Application Guide

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Sartorius Ultrafiltration Products in the Preparation of Biological Nanoparticles and Medical Nanocarriers – a Short Review

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Abstract

This short review highlights ultrafiltration of various biological nanoparticles and medical nanocarriers. Here ultrafiltration is used to purify, concentrate and separate nanoparticles from substrates. The nanomaterials discussed comprise metals, polymers, lipids (in vesicles) and proteins. You will find a guidance for the selection of an ideal performing ultrafiltration device with the optimum molecular weight cut-off (MWCO) for typical concentration applications.

Introduction

Paul Ehrlich was inspired by the idea of the "magic bullet"* when he for the first time described in theory toxic drugs assembled to so-called "Nanocarriers" in 1908.[1] Today, Nanocarriers have found multiple applications in modern medicine and biotechnology. A key application for these special nanomaterials is a targeted delivery of drugs where they act as transport modules (i. e. as nanoparticles, vesicles, or micelles) for the active ingredient.[2,3,4,5] This is assumed to be more effective and less toxic to the (human) organism compared to traditionally administered drug substances.[6] Besides drug delivery, various further fields using Nanocarriers evolved during the last decades; e. g. magnetic resonance imaging or stem cell gene therapy with metal-based nanoparticles,[7,8] or optical imaging with quantum dots.[9]

Nanocarriers can be categorized by their starting material (i. e. metal-, lipid-, polymer-, and protein-based) and by their formation after preparation (i. e. vesicles, particles and micelles). In general, the preparation of a nanoparticle suspension or a vesicle dispersion in an aqueous medium consists of three steps: a) assembly of the Nanocarriers (for example by injections, film hydration, or reverse phase evaporation), b) purification (exemplary: chromatography, dialysis or ultrafiltration), and c) concentration like ultrafiltration or evaporation.

This short review provides examples of recent literature dealing with the preparation of Nanocarriers. Particular focus is laid on the concentration and purification steps which were performed via ultrafiltration with Sartorius Vivaspin® or Vivaflow® devices with different pore sizes (respectively molecular weight cut-off, MWCO). The Vivaspin® portfolio spans a volume range from 0.5 mL to up to 20 mL, whereas the Vivaflow® system covers volumes from 0.05 liters to up to 5 liters. Thus, Sartorius offers an unrivaled wide range of processable sample volumes, membrane materials and MWCOs to meet the different requirements of their intended use. Challenges in this context are buffer exchange after synthesis, desalting and washing,[10,11] exclusion of solubilized compounds,[12,13,14] or aggregates.[15]

Purification is essential to obtain isosmotic conditions for in vivo applications to prevent aggregation or agglomeration and to remove free toxic drugs, ligands, or other substrates potentially triggering side effects. Concentration steps are essential to adjust the amount of pharmaceutical active ingredient in the drug to achieve the anticipated therapeutic or diagnostic effect.

During purification, the separation of free substances (starting material) from the desired Nanocarriers via size-exclusion chromatography (SEC) leads to an unavoidable dilution and to the necessity of a subsequent concentration step. In contrast, diafiltration purifies without significant dilution but a concentration step can still be mandatory, if higher Nanocarrier concentrations are necessary. Both separation methods require a quite extensive costly and time-consuming manual handling. This drawback is overcome by the ultrafiltration utilized by centrifugation in Vivaspin® or with a peristaltic pump for the Vivaflow® System. This technique is less expensive and quickly performed with very little manual input. Noteworthy is that purification and concentration steps are performed simultaneously.[16]

After the Nanocarrier is purified the determination of drug loading (conjugation or encapsulation efficiency) is commonly performed. The conjugation or encapsulation efficiency is one of the reference values to describe and characterize Nanocarriers. Other important properties are the zeta potential and the size distribution determined via photon correlation spectroscopy (PCS), high-resolution transmission electron microscopy (HRTEM) imaging, or via dynamic light scattering (DLS). Prior to performing these different characterizations a successful purification and concentration of the suspension or dispersion is essential.

In the following table you can find an overview of publications using ultrafiltration steps for the purification and concentration of different kinds of Nanocarriers. This table will also give you a guidance on which MWCOs to use.

* In German "Zauberkugel", opera "Freischütz" by Carl Maria von Weber

Table 1 summarizes examples of Nanocarrier ultrafiltration applications with Sartorius Vivaspin® or Vivaflow®:

Nanocarrier: Nanoparticle, Vesicle, Micelle	Size distribution obtained via (HR) TEM or DLS, Z-Average via PCS and others–if reported	Application	Sartorius Ultrafiltration Device	MWCO	Ultrafiltration purpose	Ref.
Nanoparticles from metal, metal oxides and functionalized metals						
Iron oxides nanoparticles with cisplatin- bearing polymer coating	SD: 4.5 ± 0.9 nm via X-Ray-Diffraction Analysis	Magnetic resonance imaging	Vivaspin® 20	100 kDa	Purification and concentration step	7
Functionalized iron oxide nanoparticles	SD: 38 and 40 nm via DLS	Stem cell gene therapy and tracking	Vivaspin® 20	100 kDa	Washing step	8
Gold nanoparticles	SD: 0.8 – 10.4 nm via Atomic Force Microscopy	Antimicrobial activity	Vivaspin® 20	5 kDa	Purification step	17
Protein coated gold nanoparticles	SD: 15 and 80 nm via TEM	Drug delivery	Vivaspin® 6	10 kDa	Separation of Nanoparticles | Dyes and Washing	18
Functionalized gold nanoparticles	Core-SD: 2 nm via TEM	Targeted imaging tool and antigen delivery	Vivaspin®	10 kDa	Purification step	19
Functionalized gadolinium-based nanoparticles	Z-Average: 1.1 ± 0.6 nm and 4 – 14 nm	Diagnostic and therapeutic application	Vivaspin®	5 kDa, 10 kDa	Purification and Concentration	20, 21
Functionalized nanocrystals	SD: 10 to 20 nm	Quantum dots for imaging	Vivaspin®	300 kDa and 50 kDa	Separation of quantum dots-antibody conjugates from starting material (prior to enumeration)	9
Nanoparticles from polymers, functionalized polymers and polymersomes						
Polymer based Nanoparticles		Drug delivery	Vivaspin®	30 kDa	Purification and Concentration	22
Curdlan coated polymer nanoparticles	Z-Average: 280 – 480 nm depending on the composition	Macrophage stimulant activity and drug delivery	Vivaspin® 20	3 kDa	Washing	23
Docetaxel-carboxymethylcellulose Polymer Nanoparticles	Z-Average: 118 ± 1.8 nm	Anti-cancer efficacy studies	Vivaspin®	10 kDa	Concentration step	24
Functionalized Polymersomes	Z-Average: 185 nm	Surface functionalization studies	Vivaspin® 20	10 kDa	Concentration step	3
Lipid Nanoparticles and Liposomes						
Liposomes and micelles	Z-Average: 100 nm for Liposomes and 15 nm for micelles	Ischemia-reperfusion injury	Vivaspin® 20	100 kDa	Concentration step	25
Extracellular vesicles (Exosomes and microvesicles)	Exosomes: 70 – 150 nm Microvesislces: 100 – 1000 nm	Paper provides a general protocol	Vivaflow® 50R	100 kDa	Diafiltration and Concentration	26
Bacterial outer membrane vesicles	SD: 124 nm via TRPS	Tunable resistive pulse sensing (TRPS) Analysis	Vivaflow® 200	100 kDa	Buffer exchange and concentration step	27
Bacterial outer membrane vesicles		Basic research	Vivaspin® 20 and 500	100 kDa	Buffer exchange and concentration step	28
Bacterial outer membrane vesicles	SD: 95 nm	Basic research	Vivaflow® 200	100 kDa	Buffer exchange and concentration step	29
Bacterial outer membrane vesicles	SD: 50 – 150 nm via TEM	Basic research	Vivaspin®	100 kDa	Buffer exchange and concentration step	30
Liposomes		Drug delivery	Vivaspin®	100 kDa	External buffer exchange	2
Urinary exosomes	size of exosomes <100 nm	Preparation of urinary exosomes	Vivaspin® 20 and 500	100 kDa	Concentration	31
Micelles						
Micelles		Drug delivery	Vivaspin®	30 kDa	Separation of free substrate and concentration step	4
Hydrophobic drug micelles based on polymers	SD via DLS: 39 – 165 nm depending on compound in use	Drug delivery	Vivaflow®		Removal surfactant	14
Protein Nanoparticles						
Protein Nanoparticles	SD: 20 – 40 nm via DLS	Drug carrier studies	Vivaspin® 500	3 kDa	Separation of the free from the encapsulated drug (Drug binding quantification by subsequent UV | Vis analysis)	32

SD = Size distribution

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