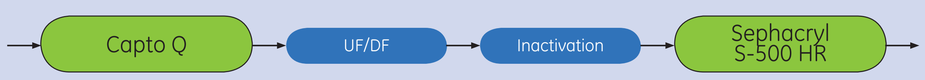
Process development

Before investing in expensive large scale purification, the process should first be developed and tested on a small scale. The small scale process must fully address all the likely performance and quality demands on the large scale process. The most important considerations include parameters such as sample load, robustness, buffer choice, throughput and scalability.

Here, the effect of sample load on the purity of VLP produced in insect cells was evaluated in small scale using a prepacked HiPrep 16/60 Sephacryl S-500 HR column.

VLP purification steps

- Harvest
- Anion exchange chromatography using Capto™ Q
- Buffer exchange and concentration using ultrafiltration/diafiltration
- Virus inactivation
- Gel filtration using Sephacryl S-500 High Resolution (HR)



Column characteristics

Column	HiPrep 16/60 Sephacryl S-500 HR
Column volume	120 ml
Matrix	Cross-linked copolymer of allyl dextran and N, N-methylenebisacrylamide
Mean particle size	47 µm
Separation range	M_r 4×10^4 to 2×10^7
Recommended flow rate	0.5 ml/min (15 cm/h)
Maximum flow rate	1 ml/min (30 cm/h)
Recommended sample volume	Up to 5 ml
Maximum pressure over packed bed	0.15 MPa

Note that larger prepacked columns with a volume of 320 ml, HiPrep 26/60 Sephacryl S-500 HR, are also available.

Conclusions

- Sephacryl S-500 HR is suitable for quality analysis of samples containing large protein structures such as VLPs or viruses
- Prepacked HiPrep 16/60 Sephacryl S-500 HR columns show good results when purifying VLPs from impurities
- Prepacked HiPrep Sephacryl HR columns are useful during the process development of large scale processes
- The results showed that sample loading volumes above 5% of the total column volume give poorer resolution

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