Title: Protein bioseparation by membrane chromatography using polyelectrolyte gel-coated adsorptive membranes

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Introduction

Protein bioseparation is an important unit operation in the food, pharmaceutical and biotechnology industry. Adsorptive chromatography has found widespread applications for high-resolution protein bioseparation. Chromatographic processes are traditionally carried out using packed-beds, which have some significant drawbacks. The pressure drop in a packed bed is normally high and may increase during a process due to bed consolidation and column blinding (by accumulated colloidal material). A major disadvantage of packed-beds particularly those using soft chromatographic media, is the dependence on intra-particle diffusion of solute molecules from the bulk solution to their binding sites. This increases the process time and consequently the process liquid volume, which in turn affects the economy of adsorptive chromatography heavily both in terms of labor costs and buffer costs [1]. The complicated transport phenomena in packed beds also make scale-up of processes based on these very difficult. By using mono-dispersed, non-porous, rigid chromatographic media some of these drawbacks can be overcome. However, these have low binding capacities and are generally expensive.

The use of membranes as chromatographic media can alleviate some of the problems linked with packed beds. In membrane chromatographic processes, solute transport to their binding sites takes place predominantly by convection, which significantly reduces both process time and recovery liquid volume. Buffer costs alone account for 70-75% of the total cost of packed-bed chromatography while it contributes only 7-8% of the total cost of membrane chromatography. Labor costs accounts for around 15% of the total cost of packed-bed chromatography while it contributes only 7-8% of the total cost of membrane chromatography. When using membranes media cost is almost double when compared to packed beds but the lower buffer and labor cost makes membrane chromatography cheaper by a factor of 2-4 [1].

Membranes behave in the same way as packed-beds of small non-porous particles from the point of view of mass transfer, hydrodynamics, binding capacity, and available surface area [2]. The real benefit of a membrane is the continuity of the solid phase. In practice, it is difficult to make a very short adsorptive bed having a large cross-sectional area using loose particles but this is achievable with membranes. Due to shorter bed heights with membranes, pressure drop compared to packed-beds tends to be low. Due to recent developments in membrane manufacturing technology, membrane chromatographic devices are now easier and cheaper to mass-produce. This makes it possible to have disposable membrane adsorbers, which eliminates the requirement for cleaning and equipment re-validation. Another major advantage of membrane adsorbers is the relative ease of scale-up compare to packed-beds. Membrane chromatography is particularly suitable for larger proteins, which rarely enter in the pores of porous chromatographic media and only bind on the external surface of such media [3].

Membrane adsorbers play important roles in current biotech processes [4]. Several examples have already demonstrated the viability of using membrane adsorbers for fast and efficient capture of biomolecules. These include the preparative purification of human serum albumin from human plasma [2], large-scale purification of oligonucleotides [5], ion-exchange chromatography of monoclonal antibodies [6], and virus purification [7]. Table 1 lists some of the commercially available membrane adsorbers with their approximate protein binding capacities calculated from the data supplied with product manuals.

Table 1: Commercial membrane adsorbers and their binding capacities

Product Name	Manufacturer	Approx. protein* binding capacity (mg/ml)
Sartobind MA15 and MA100 (S, C, Q and D type)	Sartorious	22-29
Sartobind Factor-two family	Sartorious	28-30
Vivapure centrifugal ion exchangers	Vivascience	22-30
Mustang Q	Pall	70

* In most of the instances, protein binding capacities for anion exchange membranes were reported with bovine serum albumin and for cation exchange membranes with lysozyme.

Though membrane chromatography has several advantages over packed-bed chromatography, there are some challenges too, which need to be overcome. These include flow distribution limitations in currently used membrane modules and the generally lower binding capacity (compared to packed beds) [3]. A lower binding capacity implies that membrane chromatography would be suitable only for the processing of large volumes of liquid containing low concentrations of target proteins. In other words, chromatography using membranes currently available is not suitable for the binding of major proteins in the feed streams but its use is largely restricted to the removal of small amounts of target proteins and specific impurities such as endotoxins. This work investigates the performance of a new type of ion exchange membrane with high binding capacity. This polyelectrolyte gel coated Q type membrane was developed at McMaster University. This study examines the potential for use of this membrane for bioseparation of human plasma proteins. The effects of operating parameters such as pH, salt concentration, feed protein concentration and superficial velocity on protein binding capacity were first examined. These studies were carried out in the pulse chromatographic mode. Based on these results the possible operating range was identified. The dynamic protein binding capacity of the membrane at selected conditions was then determined by carrying out breakthrough experiments.

Experimental

Materials: Purified human serum albumin (HSA) was kindly donated by the Scottish Blood Transfusion Services, UK. It has a molecular weight of ~69 kDa and a p*I* of 4.9. Sodium chloride and sodium phosphate (dibasic and monobasic) were purchased from Sigma. Glass fiber pre-filter discs (catalogue # AP2504200) were purchased from Millipore. Polyelectrolyte gel coated ion-exchange (Q type) membrane was provided by Prof. Childs' research group at the Department of Chemistry, McMaster University. All buffers and solutions used in the experiments were prepared using water (18.2 MΩ cm) obtained from a Simplicity™ (Millipore) ultra pure water purification unit. Prior to use these liquids were micro-filtered using a 0.2 μm membrane and degassed.

Experimental set-up and methodology: An AKTA™ prime liquid chromatography system (Amersham Biosciences) was used for the experiments. A custom designed membrane adsorber module containing a stack of four flat sheet membrane disks replaced the chromatographic column. The membrane disks within the membrane module were supported in between stacks of glass fiber pre-filters. The thickness of the one membrane disk was around 250 micron while its effective diameter within the module was 42 mm.

The protein samples were injected in the pulse mode for studying the effect of different operating parameters on the binding capacity of membrane. The protein solutions to be injected were prepared in appropriate binding buffers. These solutions were injected after passing 50 ml of binding buffer through the membrane. After sample injection, the binding buffer flow was maintained for another 35 ml to wash out unbound protein followed by eluting buffer to elute the bound protein. In all the experiments, the eluting buffer was 10 mM sodium phosphate buffer, pH 6.5, containing 1 M sodium chloride. The effluent stream was monitored at 280 nm to keep the track of HSA concentration. Prior to chromatographic runs, these protein solutions were centrifuged at 10000 rpm for 20 minutes and the concentration of protein remaining in solution was measured using the Bradford assay method [8].

Results and Discussion

Effects of pH and ionic strength on dynamic adsorption of HSA

Some preliminary experiments were done to get an idea about the HSA binding capacity of the polyelectrolyte gel-coated membrane. The amount of protein sample for the experiments in pulse mode was selected such that this would be in excess of the binding capacity and consequently a bound and an unbound peak would be obtained. In this way the effect of the operating condition being examined on HSA binding could easily be observed. The amount of HSA bound to the membrane was determined from the bound and unbound peak areas:

$$M_b = M_i\left(\frac{AUC_b}{AUC_b + AUC_u}\right)$$

The effect of pH was first examined using 10 mM sodium phosphate buffer of appropriate pH. HSA solutions having concentration of 20 g/l prepared in the appropriate buffer were used as the feed solutions in these experiments. All these experiments were carried out at flow rate of 7 ml/min that gave a membrane bed residence time of 11.87 seconds. Fig. 1 shows the amount of HSA bound per unit membrane volume at different pH values. For the pH range studied (i.e. 5.5-7.5), the binding capacity of the membrane increased with pH. This could be due to the effect of pH on net negative charge on HSA. However, a pH value above 7.0 would not be of much practical interest keeping in mind the isoelectric pH value (pI) of human immunoglobulin G (HIgG) which is the next most abundant protein in human plasma. In plasma protein fractionation the major goal is HSA/HIgG fractionation. As HIgG would be negatively charged above pH 7.0 (its pI) and consequently also bind to the membrane, pH 6.5 was identified for potential HSA/HIgG separations to be carried out with this membrane in the future. All subsequent HSA binding experiments in this study were carried out at pH 6.5.

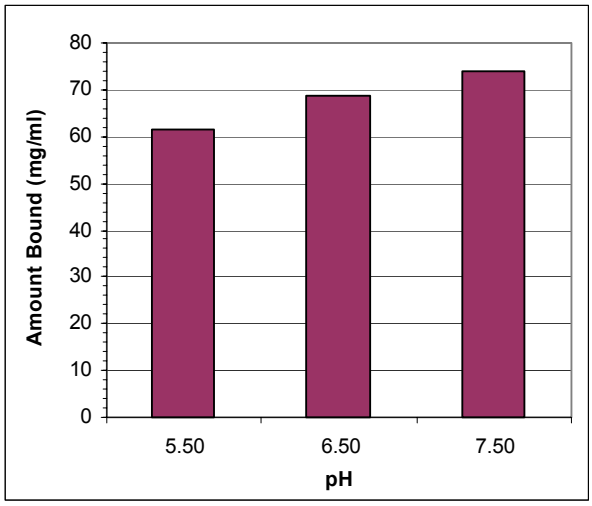

Fig.1. Effect of pH on the dynamic adsorption of HSA

Fig. 2 shows effect of binding buffer ionic strength on the binding capacity of membrane at pH 6.5. These experiments were carried out with 20 g/l HSA solutions as feed. These experiments were carried out at flow rate of 7 ml/min. From the graph, it is evident that as ionic strength of the buffer was increased, the HSA binding decreased. At low ionic strengths, competition for charged groups on the ion exchanger is expected to be low and consequently higher proteins binding resulted. Increasing the ionic strength increased competition for charged sites on the membrane and thus lowered the protein binding capacity. Hence 10 mM phosphate buffer was chosen as binding buffer for the subsequent experiments.

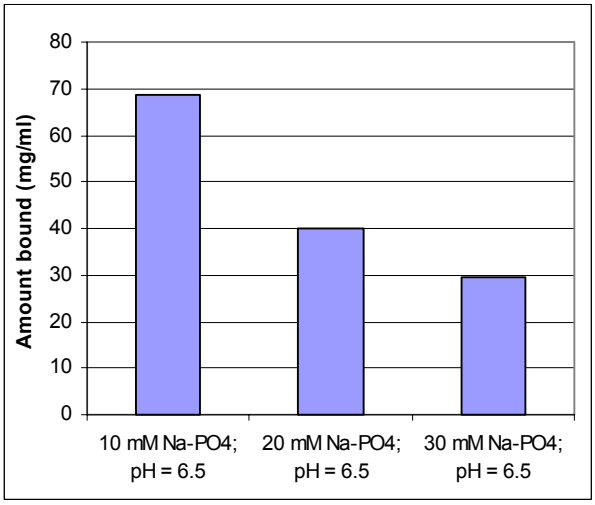

Fig. 2. Effect of binding buffer ionic strength on the dynamic adsorption of HSA

Effect of concentration on dynamic adsorption of HSA

In these experiments, the sample volume was varied in accordance to sample concentration in order to keep the total mass of protein injected in each experiment nearly constant. The total mass of protein injected in a pulse was selected such that nearly equal bound and an unbound peak would be obtained. Fig. 3 shows the amounts of HSA bound per unit membrane volume at different applied HSA concentrations. The HSA binding was

found to be nearly the same for the applied concentration range studied (i.e. 5-25 g/l). This indicated that the working concentration range was in the saturation part of the adsorption isotherm.

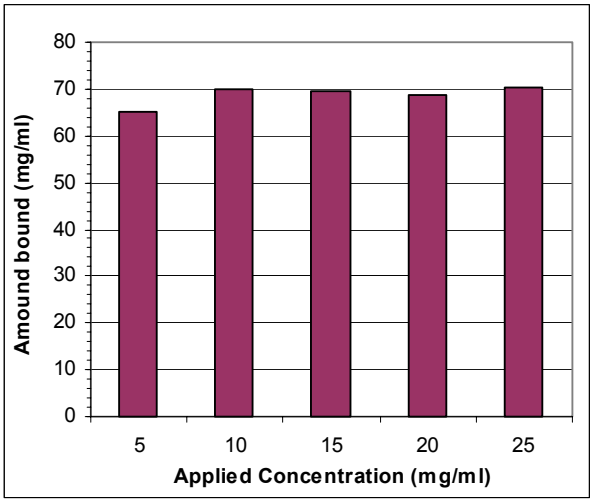

Fig.3. Effect of concentration on dynamic adsorption of HSA

Effect of flow rate on dynamic adsorption of HSA

The effect of flow rate on the binding of HSA was studied in the range of 3-11 ml/min. Fig. 4 shows the amount bound at different bed residence times. For the experimental range examined, the binding capacity was found to be independent of the bed residence time. This would suggest that even at the highest flow rate examined the protein molecules traveling through the pores had sufficient time to reach on the binding sites on the pore wall.

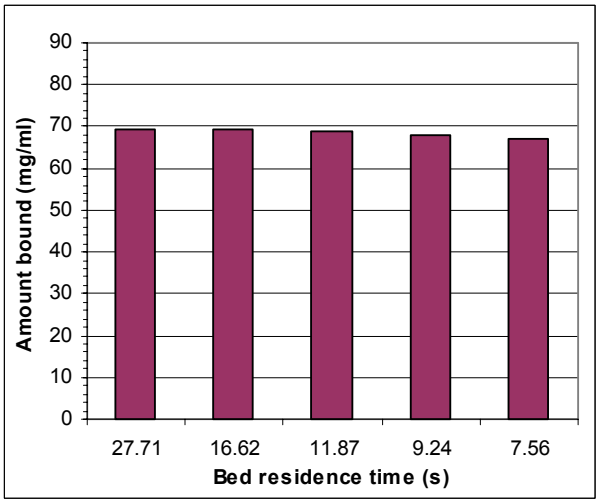

Fig.4. Effect of bed residence time on HSA binding
(HSA concentration = 20 g/l; sample volume = 10 ml)

Dynamic Binding capacity of HSA in step input mode

The dynamic binding capacity of this polyelectrolyte gel-coated membrane was studied in the step input mode at optimum conditions identified based on the results of the pulse input experiments. The step input experiment was carried out at a flow rate of 7 ml/min using 9.76 g/l HSA solution prepared in 10 mM sodium phosphate buffer (pH 6.5) as feed. From

the UV absorbance-effluent volume profile, the breakthrough curve was constructed as shown in Fig. 5 using an appropriate calibration for HSA concentration. The cumulative amount of HSA bound on the membrane was calculated from the breakthrough curve using material balance. A hydraulic correction factor was applied to correct for the void volume of the system. Using the data shown in Fig. 5, the dynamic HSA adsorption capacity at breakthrough (defined as the point at which the effluent concentration reached 10 % of the protein feed concentration) was determined to be 110 mg HSA/ml of membrane, which was considerably higher than the currently available membranes in the market.

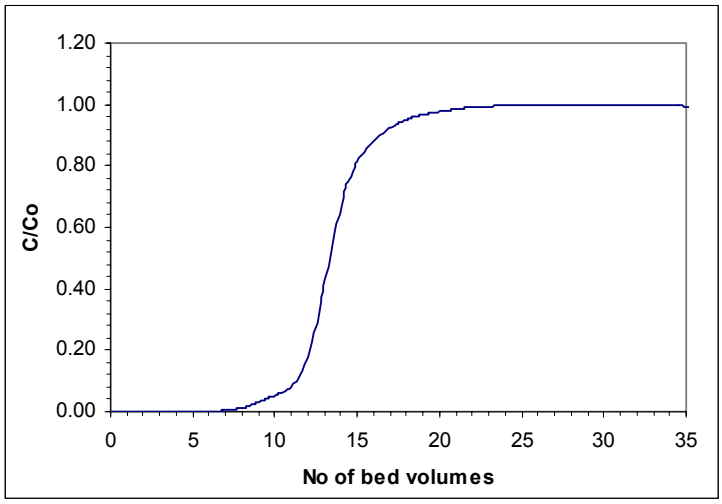

Fig.5. Breakthrough curve for HSA on polyelectrolyte gel-coated membrane

Conclusion

The protein binding capacity of the new type of polyelectrolyte gel-coated, ion-exchange membrane was significantly higher than that reported in literature. From the experimental results the following can be concluded.

1. In the pH range studied (i.e. 5.5-7.5), dynamic adsorption of HSA increased with increase in pH.
2. As the ionic strength of the buffer increased the binding capacity of the membrane decreased.
3. The amount of HSA bound was protein concentration independent in the range studied.
4. The flow rate did not affect the HSA binding in the flow rate range examined.

The low binding capacity of the adsorptive membranes reported previously meant that membrane chromatography could only be considered for certain niche applications, i.e. particularly where the concentration of the target protein in the feed stream was low. With this new type of membrane this limitation can be overcome thus facilitating the wider use of membrane chromatography in protein bioseparation and bioseparations in general.

Nomenclature:

AUC	Area under the curve (AU-m^3)
AUC_b	Area under the curve of bound peak (AU-m^3)
AUC_u	Area under the curve of unbound peak (AU-m^3)
M_b	Mass bound to membrane (kg)
M_i	Mass injected (kg)
C_o	Feed concentration (kg/m^3)
C	Concentration (kg/m^3)

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