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Controlling the polymer-nanolayer architecture on anion-exchange membrane adsorbers via surface-initiated atom transfer radical polymerization

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ABSTRACT

The immobilization of a polymer-nanolayer containing ligand sites is a widely used approach to increase the binding capacity of membrane adsorbers. In this work strong anion-exchange membrane adsorbers were produced via surface-initiated atom transfer radical polymerization (SI-ATRP) using a monomer bearing a quaternary amine group (Q-type). Additionally the architecture of the polymer-nanolayer has been controlled with respect to the length and density of the grafted polymer chains and in terms of ligand density and interchain crosslinking degree. The influence of these architecture parameters on the membrane permeability and the static binding capacity towards bovine serum albumin (BSA) as a model protein has been investigated. It could be shown that these parameters have a major impact on the performance of the produced membrane adsorbers. While the chain-length and –density significantly increase the binding capacity, a decrease in permeability is observed. The interchain crosslinking degree and a reduction of the ligand density increase the permeability, but simultaneously the static binding capacity is slightly diminished. A well-chosen combination of these architecture parameters can produce membrane adsorbers with static binding capacities >100 mg/mL membrane volume (MV) while still maintaining a specific permeability >40 mL/(min·cm^2·bar), far superior to commercially available products.

1. Introduction

Membrane chromatography offers a promising alternative to classic *packed bed* chromatography for the purification of biopharmaceutical molecules. Its feasibility on an industrial scale has been demonstrated in many cases, while ion-exchange membrane chromatography holds the biggest market share [1]. Compared to *packed bed* chromatographic media, membrane adsorbers often show a lower binding capacity towards the target molecules [2]. Therefore many attempts have been made to increase the economic efficiency by increasing the volumetric binding capacity [3–7].

Grafting polymer chains on the surface of a chromatographic support matrix is a commonly used approach to increase the binding capacity towards various target molecules. This polymer-nanolayer with adsorptive ligand sites extends into the surrounding solution and forms a three dimensional network allowing for a multilayer adsorption of the target molecules [8]. Both chromatographic beads [9,10] and macroporous membranes [11–13] have been modified this way using various synthetic strategies. Due to the often lower specific surface area of macroporous membranes, compared to chromatographic beads, a multilayer adsorption is far more essential for membranes to achieve higher binding capacities. By applying photo-grafting techniques on polypropylene microfiltration membranes Ulbricht et al. demonstrated in various studies that the hydrogel architecture can be sufficiently controlled and has a significant influence on the binding capacity and permeability of the resulting membrane adsorbers [11,14–16]. This makes the investigation of surface modification techniques, next to the membrane base material, pore size and porosity, an important field of study, if one tends to achieve a competitive industrial application of membrane adsorbers.

Since the first investigations of Husson et al. [3] many studies have shown that SI-ATRP is a versatile tool to modify macroporous membranes [3,17,18] and generate membrane adsorbers with high static binding capacities and different ligand chemistry [5,6,19]. In a two-step process an initiator is first anchored on the membrane surface from which polymer chains are grown afterwards. Due to the controlled nature of ATRP, this approach is highly suitable for the tailoring of hydrogel architectures. Wei et al. [13,20] produced strong cation-exchange and weak anion-exchange membrane adsorbers using this technique and demonstrated a positive relation between grafting degree/polymer chain length and static binding capacity. In parallel a significant decrease of the permeability could be observed. Bhut et al. [8]

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introduced an additional architecture parameter by varying the polymer chain density via the initiator density on the membrane surface, additionally to the chain length. It could be demonstrated that higher chain densities lead to higher binding capacities. The influence on the membrane permeability, however, has not been investigated.

Thus, the aim of this study was to establish a protocol using a modern SI-ATRP approach to prepare membrane adsorbers based on a regenerated crosslinked cellulose support with well-defined hydrogel architectures using 2-(methacryloyloxy)ethyl trimethylammonium chloride (METAC) as the ligand bearing monomer. In addition to the variation of chain length and chain density two more architecture parameters have been varied by the addition of suitable monomers. A surface-initiated copolymerization of METAC and *N*,*N*-methylenebis(acrylamide) (MBAm) led to crosslinked hydrogel structures, while a surface-initiated copolymerization of METAC and 2-hydroxyethyl methacrylate could be used to adjust the ligand density via the monomer ratio. The influence of all the parameters on binding capacity and permeability has been investigated and suitable combinations of these parameters have been identified to produce membrane adsorbers with properties superior to commercially available products. Furthermore, a novel method for the quantification of surface bound initiator molecules is presented.

2. Experimental

2.1. Materials

Regenerated crosslinked cellulose (RC) membranes with a diameter of 47 mm, an average pore size of 3–5 μm, a specific surface area of 1.1 m^2/g (Gemini V – Surface Area and Pore Size Analyzer, Micromeritics) and a thickness of 250 μm have been kindly donated by Sartorius Stedim Biotech GmbH (Göttingen, Germany). 2-Bromoisobutyrylbromide (2-BiBB, purity ≥97%, Alfa Aesar), copper(I)bromide (CuBr, purity ≥98%, Sigma Aldrich), copper(II)bromide ($CuBr_2$, purity >99%, Sigma Aldrich), 2,2′-bipyridine (bipy, purity ≥98%, Alfa Aesar), dichloromethane (DCM, anhydrous ≥99,8%, Sigma Aldrich), 2-(methacryloyloxy)ethyl trimethylammonium chloride (METAC, 80 wt% in water, Sigma Aldrich), methyl methacrylate (MMA, purity >99%, Sigma Aldrich), *N*,*N*-methylenebis(acrylamide) (MBAm, purity ≥99%, Sigma Aldrich), 2-hydroxyethyl methacrylate (HEMA, purity >97%, Sigma Aldrich), bovine serum albumin (BSA, lyophilized powder, purity ≥96%, Sigma Aldrich), 2-propanol (IPA, purity ≥99,5%, Sigma Aldrich), hydrochloric acid (HCl, ACS reagent, 37%, Sigma Aldrich), sodium chloride (NaCl, purity ≥99.5%, Sigma Aldrich), tris(hydroxymethyl) aminomethane (Tris–base, purity ≥99%, Sigma Aldrich). Monomers were freed from the inhibitor by passing over a column of neutral aluminum oxide. All other chemicals were used as received. Ultrapure (UP) water has been produced by an arium®pro ultra-pure water system (Sartorius Stedim Biotech GmbH, Göttingen, Germany).

2.2. Preparation of strong anion-exchange membranes

The two-step modification process is shown in Fig. 1. RC membranes were first washed with copious amounts of water and acetone to remove impurities. Prior to use the membranes have been dried for 6 h at 50 °C.

The initiator immobilization has been achieved by soaking the membrane round blanks in a solution of DCM and 0.01–5 vol% 2-BiBB, depending on the designated initiator density. The soaked membrane samples have been incubated for 5 min at room temperature before stopping the reaction by immersing them in 2-propanol and shaking for 30 min. Afterwards the membrane samples were washed with copious amounts of water and acetone, dried and weighed.

The SI-ATRP has been carried out based on a slightly modified protocol as suggested by Bhut et al. [8]. Briefly, in a typical experiment 50 mL of a solution compromised of 50 vol% 2-propanol and 50 vol% UP-water containing 2 M of the desired monomer composition (METAC, HEMA, MBAm) is deoxygenated by purging with N_2 for 30 min before CuBr (20 mg, 140 μmol, 1.0 eq.), $CuBr_2$ (5.0 mg, 23 μmol, 0.15 eq.) and bipy (75 mg, 475 mmol, 3.5 eq.) are added. The solution has been sonicated for 15 min under constant N_2 purging until a homogenous brown solution is formed. Here, the values of mass and volume are given per membrane sample, along with the final solution concentrations. To increase measurement accuracy, the batch volumes for the membrane activation and polymerization solutions were scaled-up to allow modification of 4 membranes at a time. The membrane samples were immersed in the polymerization solution at 50 °C under oxygen-free conditions for a specific amount of time to control the chain length. The polymerization has been stopped by exposing the catalyst to air. The membrane samples were afterwards washed in copious amounts of water, 2-propanol and acetone to remove trace amounts of copper and non-covalently bound polymer. The resulting samples have been dried and weighed.

2.3. Physicochemical characterization of the membrane adsorbers

2.3.1. Determination of initiator density

To determine the amount of initiator immobilized on the membrane surface a protocol has been developed based on previous works in the field of bromide quantification [21,22].

In a first step a previously weighed membrane sample has been cut in small pieces and mixed with 20 mL of a 2 M aqueous NaOH solution. The suspension has been stirred for 40 min at 50 °C to ensure a complete dehalogenation of the surface bound initiator (Fig. 2). After cooling to room temperature 10 mL of the resulting solution have been mixed with 25 μL of a 0.1 M aqueous NaOH solution containing 0.06 wt% phenol red. In addition a 1.5 M aqueous H_2SO_4 solution has been added until the color shift to yellow could be observed. UP-water was added until a total volume of 20 mL was reached (solution 1). From this worked up solution 5 mL have been withdrawn and mixed with 1.25 mL of a

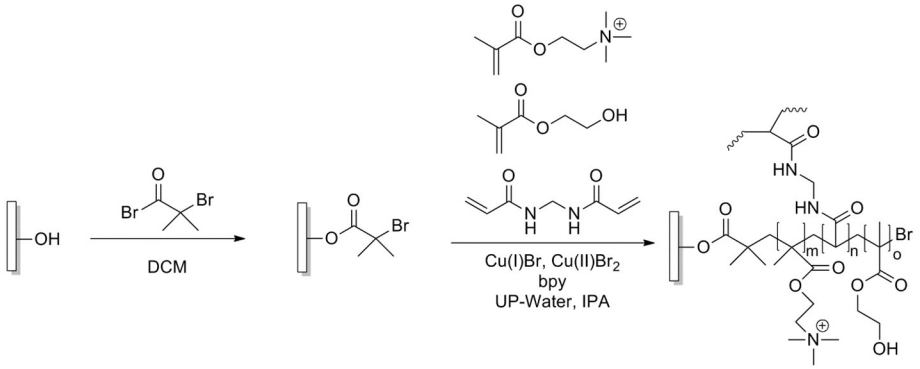

Fig. 1. Preparation of strong anion-exchange membranes via SI-ATRP using various monomer compositions.