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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 outlines 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 micelles) and proteins. Guidance is provided for selection of the ideal performing ultrafiltration devices with the optimum molecular weight cutoff (MWCO) for these typical applications and materials.

Find out more: www.sartorius.com/en/products/lab-filtration-purification/ultrafiltration-devices

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 (for example, by chromatography, dialysis or ultrafiltration), and c) concentration (for example, by 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 0.1 to 20 mL, whereas the Vivaflow® system covers volumes from 0.1 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 and achieve the anticipated therapeutic or diagnostic effect.

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

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, dialysis purifies without significant dilution but a concentration step can still be mandatory, if higher Nanocarrier concentrations are necessary. Both separation methods require quite extensive, costly and time-consuming manual handling. This drawback is overcome with 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 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 tables you can find an overview of publications using ultrafiltration steps for the purification and concentration of different kinds of Nanocarriers. Table 2 provides guidance on which devices and MWCOs to use.

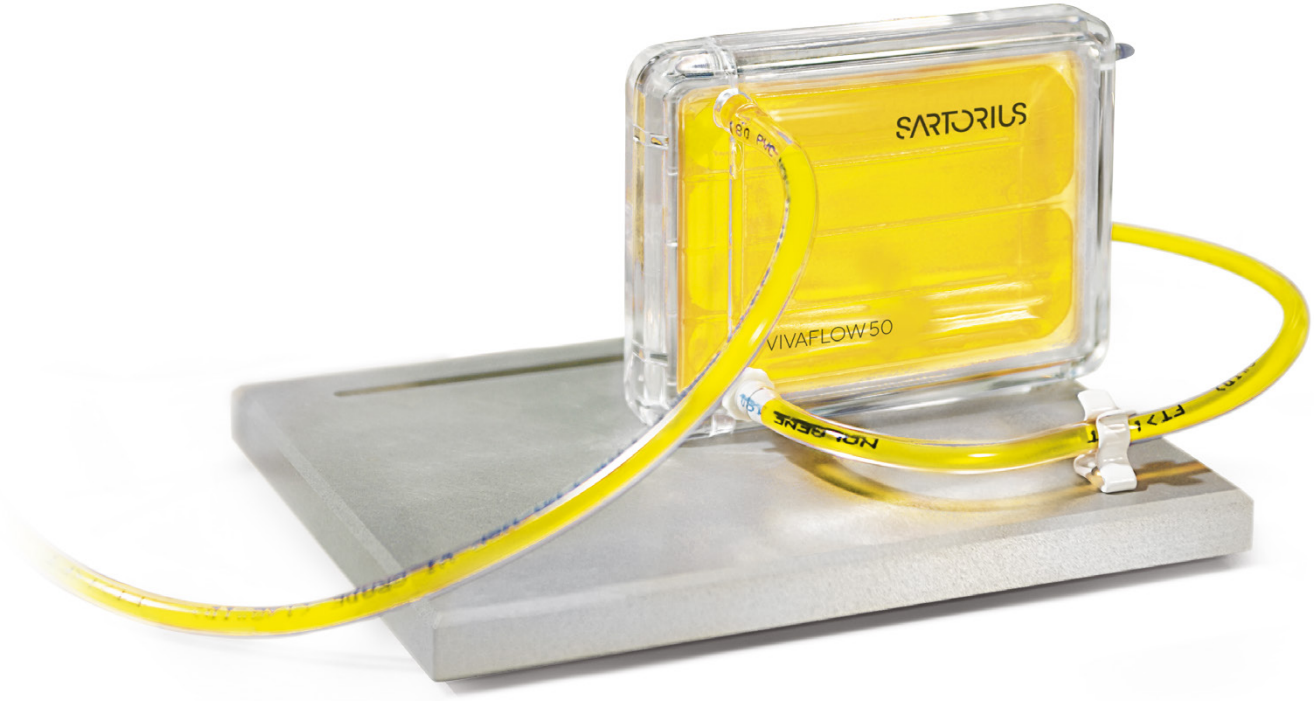

Table 1 summarizes examples of Nanocarrier ultrafiltration applications using Sartorius Vivaspin® or Vivaflow®:

Nanocarrier: Nanoparticle, Vesicle, Micelle	Size distribution obtained via (HR)TEM or DLS, Z-Average via PCS and others-if reported	Application	Ref.
Nanoparticles from metal, metal oxides and functionalized metals			
Iron oxides nanoparticles with cisplatinbearing polymer coating	SD: 4.5 ± 0.9 nm via X-Ray-Diffraction Analysis	Magnetic resonance imaging	7
Functionalized iron oxide nanoparticles	SD: 38 and 40 nm via DLS	Stem cell gene therapy and tracking	8
Gold nanoparticles	SD: 0.8 – 10.4 nm via Atomic Force Microscopy	Antimicrobial activity	17
Protein coated gold nanoparticles	SD: 15 and 80 nm via TEM	Drug delivery	18
Functionalized gold nanoparticles	Core-SD: 2 nm via TEM	Targeted imaging tool and antigen delivery	19
Functionalized gadolinium-based nanoparticles	Z-Average: 1.1 ± 0.6 nm and 4 – 14 nm	Diagnostic and therapeutic application	20, 21
Functionalized nanocrystals	10 to 20 nm	Quantum dots for imaging	9
Nanoparticles from polymers, functionalized polymers and polymersomes			
Polymer based Nanoparticles		Drug delivery	22
Curdlan coated polymer nanoparticles	Z-Average: 280 – 480 nm depending on the composition	Macrophage stimulant activity and drug delivery	23
Docetaxel-carboxymethylcellulose Polymer Nanoparticles	Z-Average: 118 ± 1.8 nm	Anti-cancer efficacy studies	4
Functionalized Polymersomes	Z-Average: 185 nm	Surface functionalization studies	3
Lipid Nanoparticles and Liposomes			
Liposomes and micelles	Z-Average: 100 nm for Liposomes and 15 nm for micelles	Ischemia-reperfusion injury	25
Solid lipid Nanoparticles	Z-Average: 100 – 120 nm depending on the used lipid	Drug delivery (Brain Targeting)	26
Bacterial outer membrane vesicles	SD: 124 nm via TRPS	Tunable resistive pulse sensing (TRPS) Analysis	27
Bacterial outer membrane vesicles		Basic research	28
Bacterial outer membrane vesicles	SD: 95 nm	Basic research	29
Bacterial outer membrane vesicles	SD: 50 – 150 nm via TEM	Basic research	30
Liposomes		Drug delivery	2
Liposomes		Encapsulated hydrophilic drugs (Drug delivery)	31
Micelles			
Micelles		Drug delivery	4
Hydrophobic drug micelles based on polymers	SD via DLS: 39 – 165 nm depending on compound in use	Drug delivery	14
Protein Nanoparticles			
Protein Nanoparticles	SD: 20 – 40 nm via DLS	Drug carrier studies	32

SD = Size distribution

Table 2 lists example Sartorius devices and typical MWCOs used for each nanocarrier ultrafiltration application.

Nanocarrier: Nanoparticle, Vesicle, Micelle	Sartorius Ultrafiltration Device	MWCO	Ultrafiltration purpose	Ref.
Nanoparticles from metal, metal oxides and functionalized metals				
Iron oxides nanoparticles with cisplatinbearing polymer coating	Vivaspin® 20	100 kDa	Purification and concentration	7
Functionalized iron oxide nanoparticles	Vivaspin® 20	100 kDa	Washing step	8
Gold nanoparticles	Vivaspin® 20	5 kDa	Purification step	17
Protein coated gold nanoparticles	Vivaspin® 6	10 kDa	Separation of Nanoparticles | Dyes and washing	18
Functionalized gold nanoparticles	Vivaspin®	10 kDa	Purification step	19
Functionalized gadolinium-based nanoparticles	Vivaspin®	5 kDa and 10 kDa	Purification and concentration	20, 21
Functionalized nanocrystals	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	Vivaspin®	30 kDa	Purification and concentration	22
Curdlan coated polymer nanoparticles	Vivaspin® 20	3 kDa	Washing	23
Docetaxel-carboxymethylcellulose Polymer Nanoparticles	Vivaspin®	10 kDa	Concentration	4
Functionalized Polymersomes	Vivaspin® 20	10 kDa	Concentration	3
Lipid Nanoparticles and Liposomes				
Liposomes and micelles	Vivaspin® 20	100 kDa	Concentration	25
Solid lipid Nanoparticles	Vivaflow® 50	100 kDa	Purification	26
Bacterial outer membrane vesicles	Vivaflow® 200	100 kDa	Buffer exchange and concentration	27
Bacterial outer membrane vesicles	Vivaspin® 500 and 20	100 kDa	Buffer exchange and concentration	28
Bacterial outer membrane vesicles	Vivaflow® 200	100 kDa	Buffer exchange and concentration	29
Bacterial outer membrane vesicles	Vivaspin®	100 kDa	Buffer exchange and concentration	30
Liposomes	Vivaspin®	100 kDa	External buffer exchange	2
Liposomes	Vivaflow® 50	100 kDa	Elimination of the free drug	31
Micelles				
Micelles	Vivaspin®	30 kDa	Separation of free substrate and concentration	4
Hydrophobic drug micelles based on polymers	Vivaflow®		Surfactant removal	14
Protein Nanoparticles				
Protein Nanoparticles	Vivaspin® 500	3 kDa	Separation of the free from the encapsulated drug (Drug binding quantification by subsequent UV-vis analysis)	32

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