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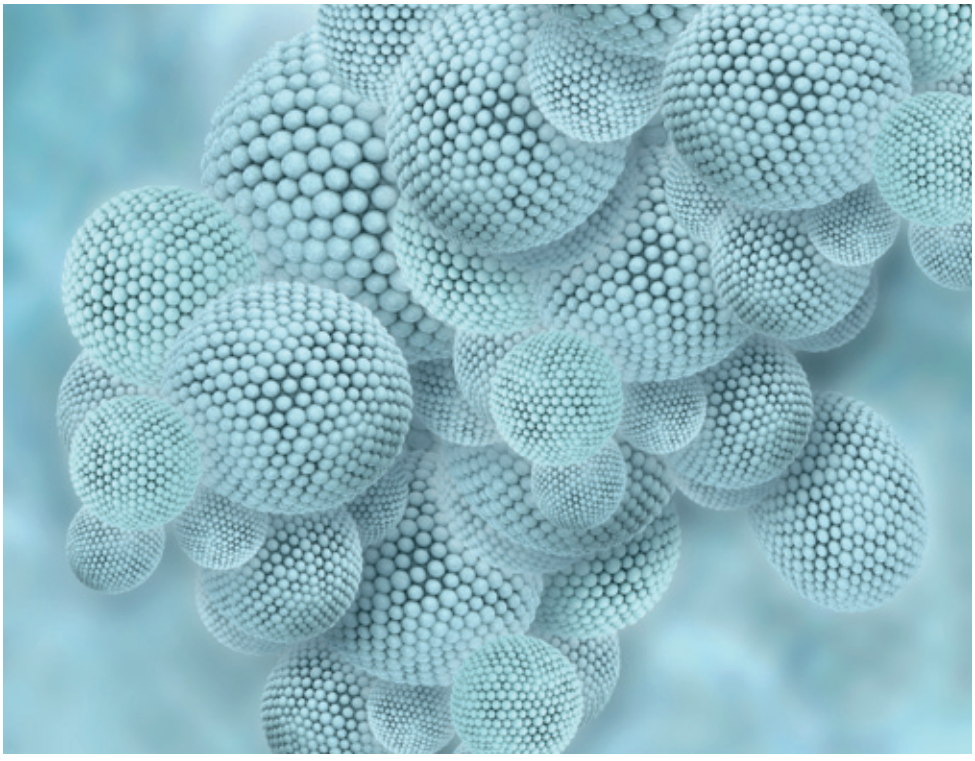
Sartorius Ultrafiltration Products in the Preparation of Biological and Medical Nanocarriers – a Short Review



Application Guide

turning science into solutions

Dr. Hannes Landmann,
Sartorius Lab Instruments,
Goettingen, Germany

Dr. Kristin Menzel,
Scientific Writer,
Goettingen, Germany

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]

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

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.

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
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
Functionalized iron oxide nanoparticles	SD: 38 and 40 nm via DLS	Stem cell gene therapy and tracking
Gold nanoparticles	SD: 0.8 – 10.4 nm via Atomic Force Microscopy	Antimicrobial activity
Protein coated gold nanoparticles	SD: 15 and 80 nm via TEM	Drug delivery
Functionalized gold nanoparticles	Core-SD: 2 nm via TEM	Targeted imaging tool and antigen delivery
Functionalized gadolinium-based nanoparticles	Z-Average: 1.1 ± 0.6 nm and 4 – 14 nm	Diagnostic and therapeutic application
Functionalized nanocrystals	SD: 10 to 20 nm	Quantum dots for imaging
Nanoparticles from polymers, functionalized polymers and polymersomes		
Polymer based Nanoparticles		Drug delivery
Curdlan coated polymer nanoparticles	Z-Average: 280 – 480 nm depending on the composition	Macrophage stimulant activity and drug delivery
Docetaxel-carboxymethylcellulose Polymer Nanoparticles	Z-Average: 118 ± 1.8 nm	Anti-cancer efficacy studies
Functionalized Polymersomes	Z-Average: 185 nm	Surface functionalization studies
Lipid Nanoparticles and Liposomes		
Liposomes and micelles	Z-Average: 100 nm for Liposomes and 15 nm for micelles	Ischemia-reperfusion injury
Solid lipid Nanoparticles	Z-Average: 100 – 120 nm depending on the used lipid	Drug delivery (Brain Targeting)
Bacterial outer membrane vesicles	SD: 124 nm via TRPS	Tunable resistive pulse sensing (TRPS) Analysis
Bacterial outer membrane vesicles		Basic research
Bacterial outer membrane vesicles	SD: 95 nm	Basic research
Bacterial outer membrane vesicles	SD: 50 – 150 nm via TEM	Basic research
Liposomes		Drug delivery
Liposomes		Encapsulated hydrophilic drugs (Drug delivery)
Micelles		
Micelles		Drug delivery
Hydrophobic drug micelles based on polymers	SD via DLS: 39 – 165 nm depending on compound in use	Drug delivery
Protein Nanoparticles		
Protein Nanoparticles	SD: 20 – 40 nm via DLS	Drug carrier studies

SD = Size distribution

Sartorius Ultrafiltration Device	MWCO	Ultrafiltration purpose	Ref.
Vivaspin® 20	100 kDa	Purification and concentration step	7
Vivaspin® 20	100 kDa	Washing step	8
Vivaspin® 20	5 kDa	Purification step	17
Vivaspin® 6	10 kDa	Separation of Nanoparticles | Dyes and Washing	18
Vivaspin®	10 kDa	Purification step	19
Vivaspin®	5 kDa, 10 kDa	Purification and Concentration	20, 21
Vivaspin®	300 kDa and 50 kDa	Separation of quantum dots-antibody conjugates from starting material (prior to enumeration)	9
Vivaspin®	30 kDa	Purification and Concentration	22
Vivaspin® 20	3 kDa	Washing	23
Vivaspin®	10 kDa	Concentration step	24
Vivaspin® 20	10 kDa	Concentration step	3
Vivaspin® 20	100 kDa	Concentration step	25
Vivaflow® 50	100 kDa	Purification step	26
Vivaflow® 200	100 kDa	Buffer exchange and concentration step	27
Vivaspin® 20 and 500	100 kDa	Buffer exchange and concentration step	28
Vivaflow® 200	100 kDa	Buffer exchange and concentration step	29
Vivaspin®	100 kDa	Buffer exchange and concentration step	30
Vivaspin®	100 kDa	External buffer exchange	2
Vivaflow® 50	100 kDa	Elimination of the free drug	31
Vivaspin®	30 kDa	Separation of free substrate and concentration step	4
Vivaflow®		Removal surfactant	14
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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Sales and Service Contacts

For further contacts, visit www.sartorius.com

Europe

Germany
Sartorius Lab Instruments
GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen

Phone +49.551.308.0
Fax +49.551.308.3289

France & Suisse Romande
Sartorius France
2, rue Antoine Laurent de Lavoisier
ZA de la Gaudrée
91410 Dourdan

Phone +33.1.70.62.50.00
Fax +33.1.64.59.76.39

Austria
Sartorius Austria GmbH
Modecenterstrasse 22
1030 Vienna

Phone +43.1.7965760.0
Fax +43.1.7965760.24

Belgium
Sartorius Belgium N.V.
Rue Colonel Bourg 105
1030 Bruxelles

Phone +32.2.756.06.90
Fax +32.2.481.84.11

Finland & Baltics
Sartorius Biohit Liquid Handling Oy
Laippatie 1
00880 Helsinki

Phone +358.9.755.951
Fax +358.9.755.95.200

Hungary
Sartorius Hungária Kft.
Kagyló u. 5.
2092 Budakeszi

Phone +3623.457.227
Fax +3623.457.147

Ireland
Sartorius Ireland Ltd.
Unit 41, The Business Centre
Stadium Business Park
Ballycoolin Road
Dublin 11

Phone +353.1.8089050
Fax +353.1.8089388

Italy
Sartorius Italy S.r.l.
Via Torino 3/5
20814 Varedo (MB)

Phone +39.0362.5557.11
Fax +39.0362.5557.99

Netherlands
Sartorius Netherlands B.V.

Phone +31.30.60.53.001
Fax +31.30.60.52.917

info.netherlands@sartorius.com

Poland
Sartorius Poland sp.z o.o.
ul. Wrzesinska 70
62-025 Kostrzyn

Phone +48.61.6473830
Fax +48.61.6473839

Russian Federation
LLC "Sartorius RUS"
Vasilyevsky Island
5th line 70, Lit. A
199178 St. Petersburg

Phone +7.812.327.53.27
Fax +7.812.327.53.23

Spain & Portugal
Sartorius Spain, S.A.
Avda. de la Industria, 32
Edificio PAYMA
28108 Alcobendas (Madrid)

Phone Spain +34.913.586.095
Phone Portugal +351.800.855.800
Fax Spain +34.913.589.623
Fax Portugal +351.800.855.799

Switzerland
Sartorius Mechatronics Switzerland AG
Ringstrasse 24a
8317 Tagelswangen (ZH)

Phone +41.44.746.50.00
Fax +41.44.746.50.50

U.K.
Sartorius UK Ltd.
Longmead Business Centre
Blenheim Road, Epsom
Surrey KT19 9QQ

Phone +44.1372.737159
Fax +44.1372.726171

Ukraine
LLS "Sartorius RUS"
Post Box 440 "B"
01001 Kiev, Ukraine

Phone +380.44.411.4918
Fax +380.50.623.3162

Americas

USA
Sartorius Corporation
5 Orville Drive, Suite 200
Bohemia, NY 11716

Phone +1.631.254.4249
Toll-free +1.800.635.2906
Fax +1.631.254.4253

Argentina
Sartorius Argentina S.A.
Int. A. Ávalos 4251
B1605ECS Munro
Buenos Aires

Phone +54.11.4721.0505
Fax +54.11.4762.2333

Brazil
Sartorius do Brasil Ltda
Avenida Senador Vergueiro 2962
São Bernardo do Campo
CEP 09600-000 - SP- Brasil

Phone +55.11.4362.8900
Fax +55.11.4362.8901

Canada
Sartorius Canada Inc
1173 North Service Road West, D4
Oakville, ON L6M 2V9

Phone +1.905.569.7977
Toll-Free +1.800.668.4234
Fax +1.905.569.7021

Mexico
Sartorius de México, S.A. de C.V.
Libramiento Norte de Tepotzotlan s/n,
Colonia Barrio Tlacateco,
Municipio de Tepotzotlan,
Estado de México,
C.P. 54605

Phone +52.55.5562.1102
Fax +52.55.5562.2942

leadsmex@sartorius.com

Peru
Sartorius Peru S.A.C.
Avenue Alberto del Campo 411
Floor 12 – The Office
15076 – San Isidro, Lima

Phone +51.1.441 0158
Fax +51.1.422 6100

Asia | Pacific

Australia
Sartorius Australia Pty. Ltd.
Unit 5, 7-11 Rodeo Drive
Dandenong South Vic 3175

Phone +61.3.8762.1800
Fax +61.3.8762.1828

China
Sartorius (Shanghai) Trading Co., Ltd.
3rd Floor, North Wing, Tower 1
No. 4560 Jinke Road
Zhangjiang Hi-Tech Park
Pudong District
Shanghai 201210, P.R. China

Phone +86.21.6878.2300
Fax +86.21.6878.2882

Hong Kong
Sartorius Hong Kong Ltd.
Unit 1012, Lu Plaza
2 Wing Yip Street
Kwun Tong
Kowloon, Hong Kong

Phone +852.2774.2678
Fax +852.2766.3526

India
Sartorius Weighing India Pvt. Ltd.
#69/2-69/3, NH 48, Jakkasandra,
Nelamangala Tq
562 123 Bangalore, India

Phone +91.80.4350.5250
Fax +91.80.4350.5253

Japan
Sartorius Japan K.K.
4th Fl., Daiwa Shinagawa North Bldg.
8-11, Kita-Shinagawa 1-chome
Shinagawa-ku, Tokyo, 140-0001 Japan

Phone +81.3.3740.5408
Fax +81.3.3740.5406

Malaysia
Sartorius Malaysia Sdn. Bhd
Lot L3-E-3B, Enterprise 4
Technology Park Malaysia
Bukit Jalil
57000 Kuala Lumpur, Malaysia

Phone +60.3.8996.0622
Fax +60.3.8996.0755

Singapore
Sartorius Singapore Pte. Ltd
10 Science Park Rd
The Alpha #02-13/14
Singapore Science Park II
Singapore 117684

Phone +65.6872.3966
Fax +65.6778.2494

South Korea
Sartorius Korea Ltd.
8th Floor, Solid Space B/D,
PanGyoYeok-Ro 220, BunDang-Gu
SeongNam-Si, GyeongGi-Do, 463-400

Phone +82.31.622.5700
Fax +82.31.622.5799

Thailand
Sartorius (Thailand) Co. Ltd.
129 Rama 9 Road,
Huaykwang
Bangkok 10310

Phone +66.2643.8361-6
Fax +66.2643.8367

www.sartorius.com

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