Separation and Purification Technology 157 (2016) 120–130

Contents lists available at ScienceDirect

Separation and Purification Technology

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

Ultrafiltration membrane-based purification of bioconjugated gold nanoparticle dispersions

Nkem Alele [a,c], René Streubel [b,c], Lisa Gamrad [b,c], Stephan Barcikowski [b,c], Mathias Ulbricht [a,c,*]

[a] Lehrstuhl für Technische Chemie II, Universität Duisburg-Essen, Essen, Germany
[b] Lehrstuhl für Technische Chemie I, Universität Duisburg-Essen, Essen, Germany
[c] Center for Nanointegration Duisburg-Essen (CENIDE), Duisburg, Germany

ARTICLE INFO

Article history:
Received 18 September 2015
Received in revised form 25 November 2015
Accepted 25 November 2015
Available online 26 November 2015

Keywords:
Ultrafiltration
Regenerated cellulose membrane
Nanoparticle
Bioconjugate
Colloid purification
Conjugation efficiency
Supernatant removal

ABSTRACT

Functionalization of nanoparticles (NP) with biomolecules to form bioconjugated systems has received large attention in biomedical applications. However, purification of these nanoparticle bioconjugates from unbound free biofunctional ligands (e.g., peptides) remains a significant challenge in the production of well-defined materials. The conventional separation methods often compromise the product's properties and recovery. In this work, removal of excess of unbound peptides after the bioconjugation step to yield functionalized gold nanoparticles (AuNP) was achieved by exploiting the sieving properties of commercial regenerated cellulose (RC) ultrafiltration (UF) membranes. The RC membrane with nominal molecular weight cut-off (NMWCO) of 30 kDa precisely fractionated the mixtures and purified gold nanoparticle–peptide bioconjugates in a pressure driven semi-continuous diafiltration process. The RC 30 kDa membrane showed absolute rejection of the bioconjugated AuNP and the recovery of AuNP–peptide bioconjugate in the retentate was >87% relative to the initial amount in the mixture. In addition, the separation efficiency and throughput results were much better compared to the centrifugal membrane filtration method using an analogous membrane. All results indicate that by choice of an appropriate membrane type and barrier pore size, and with optimized solution chemistry and filtration parameters, ultrafiltration membranes, and in particular RC membranes, can be very well suited for the purification of bioconjugated nanoparticle dispersions, and the diafiltration mode is very well suited for upscaling.

1. Introduction

Several decades of research and development in nanotechnology have led to many promising applications of nanomaterials, especially in the field of biomedicine. Some tailored biotechnological and medical applications of nanoparticles include: biosensors, targeted drug delivery to specific cells, new cancer therapy and hyperthermia treatments, magnetic resonance imaging, single cell studies, cell manipulation, and novel diagnostic tools for early stage detection of diseases [1–4]. Noble metal nanoparticles are of particular interest for bioapplication, being approved by drug administration and bearing unique photonic properties such as local surface plasmon resonance [5,6] relevant for bioimaging [7,8].

The demand to functionalize nanoparticles with biomolecules to form bioconjugated systems has continued to receive attention [9]. The functionalization of AuNP with biomolecules produces nanoscale bioconjugate systems that have improved performance concerning their properties as each component impacts the hybrid material with a unique feature. For example, the biological molecule provides the biorecognition and targeting while the NP may allow the system to be used for *in vivo* electromagnetic contrast [8,10–12].

However, purification of colloidal solutions remains a significant challenge in the production of well-defined materials for fundamental studies and new applications. Primary requirements are a uniform particle size distribution and a high degree of purity. This is pertinent because unbound ligands can compromise the specificity of bioassays and multivalent bioconjugation cannot be controlled without proper ligand removal. Also, high purity helps to gain systematic insight into structure–function relationships. In addition, there is an increasing concern about NP impurities, especially for chemically synthesized nanomaterials. These stabilization agents are required for the synthesis of the nanoparticles but are frequently classified as toxic additives; an example is cetyltrimethylammonium bromide (CTAB) [13]. Removal of excess of these reducing agents or organic peptide linkers are essential to

* Corresponding author at: Lehrstuhl für Technische Chemie II, Universität Duisburg-Essen, D-45117 Essen, Germany.
E-mail address: mathias.ulbricht@uni-essen.de (M. Ulbricht).

http://dx.doi.org/10.1016/j.seppur.2015.11.033
1383-5866/

avoid post-synthesis particle ripening (compromising size quality and colloidal stability), and unintended reducing potential provided by these residual reactants. An alternative is to employ totally ligand-free nanoparticles, allowing bioconjugation of the naked gold surface with precise sub-monolayer dosing of the ligand, omitting use of any reducing agent or excess of ligand [14]. However, this physical synthesis method is still new so that ligand excess or ligand/reducing agent exchange is still the standard synthesis method approach to bioconjugated AuNP.

Hence, the purification of the NP bioconjugate, in particular removal of reducing agent or polypeptide linkers, is essential for achieving reproducibility and well controlled performance in the intended nanoparticle application and, particularly, to rule out cross-effects in biological studies. Traditional techniques such as extraction, chromatography and electrophoresis are used for particle purification toward a functional dispersion, which comprises also ligand exchange, many washing steps, and centrifugation. These traditional separation methods are highly time consuming and limited by many drawbacks which include a low capacity, risk of particle aggregation and precipitation, loss by non-specific binding, tedious recovery and large amounts of required solvents [10].

Permeation-selective membranes have been successfully and commercially applied in a large diversity of separation processes, including bioseparations in which traditional separation methods are less convenient, undesirable or even non-applicable. Previous reports have demonstrated that membrane filtration could be used as a purification process for removing excessive surfactants in formulating stable nanoparticle dispersions and also as size fractionation/purification technique for nanoparticle dispersions in water [15–18]. In addition, macromolecule-formulated nanoparticles (PEGylated or drug loaded NP) had also been purified from surfactants (polyvinylalcohol, sodium cholate) using tangential flow filtration and diafiltration centrifugal device [19,20]. In a very recent work, the separation of model colloids, i.e. silica nanoparticles and proteins, by ultrafiltration including diafiltration mode had been studied with focus on the influences of membrane type, solution/dispersion conditions and process parameters. It had been demonstrated that quantitative separations can be achieved with low-fouling membranes, e.g. those made from regenerated cellulose, and carefully controlling pH value, salt concentration and transmembrane flux [21].

In this work, the use of polymeric membranes for the removal of unbound excessive peptides from mixtures with gold nanoparticle–peptide bioconjugates in a pressure driven process is presented. Since the purification task is separating components of different sizes, selection has been narrowed down to porous polymeric membranes whose dominant separation mechanism is based on pore size and solute size differences. The focus here was on identifying a suitable UF membrane, and the best suitable conditions for the purification of the AuNP–bioconjugate. Based on results of an earlier own study with model colloids [21], commercial polymeric UF membranes made from regenerated cellulose (RC) had been chosen. In a dead-end filtration set-up, ultrafiltration was used for flux and rejection measurements, and the concept of discontinuous or semi-continuous diafiltration operation mode was employed to reach the actual purification objectives.

To achieve clear results, a model system based on a laser synthesis process to produce nanoparticles possessing a ligand-free and bare gold surface was employed. For efficient use of this bare surface small nanoparticles (high surface to volume ratio) were used as building blocks, and the peptide was added in high excess to the nanoparticles representing standard synthesis protocols in a controlled manner; and the AuNP had before been formed and dispersed simultaneously by the laser ablation process [22]. This ensures formation of a monolayer of peptides on the surface of the nanoparticles [23] which simulates a high dose of functionalization in a small space. To the best of our knowledge, this is the first systematic study to expand the use of polymeric UF membranes to the purification of bioconjugated noble metal nanoparticle dispersions by removal of unbound biological ligands. Subsequent comparison with standard centrifugal membrane filtration process shall give quantitative performance indicators for the AuNP-polypeptide model system purification method. The gold nanoparticle properties and the pressure-driven UF and diafiltration concept are shown in Fig. 1.

2. Experimental section

2.1. Materials

Bulk gold (99.99%) was purchased from Agosi, Allgemeine Gold- und Silberscheideanstalt AG. Sodium phosphate buffer (NaPB), at a pH of 8, composed of sodium dihydrogen phosphate (NaH_2PO_4, >99% purity, Merck) and disodium hydrogen phosphate (Na_2HPO_4, Ph. Eur., Fluka), was used for the stabilization of gold nanoparticles (AuNP). The peptide dodeca (glutamic acid) was terminated at one end by cysteine for defined chemisorption via gold-thiol bond and

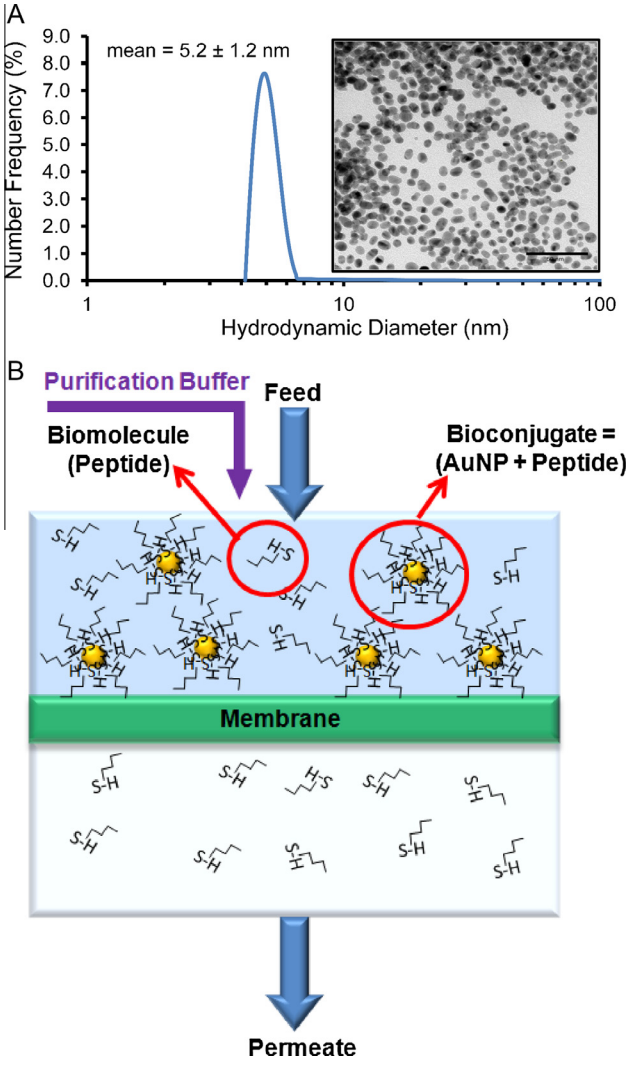

Fig. 1. (A) Number distribution of monodisperse and monomodal laser-generated ligand free gold nanoparticles measured by analytical disk centrifuge (ADC); insert shows a TEM picture of these nanoparticles (scale bar 50 nm); (B) purification concept showing the use of ultrafiltration membranes with semi-continuous diafiltration to purify gold nanoparticle–biomolecule conjugates from unbound excess peptide ligands.