Separation and Purification Technology 158 (2016) 171–182

Contents lists available at ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur

Membrane-based purification of proteins from nanoparticle dispersions: Influences of membrane type and ultrafiltration conditions

Nkem Alele, Mathias Ulbricht *

Lehrstuhl für Technische Chemie II, Universität Duisburg-Essen, 45117 Essen, Germany

ARTICLE INFO

Article history:
Received 11 September 2015
Received in revised form 23 November 2015
Accepted 23 November 2015
Available online 2 December 2015

Keywords:
Nanoparticle dispersions
Silica nanoparticles
Protein
Purification
Ultrafiltration
Diafiltration

ABSTRACT

The combination of nanoparticles with proteins to form functional hybrid systems is receiving undiminished attention because of its many biotechnological and medical applications. The separation of these hybrid materials from unbound free biomolecules has posed difficult challenges to fractionation and purification. Here, a model study has been carried out by removing proteins (bovine serum albumin (BSA) or lysozyme (LYS)) from the dispersion mixtures with silica nanoparticles (nominal size 20 nm) using ultrafiltration (UF) membranes. Regenerated cellulose (RC) and polyethersulfone (PES) membranes with nominal molecular weight cut-off (NMWCO) of 100 kDa, and a PES UF membrane (NMWCO 300 kDa) functionalized with UV-grafted amphoteric polymer hydrogel layer consisting of *N*-[3-(dimethylamino)propyl]-acrylamide (DMAPAA) and 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) and having an experimentally determined cut-off of 180 kDa (identical with the experimental data for PES 100 kDa) were studied. Membrane properties and filtration conditions, in particular pH value and flux, were selected or adapted based on data for single component feeds to achieve maximum protein transmission, complete silica retention and, hence, maximum silica/protein selectivity. Batch dead-end and continuous diafiltration processes were used for fractionation and purification. Overall, the performance of PES UF membranes was inferior compared to the other membranes because of too strong fouling. With membrane RC 100, the transmission data of LYS and BSA from the mixture with silica were 80% and 30%, respectively. With the hydrogel-functionalized PES membrane, the respective transmissions from the mixture were ~35% and ~15% for LYS and BSA, respectively. In both cases, quantitative rejection of silica could be achieved. Using continuous diafiltration, membrane RC 100 had better purification efficiency, removing a total of 91% of LYS using 6 diavolumes (DV) in 2.4 h and 84% of BSA using 10 DV in 5.5 h. With the hydrogel-functionalized PES membrane, 82% of LYS and 74% of BSA were removed using 6 and 10 DV within larger time, i.e. 4.0 and 6.8 h, respectively. Importantly, the retained silica nanoparticles remained stable in the dispersion, without any indication of aggregation. Overall, this study will add valuable knowledge to the most efficient use of ultrafiltration sieving properties for the removal or purification of proteins from systems comprising other colloidal particles having a size which is larger by a factor of only 3–10.

1. Introduction

Membrane based separation processes such as ultrafiltration (UF) and microfiltration (MF) are established in various applications, including water and wastewater treatment, purifications in biotechnological and pharmaceutical industries, food and beverage processing, and medical applications [1–4]. Membranes have traditionally been used for size-based separations with high-throughput but relatively low-resolution requirements [5]. The most common applications of UF in biotechnology downstream processing are protein concentration, buffer exchange and desalting, virus removal and product clarification [6]. Current research and development efforts are directed towards large improvements in selectivity while maintaining the inherent high-throughput characteristics of membranes. Conflicting with those increasing demands are problems like concentration polarization and fouling which cause significant loss in performance with respect to flux and selectivity. This is critical if membrane processes are to satisfy the new purification process and economic challenges, especially in biotechnology applications.

On the other hand, nanotechnology is presently attracting rapidly growing interest. The combination of metal or metal oxide

* Corresponding author.
E-mail address: mathias.ulbricht@uni-essen.de (M. Ulbricht).

http://dx.doi.org/10.1016/j.seppur.2015.11.031
1383-5866/

nanoparticles (NP) with biomolecules to form bioconjugated systems is of great relevance for many applications, including drug delivery, target-specific therapy, as well as bio-imaging and sensing for diagnostic and therapeutic purposes [7]. However, purification of these NP-bioconjugates from free ligands such as proteins still remains a challenge to achieve reproducible and well controlled performance in the intended applications. Furthermore, depending on the synthesis route for NP production, potential impurities which may be harmful must also be removed from the final product. These impurities include organic solvents, surfactants, emulsifiers or stabilizers, monomer residues, polymerization initiators, large polymer aggregates and salts [8]. There have been various approaches employed for the purification of nanoparticles in general. Traditional techniques such as ultracentrifugation, extraction, size exclusion chromatography and dialysis are still being used for NP purifications which comprise ligand exchange, and many washing steps. These separation methods are limited by many draw backs that include: low capacity, highly time consuming, product loss by non-specific binding, tedious recovery and large amounts of required solvents [9,10]. Earlier studies have investigated the use of cross-flow MF, UF and diafiltration for the purification of nanoparticle suspensions [8,11–14]. Dalwadi et al. have compared diafiltration and tangential flow filtration and shown that it is possible to remove surplus surfactant to achieve stable nanoparticle dispersions [15]. Most recently, Zhao et al. have also demonstrated the use of stimuli-responsive membranes for the separation of BSA and polystyrene nanoparticles [16]. While most of these studies where done with the purpose to improve non-uniform size distribution of NP, or to remove unwanted impurities from NP synthesis/production processes, there was a single work devoted to the purification of proteins from polymeric NP (cf. above), and no work has been done to purify biomolecules (e.g. BSA) from metal oxide nanoparticle dispersions.

In this study, the sieving properties of UF membranes were employed to remove proteins (BSA and LYS) from colloidal silica nanoparticle dispersions. The purification concept is shown in Fig. 1. In this model study, RC and PES UF membranes with different NMWCO were used. To investigate an alternative to possible chemical instability problems associated with RC membranes and fouling problems with PES membranes, a hydrogel composite PES UF membrane was prepared using UV-initiated graft copolymerization ("grafting-from"; analogous to previous published work [17]), here yielding an amphoteric polymer hydrogel layer consisting of DMAPAA and AMPS. This work will expand the knowledge about the efficient use of polymeric UF membranes in solving separation challenges posed in the purification of unbound biomolecules from bioconjugated metal nanoparticle dispersions.

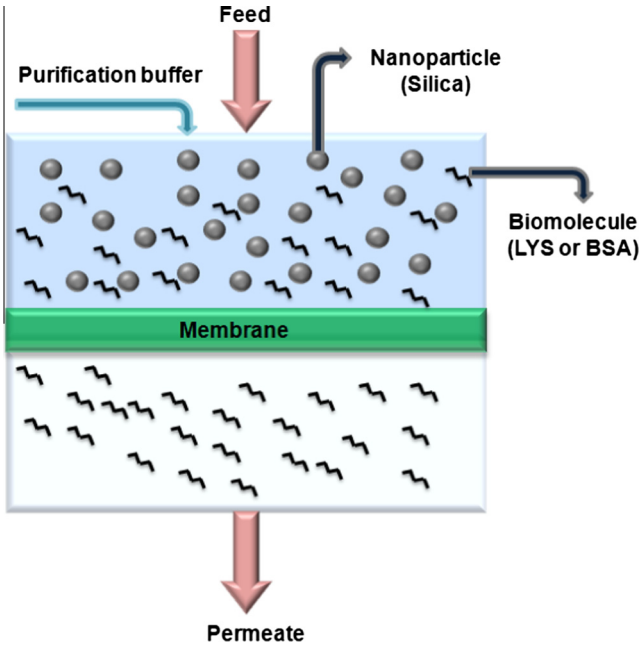

Fig. 1. Schematic concept for the purification of metal oxide nanoparticle dispersion by removal of proteins through the membrane.

2. Experimental section

2.1. Materials

Commercial RC and PES UF membranes with NMWCO of 100 kDa, donated by Sartorius-Stedim (Göttingen, Germany), were used for the filtration studies. In addition, a PES membrane from Sartorius-Stedim with NMWCO of 300 kDa was functionalized with a grafted amphoteric polymeric hydrogel and used for performance comparison. *N*-[3-(Dimethylamino)propyl]-acrylamide (DMAPAA) from Tokyo Chemical Industry Co., Ltd., Tokyo, Japan, and 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS) from Sigma–Aldrich Co., St. Louis, USA, were used as hydrogel monomers. Analytical reagent grade (99.99%) ethanol (Fisher Scientific, UK) was used for washing of membranes before use. Sodium azide (NaN_3) used for membrane storage was purchased from Sigma–Aldrich. In order to determine the actual molecular weight cut-off, dextrans with molecular weights of 10, 40, 70 and 500 kDa were obtained from Pharmacia Fine Chemical AB, Uppsala, Sweden, while dextrans with molecular weights of 4 and 2000 kDa were procured from Serva Feinbiochemica GmbH & Co (Heidelberg, Germany) and Sigma–Aldrich, respectively. The chemicals used during zeta potential measurement include hydrochloric acid (HCl), potassium chloride (KCl) and potassium hydroxide (KOH), all from Bernd Kraft GmbH, Duisburg, Germany. Buffer chemicals purchased for this work include sodium hydroxide (NaOH) from Bernd Kraft GmbH, potassium dihydrogen phosphate (KH_2PO_4) from AppliChem GmbH, Darmstadt, Germany, and disodium hydrogen phosphate (sodium phosphate, dibasic, 99+ %, anhydrous, Na_2HPO_4) from Acros Organics, New Jersey, USA. The model proteins, BSA and LYS, were obtained from GERBU Biotechnik GmbH, Heidelberg, Germany, and Sigma–Aldrich, respectively. Ludox HS-50, the colloidal silica nanoparticles were purchased from Sigma–Aldrich Co. Milli-Q quality ($\geqslant 18.2\ M\Omega\ cm^{-1}$) water, produced with a system from Millipore, USA, was used in all experiments. The buffers used for all ultrafiltration and diafiltration experiments had been made up from Na_2HPO_4/KH_2PO_4 (20 mM phosphate; pH 8) and $NaOH/Na_2HPO_4$ (20 mM mM phosphate; pH 12).

2.2. Membrane pre-treatment and membrane surface functionalization

The membranes were cut in circular discs of required size with separation area of 4.16 cm^2. The samples were pre-treated with pure ethanol by placing them in a beaker and on a shaker for one hour, with 150 rpm at room temperature. This was done to remove impurities left from the manufacturing process or additives used for stabilization. Thereafter, the membranes were rinsed three times and washed overnight with Milli-Q water and stored in aqueous 0.01 M NaN_3 before use. Freshly pre-treated membranes were used in all experiments unless indicated otherwise. Following the same pre-treatment as outlined above, PES 300 kDa membranes were photo-grafted using the UVACUBE 2000 (Dr. Hönle AG, Gräfelfing, Germany) equipped with a high-pressure mercury lamp as source of radiation emitting wavelengths above 300 nm.