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Preparation and characterization of grafted cellulosic fibers and their applications in protein purification

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ABSTRACT

Fibrous adsorbents were subjected either to chemical treatment or gamma irradiation to introduce epoxide groups onto their cellulosic backbone using glycidyl methacrylate. These epoxide moieties were modified to have diethylaminoethanol (DEAE) as well as quaternary ammonium (Q) functionalities. The resulting anion-exchange adsorbents were characterized by their FTIR spectra and ionic capacities. The fiber-based adsorbent systems showed similar packing efficiency to the commercially available adsorbents, where the Péclet number values were $\geqslant 60$, suggesting near-plug-flow conditions. The total ionic capacities obtained for these chemically grafted adsorbents were *ca.* 400 mmol/L. These adsorbents showed dynamic binding capacities (DBC) of *ca.* 48 mg/mL for bovine serum albumin (BSA). Protein binding capacities obtained from chemical grafting initiation techniques were 18-fold-higher than radiation-induced techniques. The advantage of these adsorbents lies in their high operational flow rates while maintaining their high binding capacities.

1. Introduction

Most downstream bioprocesses include several chromatographic steps amongst other traditional purification steps in order to produce highly purified bioproducts like plasmid DNA, virus-like particles, and monoclonal antibodies. With recent advancements in technology and increased demand for therapeutic biomolecules, the market value of the biopharmaceutical industry is expected to reach nearly $166 billion USD by 2017 [1]. Consequently, there is an enormous demand for developing innovative and cost-effective bioprocessing techniques for reducing production costs, which mostly lie in downstream bioprocessing (up to 80% of the entire production cost) [2–4]. Ion-exchange chromatography is mostly preferred for downstream bioprocessing and porous resins with anion-exchange functionalities (DEAE and Q) have been extensively used for the separation and purification of biomolecules [5–9]. However, separation of biomolecules using these adsorbents is often hampered by restricted flow rates and low operating pressures [10]. Fiber-based materials show excellent physico-chemical properties and offer several advantages, including large surface areas, high swelling capacities, mechanically robustness, and convenient usage [11]. These materials have been extensively used for the removal of metal cations [12], extraction and separation of analytes from aqueous media [13,14], and also for capturing the trace elements [15,16]. Recently, Gavara et al. [17] successfully demonstrated the potential use of cation-exchange fiber-based material for the separation and purification of biomolecules. In the current study, we examine the synthesis and performance of anion-exchange fiber-based variants. These adsorbents are easy to functionalize and offer very flexible operational conditions. Furthermore, their scale-up has previously been demonstrated up to the pilot scale without any pressure drop or bed compression issues [9,17].

Various kind of grafting techniques, like chemical, photo-initiation [18] and gamma irradiation grafting [19,20], have been used for introducing epoxy groups onto cellulosic substrates [21,22]. Graft polymerization of glycidyl methacrylate (GMA) through the chemical-initiation techniques onto substrates like nylon [23], acrylic [18], gelatin [24], polypropylene, poly (alginic acid), and cotton fabric has been reported for improving their properties for different applications [25]. In the present study, the cellulosic fiber backbone was graft-polymerized with GMA, both chemically as

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well as by gamma-irradiation. The free epoxy groups were further modified to have Q and DEAE functional moieties. The physico-chemical characteristics of these adsorbents were evaluated in terms of grafting percentages, swelling, porosity, as well as ionic capacity. The binding capacities of these adsorbents were assessed using BSA as a model protein.

2. Material and methods

2.1. Chemicals and reagents

Glycidyl methacrylate (GMA), diethylamine (DEA 99.5%), dimethylamine (40%), diethyl sulfate, ammonium cerium (IV) nitrate (CAN), *N*,*N*-dimethylacrylamide (DMA), and bovine serum albumin (Fraction *V* > 96%; Molecular Weight: 66.5 kDa; isoelectric point: 4.7) were purchased from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). Acetone, acetonitrile, ammonium persulfate (APS), sodium dihydrogen phosphate, di-sodium hydrogen phosphate, sodium chloride (NaCl), sodium hydroxide (NaOH), and nitric acid (HNO_3, 65%) were from AppliChem GmbH (Darmstadt, Germany). Tris (99.3%, buffer grade) and nitromethane were purchased from Carl Roth (Karlsruhe, Germany). Alexa Fluor 488-labeled BSA was purchased from Molecular Probes, inc (Eugene, OR, USA). Ethanol absolute was purchased from Honeywell specialty chemicals Seelze GmbH (Seelze, Germany). Natural cotton was purchased from Gebrüder Otto GmbH & Co. Kg (Dietenheim).

All the buffer solutions were filtered with 0.45 μm filters (Sartorius, Goettingen, Germany).

2.2. Instrumentation

An ÄKTA explorer 100 system running on Unicorn 4.10 software and Tricorn chromatography column 5/50 (5 mm internal diameter (ID) × 55 mm length), were purchased from GE Amersham Bioscience (Uppsala, Sweden). Finite bath adsorption capacity was determined by measuring the absorbance at 280 nm using a Shimadzu UV-1700 PharmaSpec spectrophotometer. The fibrous adsorbents were scanned (averaged over 32 scans) from 4000 cm^{-1} to 500 cm^{-1} and analyzed with IRsolution Shimadzu 1.50 software (Shimadzu Corporation, Kyoto, Japan). Confocal microscopy images were taken using a Carl Zeiss LS510 laser scanning microscope (Jena, Germany; software version 3.0).

2.3. Adsorbent grafting and functionalization

2.3.1. Pre-treatment of the fibers (Mercerization)

The fibers were pre-treated with 30% (w/v) NaOH. The mercerization treatment was modified from the method as described in Rousselle et al. [26]. The fiber-containing solution (13 mL/g of fiber) was placed in a water bath at a constant temperature for 1.5 h. The treated fibers were washed with copious amounts of water followed by 0.1 M acetic acid, and once again with copious amount of water until neutral pH was achieved. The fibers were dried and used for further functionalization.

2.3.2. Chemical grafting (CG)

A 0.5 g of dried pretreated cotton was soaked in 33.75 mL of nitrogen-purged water containing 2.5 mL of GMA in 0.1 M nitric acid and 50 mg of CAN as an initiator for 3 h at 40 °C. The grafted material was washed with copious amount of water until neutral pH was achieved and then dried at 50 °C [27,28]. The concentration of the CAN (2–6 mM) was varied to achieve a different grafting percentage (12%, 45%, 100% and 112%) by this method.

2.3.3. Gamma irradiation (GIR) induced grafting

0.5 g of dried pretreated cotton was enclosed with 50 mL of nitrogen-purged grafting solution containing 3.2% (v/v) GMA and 7.6% (v/v) DMA in 1:1 v/v ethanol/water in a falcon tube. Gamma-irradiation was carried out at room temperature with a 10 kGy dose of gamma rays (Beta Gamma Service GmbH and Co. KG, Wiehl, Germany). After irradiation, the epoxy-grafted fibers were washed with 50% ethanol (v/v) and then with absolute ethanol and then dried at 50 °C [29].

2.3.4. Surface functionalization

Epoxide-functionalized fibrous adsorbents (0.5 g) were immersed in a 20 mL solution containing 5 mL each of diethylamine and dimethylamine and 10 mL of ethanol at 40 °C for 15 h under constant shaking to prepare weak anion-exchangers functionalized with DEAE. Subsequently, the adsorbent material was extensively washed with copious amount of water until neutral pH was achieved and then vacuum dried at 50 °C in an oven [30].

These DEAE-functionalized fibers were further alkylated into quaternary ammonium functionality using 4 mL of diethyl sulfate in 20 mL of nitromethane at 40 °C for 15 h under constant shaking [31]. Finally, the functionalized adsorbent was flushed with acetonitrile and water until a neutral pH was obtained and then dried at 50 °C in a vacuum oven.

2.3.5. Physico-chemical and functional characterization

The chemical changes involved in the backbone of dried fibrous adsorbents at each stage of functionalization were evaluated using IR Spectroscopy. The DEAE-fibers were investigated by confocal laser scanning microscopy (CLSM). Briefly, a known amount of the CG-DEAE fiber sample was incubated with Alexa Fluor 488-labeled BSA in 0.1 M phosphate buffer, pH 7.4 for 2 h at room temperature on a shaker. After incubation, the fiber samples were thoroughly washed with 0.1 M phosphate buffer, pH 7.4 and then observed directly by confocal microscopy. The obtained images were collected using the laser excitation sources at 488 nm. The percentage degree of grafting (%DG) was evaluated from the difference in weight before (W_0) and after (W_1) grafting [27] as shown in Eq. (1).

$$\%\text{DG } (g/g) = \frac{(W_1 - W_0)}{W_0} \times 100 \quad (1)$$

Additionally, the degree of swelling (DS) for functionalized fibrous adsorbents was evaluated by immersing them in water for an hour and then weighing them in their wet (m_{wet}) and dried (m_{dry}) form till constant weight was achieved at 50 °C under vacuum. DS was measured using Eq. (2) [17].

$$\text{DS } (g/g) = \frac{(m_{wet} - m_{dry})}{m_{dry}} \quad (2)$$

Finally, the porosity of fibrous adsorbents was estimated using Eq. (3) [17], where 1 g of adsorbent was immersed in deionized water for an hour. The swollen weight of fibers was denoted as $m_{swollen}$. The excess water present in the pores of the fibers was removed by squeezing the fibers and denoted as $m_{squeezed}$.

$$\text{Porosity } (\%) = \frac{(m_{swollen} - m_{squeezed})}{m_{swollen}} \times 100 \quad (3)$$

Finite bath adsorption measurements were performed using 10 mg/mL of BSA as a model protein. The fibrous adsorbents were first equilibrated with 20 mM phosphate buffer (pH 7.4) and then further incubated for 3 h with BSA under mild shaking. The exact amount of BSA bound to the adsorbents was determined by measuring the differences in the absorbance (A_{280}) of BSA before and after incubation.