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Preparation, characterization, and process performance of composite fibrous adsorbents as cation exchangers for high throughput and high capacity bioseparations

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ABSTRACT

Fibrous materials are proposed as novel chromatographic supports depicting high throughput and high product capacity. In this work, a composite fiber harboring strong cation-exchange moieties has been investigated. Such materials were characterized by a plethora of physical methods including degree of swelling (DS), scanning electron microscope (SEM), confocal laser scanning microscopy (CLSM), and Fourier transform infrared spectroscopy-attenuated total reflection (FTIR-ATR). The composite showed a high degree of grafting (~30%) and exhibited a high swelling ratio (~300%). Moreover, homogenous grafting and the development of an internal (functional) hydrogel were observed. The fibrous adsorbent was packed utilizing a designed "double roll" supported-structure and subsequently tested for packing efficiency and chromatography performance. The mentioned system showed similar packing efficiency of height equivalent to a theoretical plate (HETP) value and higher permeability coefficient (0.92×10^{-7} cm^2) than commercial resins. Experimentally determined Peclet number (Pe) values were within the range 60–90, suggesting a close-to-plug-flow condition. Total ionic capacity of the fibrous adsorbent was determined by the transition pH method. A capacity of 6.5 mequiv./g was obtained. Moreover, a high dynamic binding capacity for lysozyme was found to be 283 mg/g. On the other hand, a bed of randomly packed fiber also demonstrated high-resolution ability when a mixture of model protein was utilized to that end. Resolution was maintained at high flow rates (up to 900 cm/h) and utilizing shorter gradient development routines. Direct sequestration of a model protein (lysozyme) was also possible from an artificial mixture containing 1.5% yeast homogenate. Summarizing, the composite fibrous adsorbents exhibited superior performance during early protein capture and intermediate-resolution applications.

1. Introduction

Conventional multistep downstream processing of bioengineered therapeutic production is often time consuming, labor intensive, requiring huge hold-up volumes to operate in batch or semibatch mode and usually accounts for up to ≥80% of the total process costs [1–4]. Hence the biopharmaceutical industry has begun to target on process intensification and integration to improve economics by merging two or more separation schemes into one, thus reducing process times, cost of goods, and capital investments [5–7]. However, the introduction of expanded bed adsorption (EBA) has allowed early process integration by sequestration from a crude feedstock [8], but reduces the overall system dynamic binding capacity for the target product due to drawback in maintaining an appropriate (close to plug-flow) hydrodynamic condition of the fluidized bed [9]. The chromatographic resins exhibit several major limitations due to slow intra-particle diffusion within the porous beads which tend to limit the dynamic binding capacity of the resins to capture desired target product, the column design reduces throughput as a result of the increased pressure drop at higher flow rates and high material and operational cost. As a consequence, the separation and purification schemes applied in the biopharmaceutical industry for the purification of biotherapeutics require alternative materials to replace traditional packed-bed column resins [10–14]. As a potential solution for this challenge, a variety of chromatographic systems for bioseparations have been reported and the use of chromatographic supports with a polymer based fibrous geometry is prominent among the novel systems. They give a packing configuration that enables higher liquid flow rates and the potential for significantly higher mass transfer rates translates into product throughput in developing

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for scaled-up separations. However, these fibrous substrates have not been reported for high throughput and capacity due to low porosity, irregular packing and low pressure drops. And more significantly they are not suitable for process bioseparation due to lack of biomass compatibility [15,16].

In the literature, numerous methods concerning graft polymerization onto cotton and cellulosic materials using chemical initiation, ultraviolet light (UV), cold plasma, electron-beam and gamma rays have been described in which all made use of preirradiation and peroxidation techniques. None of these methods, however, are appropriate to the production of potential chromatographic supports, since grafting is in-homogeneous and limited to substrate external surface, swelling properties are difficult to control, ligand immobilization is complicated due to steric hindrance and the mechanical and chemical stability are poor for the resultant composite. Moreover, there is a lack in internal hydrogel which reduces the swelling ability and concomitantly the specific adsorption properties [17–24]. In the recent years, only a few limited studies can be found on the activation of membrane fabrics by surface grafting as an alternative to cotton fabric for bioseparations with low throughput and low dynamic binding capacities which are equal to or lower than commercial bead packed columns [25–28]. A composite fiber (gPore) which was synthesized by the mentioned procedure [29] has the advantages to act as a potential chromatography media. The composite fibers are having irreversible open compact structure with internally grafted homogeneous hydrogel, where reactive groups could be directly introduced to perform ion exchange or affinity ligand immobilization to the fiber surface, and thus reducing diffusion limitations as the process fluid is transported across the fiber. The composite fiber with hydrogel provides mechanical and chemical stability to the medium and also exhibits high swelling ratio, particularly good flow and adsorption properties can be ascertained. Composite fibers also offer promising prospects in developing single use disposable bioseparation devices in process scale operations due to low production cost and relative inexpensiveness of cotton fabric materials. In earlier works, the issue of column packing, high throughput and high capacity for purification factor and process bioseparation, has not been addressed properly, so this is the first research to deal with these issues directly.

In this research paper, the synthesized gamma irradiated simultaneously grafted composite fiber was further immobilized with functional sulfonate groups with proper surface activation thus being a strong cation-exchange adsorbent (SP gPore) and characterized for physical and functional properties. The fibrous columns, packed double roll form either randomly for purification or non-randomly for process bioseparation, have been evaluated. Besides, permeability coefficient and the total ionic capacity of the SP gPore were measured. For the column characteristics of the packed fibers, breakthrough analysis has been performed for operation in the frontal mode. The performance of the mentioned composite fiber as a chromatographic support was explored during early protein capture and intermediate purification applications utilizing a model protein.

2. Experimental materials and methods

2.1. Materials

Glycidyl methacrylate (GMA), dimethyl acrylamide (DMAA), 2-(N-Morpholino) ethanesulfonic acid (MES), were obtained from Sigma–Aldrich Chemie GmbH (Steinheim, Germany). Acetone, sodium sulfite anhydrous, isopropanol, ethanol, sodium hydroxide and lysozyme were purchased from AppliChem GmbH (Darmstadt, Germany). Low molecular weight protein marker was purchased from SERVA Electrophoresis GmbH (Heidelberg, Germany). Immunoglobulin (IgG) was obtained from Octapharma GmbH (Munich, Germany). Fluorescence dye Cyanine 3 (Cy3), chromatography columns, Tricorn [0.5 mm internal diameter (ID) × 5.5 cm length (L)], XK16 (16 mm ID × 20 cm L), SP Sepharose FF and the ÄKTA explorer system equipped with Unicorn 4.10 software for data collection and analysis were obtained from GE Amersham Bioscience (Uppsala, Sweden).

2.2. Sample preparation and characterization

2.2.1. Preparation of composite fiber and ligand immobilization

gPore – an innovative composite fibrous material was developed according to the mentioned procedure [29]. Briefly, a known amount of the dry mercerized hydrophilic polymeric material (TN & Platex, S.A., Buenos Aires, Argentina) was soaked in a monomer solution (GMA, 1.6% (v/v) and DMAA, 5% (v/v) in methanol/water, 1/1 (v/v)), previously degassed by nitrogen gas bubbling. Samples were irradiated with a 10 kGy dose at room temperature employing a ^{60}Co PISI semi-industrial irradiation source (CNEA, Ezeiza, Argentina). The resulting material was washed with an ethanol/water mixture (1/1) and subsequently with 96% ethanol. Washed gPore material was dried for 24 h in a vacuum oven at 40 °C until constant weight was reached. Pendant epoxy groups from grafted polyGMA were reacted with a mixture of sodium sulfite/isopropanol/water (10:15:75 by weight) at 37 °C overnight. After extensive washing with water, the functionalized sulfonate group composite fibrous adsorbent (SP gPore) was dried and then the degree of grafting (DG) was determined by gravimetrically [5]. The material porosity by water uptake (porosity, expressed as %) and the degree of swelling (DS) of the SP gPore material were determined by the mentioned procedure [30].

2.2.2. Scanning electron microscopy

All samples were equilibrated in 3 M KCl in phosphate buffer and rinsed with distilled water. Then the samples were dried in an oven at 45 °C up to constant weight. Gold sputtered samples were examined at different magnification using a Joel JSM 5900 (Peabody Inc., USA) scanning electron microscope (SEM).

2.2.3. Confocal laser scanning microscopy (CLSM)

The visualization of internal localization of the SP gPore morphology with protein adsorption was investigated by confocal laser scanning microscopy (CLSM). Briefly, a known amount of the SP gPore sample was incubated with an IgG labeled amino-reactive fluorescence dye Cy3 in adsorption buffer (0.1 M potassium phosphate pH 7.4) for 2 h at room temperature on a shaker. Then the samples were thoroughly washed with adsorption buffer and observed under confocal microscopy. SP Sepharose FF was utilized as a control cation exchange material. Confocal images were taken using a Carl ZeissLS510 (Jena, Germany; software version 3.0) with an oil immersion (63×/NA1.2 C-Apochromat objective lens). The obtained images were collected using the laser excitation sources ~550 nm, ~570 nm and recorded using a filter BP530-550.

2.2.4. FTIR

To confirm the introduction of functional groups onto the composite fiber, the dried samples were measured directly using Fourier transform infrared spectroscopy affinity instrument equipped with attenuated total reflectance (FTIR-ATR) accessory, PIKE GladiATR diamond single reflection (Shimatzu Corporation). The FTIR-ATR spectra were acquired by scanning the specimens for 32 times in the wave number range from 550 to 4000 cm^{-1} with a resolution of 0.4 cm^{-1} and analyzed with IRsolution Shimatzu 1.50 software.

2.3. Column packing

In or as chromatography media, the composite fibers are preferably physically arranged in the form of stacked discs, simple rolls, or in parallel or twirled arrangement either in packed or open form by randomly packed bed (RPB) and non randomly packed bed (NRPB). In this work, a unique packing technique was utilized where a defined amount of fibrous material was designed in a double roll on a thin flexible plastic net support structure considering proper aspect ratio and expansion factor. This double roll SP gPore material was randomly packed for the resolution applications and non-randomly for the process bioseparation applications in lab scale columns. The packed fabric bed was then swollen in place with 20 mM phosphate buffer (pH 7.4) at very low linear flow velocity (~75 cm/h) and the bed height of the column was fixed so that there was no visible head space in the column after swelling of the medium. The column packing efficiency was evaluated in terms of HETP and peak asymmetry factor (As) by acetone pulse experiments.

2.4. Flow permeability experiments

A fixed adaptor column (5 mm ID × 5.5 cm L), with 0.2 g of SP gPore material was prepared for flow permeability experiments. The experiments were conducted at room temperature on an ÄKTA explorer FPLC system with an internal pressure monitor. The height of the packed bed was 5.5 cm and pressure drops along the length of fibrous packed column were measured at different linear flow velocities between 150 and 600 cm/h of phosphate buffer. The extra-column pressure drop was accounted for by conducting the same measurements by passing the fibrous column in the system. The measurements of pressure drops were determined by the difference in the pressure at the inlet and the outlet of the column observed in the pressure monitor.

2.5. Determination of the ionic capacity

Transient pH phenomenon can be employed to determine the ion exchange capacity of porous media [31] using two buffer solutions with different ionic strength yet the same pH value. For the measurement of the cation exchanger capacity the following solutions were utilized: 20 mM Tris–HCl pH 7.4 (buffer A) and the same buffer containing 1.0 M sodium chloride (buffer B). The SP gPore material in a glass column (5 mm ID × 5.5 cm L) was mounted on ÄKTA explorer FPLC system, and a control bed of packed SP Sepharose FF beads was equilibrated with buffer A. The mobile phase composition was then switched to buffer B. The time interval Δt(pH) recorded between the switching point of the mobile phase and the point at which 50% of the maximum reachable pH value was actually obtained was recorded. The ionic capacity was calculated from the relative elution volumes, normalized by column volume, as described [31,32] with the following expression:

$$K = \left[\frac{\Delta t(\mathrm{pH})\Phi_v}{V_c}\right] = a \times q + b \quad (1)$$

where Φ_v is the volumetric flow rate, V_c is the column volume, and Δt(pH) was calculated from the pH transition profile experiments. For cationic systems, $a = 1.57$ and $b = 0.5$. These constants were previously calculated by Nika Lendero et al. [32] for similar systems.

2.6. Dynamic breakthrough by frontal analysis

The dynamic binding capacity (DBC) at 10% breakthrough for the lysozyme with SP gPore material was determined by frontal studies. The packed column (Tricorn 5/50) with double roll structure fibers was mounted to an ÄKTA explorer FPLC system and equilibrated by passing the phosphate buffer pH 7.5. Lysozyme solutions of concentration (2 mg/mL) were loaded through a super loop at linear flow velocities of 75–600 cm/h. When the initial protein concentration was reached, the column was washed with equilibration buffer until the effluent concentration fell back to a baseline value. The effluent concentration of lysozyme was measured with UV absorbance at 280 nm by internal UV detector. Bound lysozyme was eluted with 1 M NaCl in phosphate buffer and the column can be reused for next cycle. The dead volume of the system was determined by acetone injection. Complete breakthrough curves were obtained essentially according to Plieva et al. [31]. Calculations were performed as follows:

$$\mathrm{DBC} = C_o \frac{V - V_o}{\mathrm{CV}} \quad (2)$$

where C_o represents the feed protein concentration (mg/mL); V is the volume of protein solution required to reach the 10% breakthrough (mL); V_o is the chromatographic system dead volume (mL); and CV is total column volume (mL).

2.7. Purification of lysozyme from a complex mixture of proteins

The SP gPore elution behavior of lysozyme from a mixture of proteins was compared with commercial beads under three different superficial velocities. These experiments were performed with the columns (Tricorn 5/50) mounted to an ÄKTA explorer FPLC system. A defined amount of SP gPore material and 1 mL of Sepharose FF beads were packed inside the columns and loaded with injection of 200 μL loop of complex mixture proteins having concentrations of lysozyme (0.5 mg/mL, pI 11.0), concanavalin A (~0.2 mg/mL, pI 5.5) and α-Chimitripsinogen (~0.2 mg/mL, pI 9.0). The unbound protein was washed out with equilibration buffer A (MES 50 mM, pH 6) and the target protein was separated by passing elution buffer B (MES 50 mM + 1 M NaCl, pH 6) in a linear gradient of 0–90% B in 20 column volumes (CVs). The eluted purification profiles were analyzed and compared with an UV detector.

2.8. Lysozyme separation from crude feedstock

The biomass tolerance experiments were performed with an artificial mixture as a model process. The open structure fibrous material, double roll packed column (XK16) was mounted to a system (Pharmacia LKB Pump 500 Control LCC-501 plus with UV external AD 900 detector). The bed height was 6 cm and the linear flow rate was 150 cm/h (5 mL/min). The column was loaded with biomass containing disrupted 1.5% yeast and 5 g/L lysozyme. This artificial biomass mixture, corresponded to a stationary growth phase cell density of 1.2×10^{-9} cells/mL (a final optical density of ~1.0 at 600 nm). Then washing was done with the equilibration buffer A (20 mM PO_4 + 0.1 M NaCl, pH 7.5) and elution of the lysozyme with elution buffer B (20 mM PO_4 + 1 M NaCl, pH 7.5) by applying a step gradient of 10%, 60% and 100% B. The eluted fractions were collected and analyzed on SDS-PAGE.

3. Results and discussion

3.1. Fiber characterization

3.1.1. Scanning electron microscopy

The SEM images of untreated, gPore (gamma irradiate grafted) and SP gPore are shown in Fig. 1, respectively. Though not a high resolution is shown, it can be noticed that the images obtained by SEM of a cross section of the material at high magnification, evidence the presence of a polymethylmethacrylate (PGMA) grafted layer, that nearly homogenously covers the surface of the fibers and also

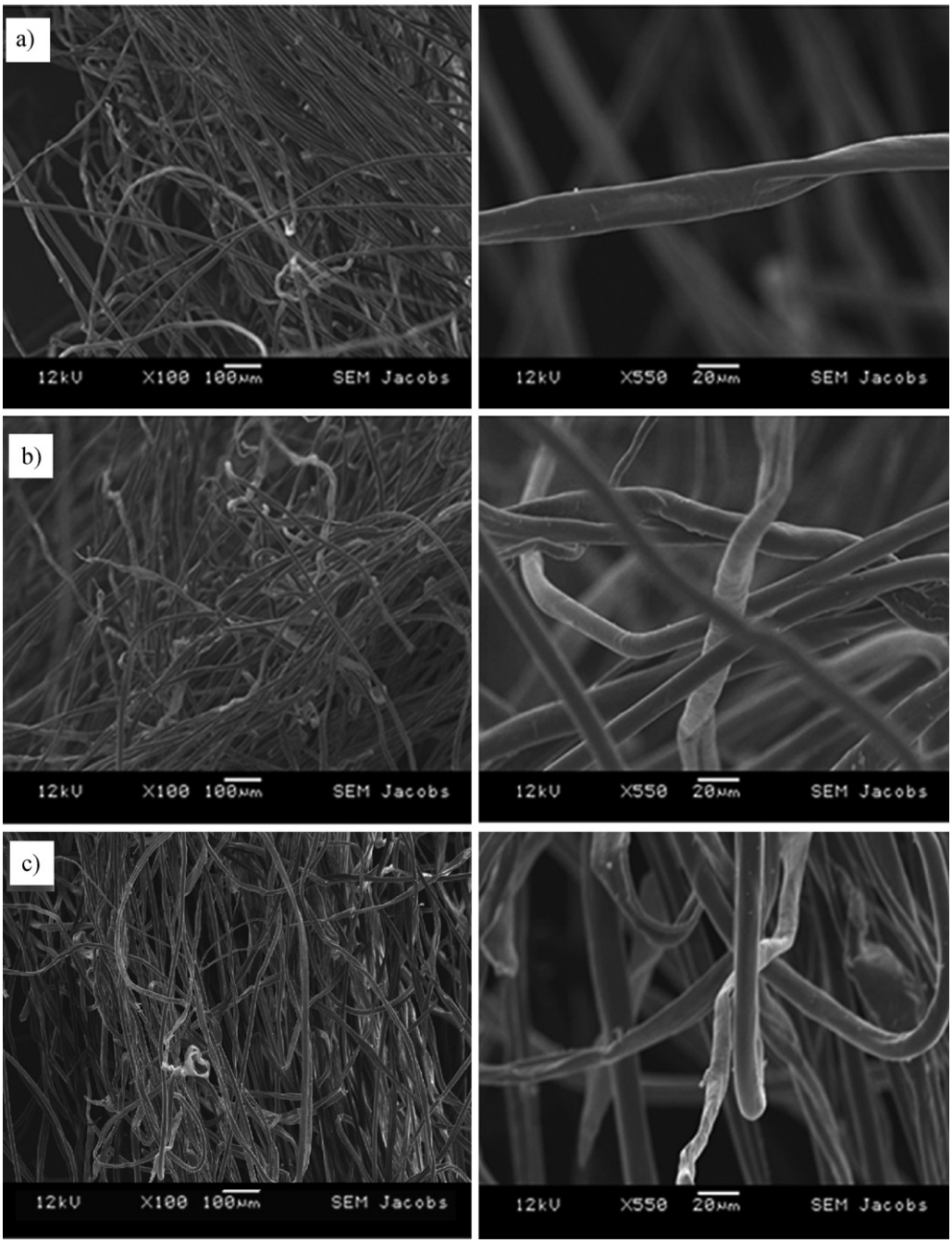

Fig. 1. Representative SEM images of (a) untreated, (b) gPore and (c) SP gPore in different scales.

the irradiated sample indicates no considerable deterioration as compared to the untreated sample. This, inside formed layer of polymer is, providing good dynamic binding capacity and good convective flow and presents mechanical support and chemical stability to the fiber to act as a cation-exchange chromatographic adsorbent. The degree of grafting yield, as measured by gravimetry, for the gPore fiber was obtained with 30%. The substantial difference between the modifications made in this work and other grafting techniques is that the same radiation dose of 10 kGy applied is 10–20 times lower because the polymerization is carried out simultaneously [33]. However, yields obtained in the simultaneous grafting technique are higher due to the avoidance of side reactions and the decomposition generated free radicals [34,35]. The degree of grafting, depending on the concentration of monomer used, radiation dose, solvent type and the porosity of the base polymer [36], otherwise resulted in polymeric bodies with undesirable characteristics such as low porosity, poor swelling, or lack of physical robustness. Additionally, a study by microspectroscopy Raman of styrene grafts on various substrates by pre-irradiation confirms that the modification occurs not only on the surface but also inside the substrate, and the increase in total degree of grafting is accompanied by a proportional increase in the number of grafts within the mass of the substrate [37]. The structural changes in the support material produced by high grafting degree can affect the mechanical characteristics so the tested polymers are attractive for use as supports for immobilization. Therefore, it is desirable that the modification in base polymer is only skin deep and this is achieved through technique utilized in this work. The swelling of cellulose in water is limited as a consequence of the presence of crystalline regions which restrict

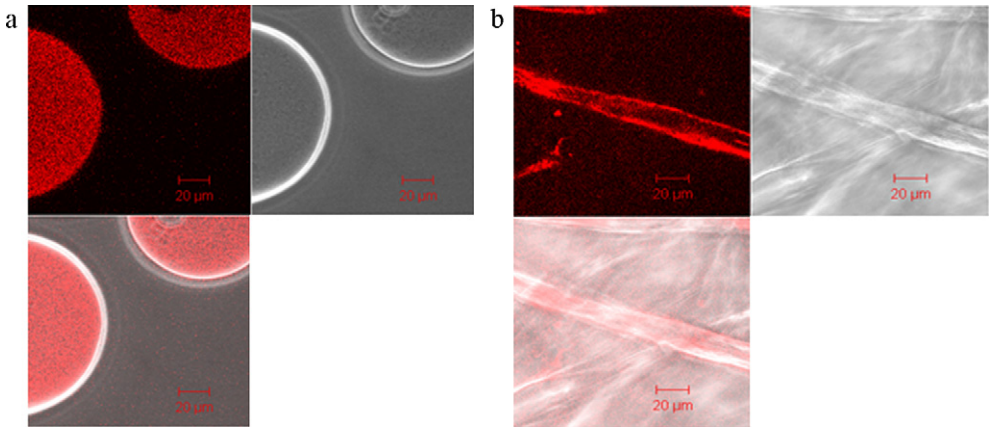

Fig. 2. Representative confocal microscopy studies on IgG adsorption: (a) SP Sepharose Fast Flow and (b) SP gPore.

the mobility of the polymer chains [20]. The degree of swelling was observed as 2.95 g/g (~300%) in distilled water at 24 °C and having a substantially cross section; the fiber diameter increases upon swelling compared to the fiber in its completely dry state. The resulted composite fibers were having a thickness of 5–80 μm in their swollen state and a length of 0.5–10 cm [29]. With this swelling ratio, particularly good convective flow properties can be ascertained for the chromatography adsorbent. Water uptake experiments revealed a 75% porosity, which indicates that, the grafting procedure is not affecting the physical integrity of the pores present within the material. This is due to highly penetrative nature of gamma rays permitting their use in the homogenous and efficient modifications of the material.

3.1.2. Confocal laser scanning microscopy (CLSM)

The confocal images in Fig. 2b illustrate the typical morphology of hydrogel inside the composite fibrous structure, consisting of relatively thick cellulose-based fibers connected to a network of interconnected fibers and forms the macropores on the outer surface. The CLSM image (Fig. 2b) of an IgG labeled with Cy3 fluorescence-stained fiber, reveals the very coarse structure containing the macroporous cellulose fiber network approximately, ~20 μm from the outer surface. The diameter of the larger pores is larger than ~15 μm. It had been found that fiber morphology in buffer can be visualized using CLSM in higher magnification, however in such conditions the complete profile cannot be obtained. Only a little part of matrix structure was visible as scale bar indicated in Fig. 2a and b. The control CLSM image of an IgG labeled with Cy3 fluorescence-stained commercial SP Sepharose FF is shown in Fig. 3a. The adsorbent gradually fills with the protein as expressed by penetration of fluorescing protein molecules into the internal regions of the beads. Protein binding as a function of concentration and incubation times was also accounted by CLSM and related to the binding isotherms for these samples but needs further investigation. A study of the flow-through property and protein binding of the fiber can be monitored with CLSM, which might give important information on the dynamic behavior of porous fibrous absorbents during separation.

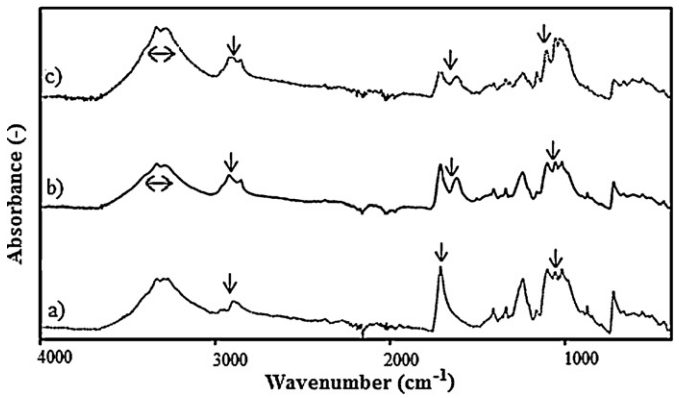

Fig. 3. FTIR-ATR spectra of (a) untreated, (b) gPore and (c) SP gPore.

3.1.3. FTIR

To better understand the chemical changes involved in the sample preparation, the IR spectra of untreated, gPore and SP gPore are shown in Fig. 3. The appearance of characteristic peaks at 1720 cm^{-1} corresponding to the carbonyl of the GMA is not observed due to the overlap of this with characteristic peak of the cellulose base material and also epoxy signal (900 cm^{-1}) is masked due to many signals in that zone in the gPore and SP gPore. However, there are some indications that peak broadening (2900 cm) and a new signal at 2850 cm^{-1} both are attributed to the stretching of C—H bond in pGMA–DMAA. In gPore and gPore SP, the peak at 1640 cm^{-1} is corresponding to carbonyl of the DMA. There is an increase in absorption signal at 3400 cm^{-1} due to the stretching of C—N bond in DMAA. The characteristic absorption signals at 1000–1100 cm^{-1} and the observed area which show changes in the peak shape and stretching of some characteristic bonds in sulfonic groups, are clear indications about the modifications in the SP gPore with respect to the untreated samples.

3.2. Functional characterization

3.2.1. Column packing

Fig. 4 illustrates a unique column packing technique with the SP gPore material as a chromatographic support in a packed column. It has been known that while conditioning with equilibration buffer, the chromatographic adsorbent can expand 3–4 times its dry packing density (1.0 mL/0.2 g) due to its high swelling ratio. In order to ensure uniform column packing, swelling of the bed is done at very low linear flow velocity (75 cm/h), and therefore, hydrodynamic forces are not a factor in packing efficiency. It has been shown that the effect of packing heterogeneity in chromatographic columns might seriously affect separation efficiencies and lead to poor performance of chromatographic materials [38–41]. Fig. 1c evidences that the gPore fiber generated by gamma irradiated polymerization has nearly homogenous distribution with internal hydrogel for mechanical support and good swelling properties. It is of utmost importance to realize that the calculated plate number will vary according to the test conditions like changes in solute, solvent, eluent, sample volume, flow rate, liquid pathway, temperature, etc.

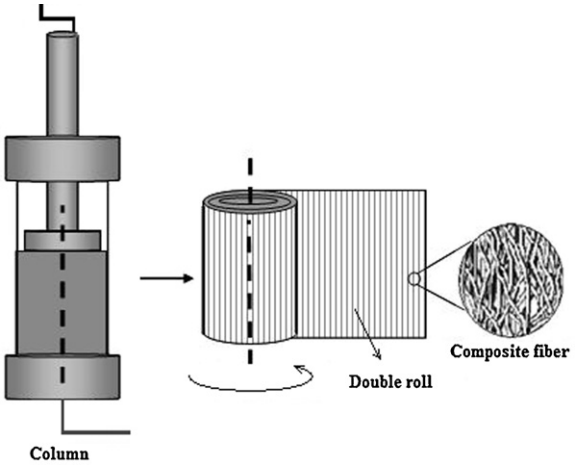

Fig. 4. Demonstration of a unique designed double roll column packing technique.

and it should therefore be used as a reference value only. In this paper, acetone was used as a probe solute under the non-retained condition in a fibrous packed 1 mL column volume, HETP and As were estimated at a linear flow velocity of 300 cm/h. The resulted acetone peak shows a slight asymmetry (As ratio 1.5) indicative of some slight tailing with an HETP of 0.0647 cm/plate and a plate count of 1574 N/m. The plate count for this column is slightly less than the typical range and may reflect a slight loss in efficiency by operation of the column at a high flow rate (300 cm/h). Initial studies are usually carried out at 75 cm/h where the HETP and plate count will be optimal. The measured peak asymmetric (As) values are in a quite comparable range to commercial beads packed-bed columns (As: 0.8–1.8) which indicated that the uniform packing density of the resulting fibrous packed column is known to have good packed performance. Nevertheless, the resulted values for this column would be acceptable for use in an ion exchange purification process.

3.2.2. Pressure drop measurements

The pressure drop in a porous medium which follows Darcy's Law at low Reynolds number is given by the expression [10,42]

$$u_0 = \frac{k}{\mu}\frac{\Delta P}{L} \quad (7)$$

where u_0 is the superficial velocity; k is the permeability coefficient; μ is the viscosity of the mobile phase, which is 0.01 P for the phosphate buffer; ΔP is pressure drop across the medium and L is the height of medium. Fig. 5 illustrates the plot of superficial velocity versus the measured $\Delta P/L\mu$ value for a column packed with 0.2 g composite fibrous material. The slopes of the curves are the permeability coefficients, which are equal to 0.92×10^{-7} cm^2. This obtained value is 1–3 times higher than the literature values of membranes developed for the similar purposes [43] and 1–2 times higher than the values of columns packed with ion exchange beads [44,45]. Many factors such as pore structure, shape, size, swelling degree and compatibility with mobile phase can change the back pressure of column.

3.2.3. Total ionic capacity

The fibrous composite material may have built-in functionality as a strong cation exchanger when the monomer utilized is selected accordingly i.e. the utilization of MAA during polymerization resulted in pendant carboxylic groups within the structure of the porous material. In this regard, total ionic capacity as measured by the pH transient method was reached with 6.45 mequiv./g and the pH profile of the fibrous packed column is shown in

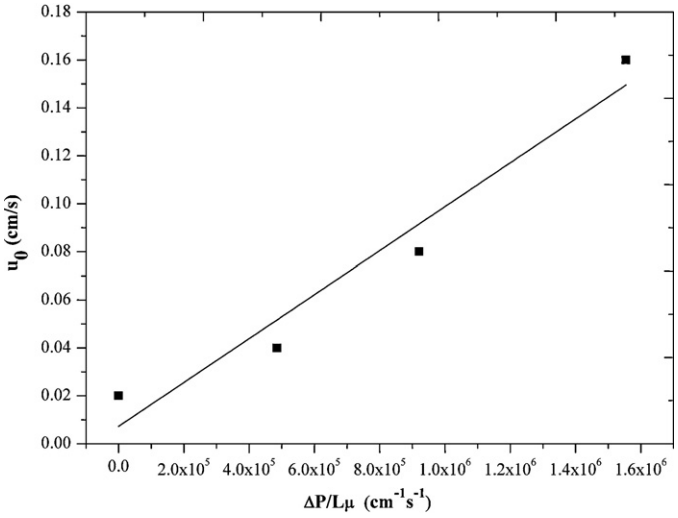

Fig. 5. Superficial flow velocity versus pressure drop for a column packed with SP gPore.

Fig. 6. The obtained parameter is comparable to that of commercially available cation-exchanger prepared by gamma radiation grafted membranes [46]. For comparison, similar frontal analysis performed for the commercial bead cation exchanger SP Sepharose FF showed an ionic capacity of 1.8 mequiv./g which is similar to the value mentioned in the literature. However, the strategy of build-in function is known to produce surface modification of composite fibrous structures with high number of charged groups on the ion exchangers. This is apparently due to the existence of internally grafted homogeneous hydrogel where reactive groups are easily introducible to perform effective ligand immobilization.

3.3. Chromatography performance

3.3.1. Dynamic binding capacity

For any adsorption system, the evaluation of dynamic breakthrough analysis is a combination of equilibrium binding capacity, adsorption kinetics, and system dispersion [27]. The DBC parameter can change, for the same adsorbent, as a function of the molecular weight and the system superficial velocity. The most challenging

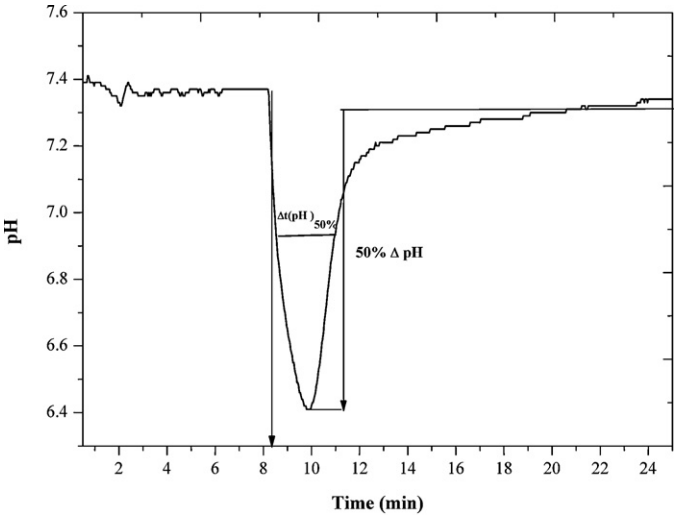

Fig. 6. Determination of $\Delta t(\mathrm{pH})_{50\%}$ from pH profile for SP gPore packed column. Conditions: buffer A: 20 mM Tris–HCl, pH 7.4; buffer B: 20 mM Tris–HCl + 1 M NaCl, pH 7.4; column (5 mm ID × 5.5 cm L); Method: 100% buffer A (8 min) step to 100% buffer B (24 min). Flow rate: 150 cm/h. Detection: pH.

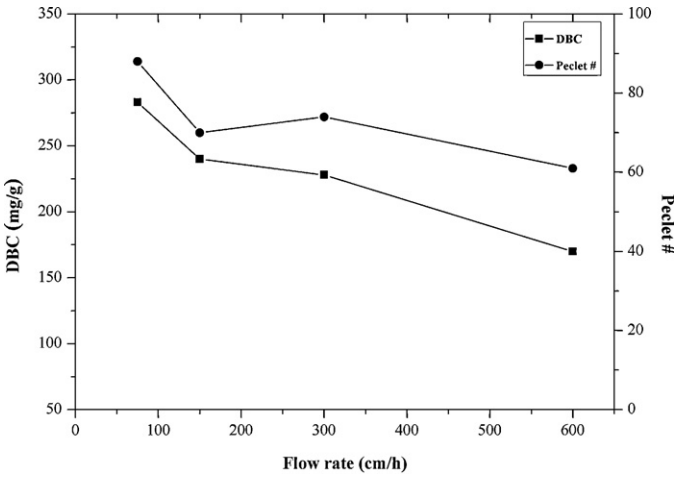

Fig. 7. Dynamic binding capacity (■) and Peclet number (●) of SP gPore, at different flow rates. Conditions: 20 mM phosphate buffer pH 7.4 was used as equilibration buffer and sample (lysozyme 2 mg/mL) was dissolved in running buffer, column: 5 mm ID × 5.5 cm L. Acetone was used as tracer for Peclet number determination. Different flow rates were applied: 75 cm/h, 150 cm/h, 300 cm/h, and 600 cm/h.

applications are situations in which macromolecules have to be sequestrated rapidly i.e. at high flow rates. To determine the existence of combined mass transfer resistance and dispersion effects and their potential influence on performance, dynamic binding capacity was evaluated via breakthrough curve (BTC) analysis as a function of superficial velocity. Moreover, residence time distribution (RTD) experiments utilizing acetone as an inert tracer were performed also as a function of flow velocity [30]; normalized profiles were interpreted by the dispersion model. The results of the breakthrough experiments carried out in a 1 mL column (5 mm ID × 5.5 cm L) as expressed by the quantities DBC and Peclet number (Pe) are shown in Fig. 7. It can be observed that DBC is decreasing with increasing the flow velocity and the maximum capacity 283 mg/g was obtained at 75 cm/h. At higher flow rates, there is noticeable, however not dramatic reduction of the DBC (ca. 20%) up to a linear flow rate of 300 cm/h. The other parameter, Pe remains almost constant at values (≥70) except reaching a maximum value at a linear flow rate of 600 cm/h, Pe suggesting a close-to-plug-flow condition (i.e. negligible axial mixing). The transport of molecules by convective flow is aided by the existence of the through-pores but limited by diffusion at the pore side and this pore architecture permits retaining a loading level when flow rate increases [27]. On

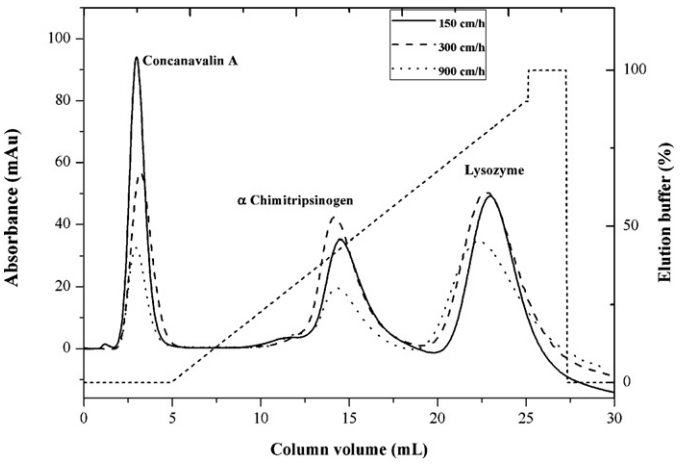

Fig. 8. Resolution behavior of SP gPore at an absorbance of 280 nm at three different flow rates.

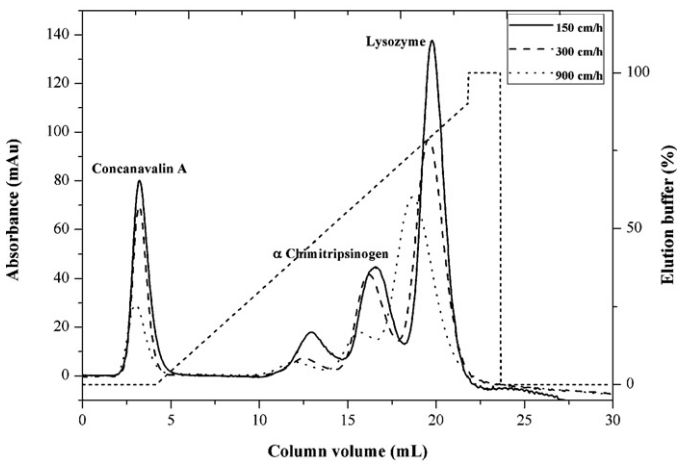

Fig. 9. Resolution behavior of SP Sepharose FF at an absorbance of 280 nm at three different flow rates.

the other hand, a reduction of DBC at very high flow velocity can be explained by a decreased Pe, which translates into an increased axial dispersion within the system [47].

3.3.2. Resolution behavior of the gPore

The elution of a protein mixture in the use of SP gPore and SP Sepharose FF, under different flow rates is shown in Figs. 8 and 9 respectively. SP gPore shows a higher resolution than the commercial Sepharose, since the three proteins of the protein mixture at the elution show clear dividing peaks, while the Sepharose displays four peaks. It can be clearly observed that resolution was maintained with the fibrous adsorbent even at high flow rates up to 900 cm/h. So increasing the flow rate by a factor of 10 is possible when using the gPore, as a high resolution is still valid. In contrast, SP Sepharose FF shows already a loss of resolution with an increase of factor 3. The increased resolution also affects the trend of gradients for the gPore; the elution of the proteins already allows a use of 50% of elution concentration. This clearly shows that the use of gPore allows a reduction of the buffer volume and the process due to time saving.

3.4. Bioprocess applications

The open structure non-randomly packed double roll SP gPore material, was tested in the commercial column to demonstrate the

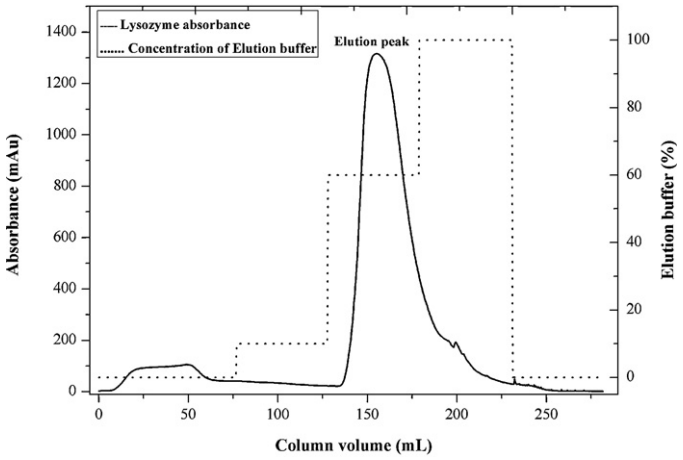

Fig. 10. Experiment for biomass tolerance of SP gPore under simulated process conditions. Lysozyme was mixed with yeast cells to the column with SP gPore. Subsequently, the column was washed and eluted the lysozyme (peak at 150 mL).

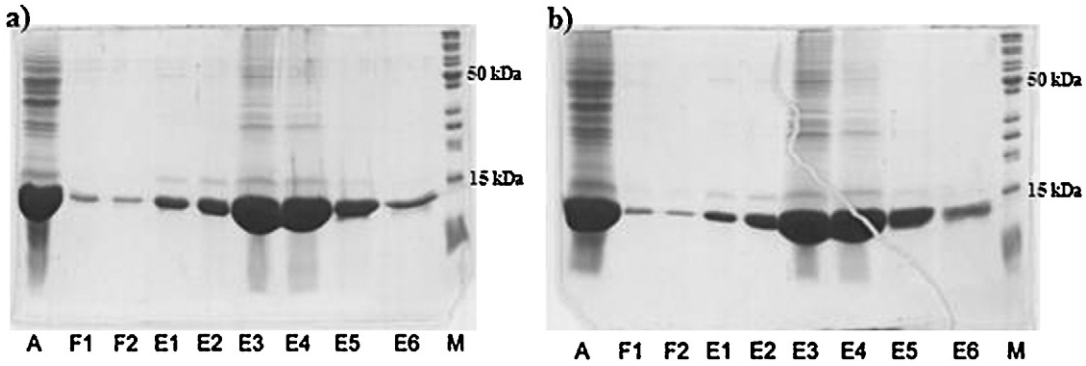

Fig. 11. Elution fractions from SP gPore column (a) filtered biomass and (b) ununfiltered biomass (A – load, M – marker, F – flow-through, E – eluted fractions).

ability of these fibrous composite to pass whole cell feed streams and the binding specificity, and observation of any blockages, back-pressure-related development. To simulate the prototype with the established expression systems of uncentrifuged and unfiltered yeast cell extracts, an artificial mixture of 5 g/L lysozyme (as a model protein to purify) and 1.5% yeast cells was produced, which realistically simulates the feedstock solution to a purification of macromolecules. The whole cell extract was given after the cell disruption on an FPLC system at a linear flow velocity of 150 cm/h. The material shows almost no pressure and no clumps, indicating high biomass compatibility. The elution of the model protein lysozyme was carried out and the resulted chromatogram elution profile is shown in Fig. 10. Lysozyme is selectively bound to gPore and then recovered as a very pure protein from unfiltered biomass as shown in Fig. 11b (gel photo: band E5). The biomass does not affect the adsorption of protein to SP gPore. The very high biomass compatibility and good binding properties allow high process speeds. These experiments demonstrate the strong tolerance of the biomass material, despite large cell density and without pre-filtration, expressed proteins can be purified with high flow rates and are eluted specifically. This in turn makes drastic reduction of process buffer volume and energy expenditure. The macro porosity would allow for the capture of target soluble species while cells or cell-derived debris present in a raw fermentation/cell culture broth may be easily elutriated with the system outflow. A more detailed study will be carried out to investigate for its performance as a potential chromatography support, with real time applications which could be particularly useful in the capture of proteins from a non-clarified complex feed stream.

4. Conclusions

A novel type of composite fibrous cation-exchange adsorbent has been prepared by gamma radiation graft simultaneous polymerization with subsequent ligand immobilization. This is the first study that demonstrates the utilization of composite fibers as a chromatography support for high throughput and high capacity bioseparation. The SP gPore was physically characterized by gravimetry, SEM, FTIR and the results indicated that a homogeneous internal hydrogel with high degree of grafting and swelling ratio was achieved which provides the mechanical and chemical stability to the chromatographic support. In this study, the potential of using column packing with fibrous support in chromatographic applications has been investigated and the results demonstrate its high column efficiency and high throughput. The results of the pressure drop experiments show that the modified fibrous adsorbent maintains very high flow permeability compared to other membrane types and to packed beds of chromatographic resins.

The total ion-exchange capacity of the synthesized fiber was determined by pH transition method and the obtained value is comparable to that of commercial available cationic-exchange based membranes. These modified composite fibers exhibited a high breakthrough performance for lysozyme at higher flow rates, indicating good mass transfer characteristics. The results obtained from the elution behavior of lysozyme indicate that high protein resolution at high flow velocities and reduction of buffer volume and processing time is possible. However, the potential with different systems such as antibody proteins (monoclonal antibodies) could be explored when the composite support is immobilized with appropriate affinity ligands. The experiments with feed stream demonstrated almost perfect compatibility with biomass and air, which is achieved by the structure of the material and eliminates the need for pre-filtration. In summary, all these results support that the composite fibrous materials have great potential for high throughput and high capacity in separation and purification of biotherapeutics and also may become an attractive alternative to other types of chromatography beads, membranes and particles currently being used.

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