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Purification of immunoglobulins from Serum Using Thiophilic Cellulose Beads

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This study evaluates the chromatographic performance of a support obtained by the reaction of mercaptoethanol with cellulose beads activated with divinyl sulfone. Cellulose beads 500-800 microns (pm) in diameter and with a solids content of 3.5% were selected for this study. A two-step sequence of permeation and reaction was used to install thiophilic sites throughout the cross section of the bead. The distribution of thiophilic sites was visualized by immobilizing fluorescent antibodies. Human and porcine serum proteins were separated on the thiophilic support at different linear velocities. Thiophilic cellulose beads were observed to bind human and porcine immunoglobulins (IgG) selectively from serum. Overall total protein recoveries in the range of 85%-99% were obtained with human serum, porcine serum and cell supernatant. Human TgG yields of 75% and 50% were obtained at linear velocities of 1 cmlmin and 3 cmlmin, respectively. Thiophilic cellulose beads were observed to bind monoclonal antibodies from cell culture supematant but yields in the range of 40-508 were obtained. Purity of the products, obtained from a single chromatographic tep, as judged by electrophoretic analysis was estimated to be greater than 80%.

INTRODUCTION

Recent unexpected shortages of immunoglobulins (IgG's) fractions from human plasma, in the United States, have contributed to a growing health care crisis and has forced doctors to cut dosages for some patients, postpone treatment for others or switch brands, which all affect

patients differently. There is a demand in the current market place to design and develop methodologies for the purification of human IgG (hIgG) from plasma or from genetically engineered sources. Purification schemes for IgG's from the cell culture supernatant matrix include precipitation with ammonium sulfate [1,2], ion-exchange chromatography [3-61, thiophilic chromatography [7-101, and affinity separations using immobilized protein-A [2,3,11-131. Affinity based methodologies are scaleable technologies important to protein purification. Of particular interest is the use of protein A or protein G immunosorbents to purify IgG's. Protein A/G columns have been widely used to purify mouse IgG's from ascities fluid and cell cultitre supernatants on a laboratory scale [ll]. This method usually results in a product with high purity and in good recovery, but the varying avidity of protein-A for IgG from different species and the possible contamination of the IgG product with leached protein-A makes it less attractive for preparative applications [3,11,12]. Moreover, the low pH (pH -3.0) often used to elute the bound IgG's from a protein- A column can induce denaturation and loss of biological activity. The limitations of protein AIG technology necessitate the development of novel pseudo-bioaffinity chemistries that will yield a product with high purity and functionality with minimized operating complexities like ligand leakage and proteolytic degradation. Thus there is a need for the development of new matrix technologies or purification protocols which are amenable to scale up without presenting excessive operational complexities. Thiophilic adsorption has been shown to selectively purify IgG's from complex biological fluids like serum, cell culture supernatant, and colostrum milk under mild elution conditions [4,7,8]. In addition, thiophilic matrices (T-gels) have shown to bind three major classes of IgG's and their sub-classes 1101. The mechanism of thiophilic adsorption can be used to selectively deplete and purify IgG's from biological fluids. Thiophilic matrices show selective binding of IgG's in the presence of lyotropic salts like ammonium or potassium sulfate [l 4-1 71. In comparison to typical hydrophobic matrices like octyl-sepharosei, thiophilic matrices have a greater affinity for IgG's than albumin [17-

191. Usually T-gel matrices are beta-mercaptoethanol derivatives of divinyl sulfone (DVS) activated agarose and have the following general formula: (Matrix)-O-CH_2-CH_2-SO_2-CH_2-CH_2-S-CH_2- CH_2-OH.

Current commercially used beaded matrices range in 20-80 microns (pm) in diameter thus providing high surface area beads. In this study we seek to evaluate a macroporous matrix derived from cellulose, a naturally occurring polysaccharide. The mechanical and flow properties of cellulose beads have been detailed elsewhere [20, 21]. In brief, cellulosic beads have shown to possess greater mechanical strength, offer low pressure drops, resist crushing under high column flow rates and are cheaper to produce than agarose or dextran beads [20]. Here we seek to investigate the use of a new porous cellulose based adsorbent for the efficient purification of antibodies from serum and cell culture supernatants. Cellulose beads with a nominal diameter in the range of 500-800 pm and a solids content of 3.5 % (wtlwt) were chosen for this study. The aim of this study is to show the utility of this phase in the separation and purification of antibodies. The understanding of how fundamentally simple and inexpensive materials like cellulose and cliitosan can be fashioned into matrices needed before true leaps in process technology will be made in the production of therapeutic proteins.

MATERIALS AND METHODS

Materials

Ligochem ^{TM} Provided cellulose beads as a generous gift. Lyophilized, 95% pure human IgG (1-4506), lyophilized 11~1man serum (S-2257). rabbit antiserum against mouse IgG's (M-8645), affinity purified goat-anti-mouse (whole molecule) IgG's conjugated to horseradish peroxidase (A-4416) and fluorescein isothiocyanate (FITC) labeled hIgG (F-9636) were purchased

from Sigma Co. (St. Louis. MO.) Immulon II microtiter plates were purchased from Fisher Scientific (Itasca, IL). Commercial T-gel was purchased from Pierce Chemical Company (Rockford, IL). 0-phenylenediamine-2HCl tablets were purchased from Abbott Laboratories (Chicago, IL.). Divinyl sulfone and β-mercaptoethanol were obtained from Fluka (Ronkonkoma, NY). Immunoaffinity separations were performed with Pharmacia C-10 columns (15 cm X I cm), a Masterflex peristaltic pump, a Knauer spectrophotometer, and a Rainini data acquisition system was used to lllonitor chromatography. Columns were kept at 4°C with a Lauda Supper RMT water cooler. 8-16% Tris-glycine gels were purchased from Novex (San Diego, CA) and gel electrophoresis was carried out with a X-Cell II Novex unit.

METHODS

Divinyl Sulfone Activation

Divinyl sulfone (DVS) activation of the cellulose beads were carried by two different methods; method A and method B and the description is are provided below:

Method A

Cellulose beads were activated according to the method described elsewhere [3,22]. In brief, decanted cellulose beads were suspended in an equal volume of I M Na2C03, pH l 1.3 in a 50 rill conical flask and DVS stock solution was added slowly to the flask to yield a final DVS concentration of 5% or 10% (v/v). The activation reaction was carried out at room temperature (RT) for 24 hours.

Method B

Decanted cellulose beads were suspended in an equal volume of 1M Na,CO,, pH 11.3 in a 50 m1 conical flask and DVS stock solution was added slowly to the flask to yield a final DVS concentration of 5% or 10% (vlv). The activation reaction was carried out in the cold room for 30 rnin (step 1). Upon completion of step 1, the pH of the solution was increased to 11.3 by the addition of sodium hydroxide and the reaction was allowed to continue at room temperature for 24 hours. The beads were then washed extensively with distilled water until the pH of the solution was neutral.

Determination of Vinyl Groups

The amount of vinyl groups was determined by sodium thiosulfate titration method described elsewhere [23]. In brief, 1 m1 of decanted DVS activated beads were mixed with 3 m1 of IM sodium thiosulfate solution. The mixture was rotated for 24 hr at room temperature to release the reactive vinyl groups. Supernatant was titrated with 0.1 N HC1 to a pH of 7.0. Divide the amount of HCI acid added by 10 to calculate the amount of reactive vinyl groups in pmole vinyl Groups lml of gel.

T-gel Modification of DVS Activated Beads

DVS activated beads prepared by method A and method B were incubated with an equal volume of 10% P-mercaptoethanol that had been titrated to pH 9.5 with 2M NaOH. The activation reaction was carried for 24 hrs at room temperature on an end-to-end rotator. Upon completion of the T-gel modification, the beads were washed with deionized water and stored at 4°C until further use. Extent of the modifications was determined by the indirect titration of active vinyl groups before and after ligand coupling.

Determination of Percent Sulfur

10 m1 of cellulose beads activated by method A and B were washed 3-times with de-ionized water and the supernatant was drawn off. The beads were then lyophilized to dryness and submitted to the Research and Soil Test laboratories at the University of Minnesota for percent sulfur (% S) analysis. Analysis was per formed on a Leco SC-132 sulfur system from Leco Corporation, St. Joseph, Michigan. We also provided a sample of commercially available T-gel from Pierce Chemical Company and a reference sample of bald or inactivated beads. Typically, 200 mg of dried beads were placed in a sample container and 500 mg of vanadium pentoxide was added on top of the sample. Samples were then heated to 2600 "F and an infra-red detector quantified the vapors of sulfur dioxide.

Ligand Binding Isotherms

Small-scale experiments were conducted to obtain ligand-binding parameters of thio-philic cellulose beads (TC-beads). 600 p1 of 50% (v/v) slurry of cellulose beads were trans-

ferred into 1.5-m1 microcentrifuge tubes to yield 300 p1 of cellulose beads. The beads were allowed to settle for 5 minutes and the liquid overlay was pipetted off. A l .0 m1 of 0.0, 0. l , l .O, 2.0,4.0, and 8.0 mglml of human IgG (hIgG) in washing buffer (0.5 M K2S04, 50 mM NaH2P04, pH 8.0) was added to the microcentrifuge tubes. Experiments were carried out in duplicate. Tubes were placed on a end-to-end rotator and rotated for 24 hrs at RT. An identical experiment was also carried out with thiophilic-gel (T-gel) obtained from Pierce Chemical Company. At the completion of the experiment, the tubes were centrifuged at 400 rpm and the supernatant was pipetted off and saved for protein determination. The protein concentration was measured spectrophotometrically at OD 280 nm. The difference in the amount of hIgG in the feed and the amount of hIgG in the supernatant yielded the amount of hIgG bound. Equilibrium binding capacity (Q,,,) was obtained from isotherm analyses and double reciprocal plots.

Purification of IgG from Serum and Cell Supernatants

100 mg of lyophilized human serum was re-suspended in 5 m1 of loading/washing buffer (0.5 M K2S04, 50 n1M NaP04 pH 8.0) and the contents were allowed to come into solution. **A** 1.5 m1 of the human serum sample was loaded to a column (l cm i.d. X 12.2 cm in length) packed with TC-beads at a linear velocity (U) of 1.0 cmlmin. Unretained proteins were collected as the column fall through and the non-specifically bound proteins were washed with the loading buffer till the OD 280 nm returned to the baseline. The bound h1gG was eluted by making a step change to the elution buffer; 50 mM NaP04, pH 8.0. Upon elution, the column was washed with 50 mM NaP04, IM NaC1, pH 8.0 to strip any tightly bound proteins and columns were re-equilibrated in washing/loading buffers. 'The chromatographic fractions were assayed for total protein content by measuring absorbance at OD 280 nni and hIgG content by specific ELISA assays. The purity of the product was judged by elcctrophoretic analysis. Similar ex-

periments were carried out at linear velocities *of'* 3 and 6 cmlmin. In separate experiments pig serum and cell culture supernatants containing monoclonal antibodies (Mabs) were also chromatographed on the column sing the procedure described above.

Determination of Human 1gG by ELISA

Immulon I1 micro titer plates were incubated with 100 µ/well of 1 : 1000 diluted rabbit anti-hIgG whole molecule in coating buffer for 34 hrs at 4°C. Wells were washed with washing buffer and residual sites were blocked with blocking buffer for 30 minutes at room temperature. Various dilutions of standard and samples in dilution buffer 20 mM Tris-HC1, 50 mM NaCl, pH 7.0, 0.5% casein) were added to the wells, 100 111 in each well and incubated for 30 minutes at 37°C. LJpon incubation, wells were washed four times and 1:2500 diluted HRP conjugated rabbit anti-hIgG was added to the wells and incubated for 30 minutes at 37°C. Wells were washed and 100 111 of OPD substrate was added to each well. The colorometric reaction was stopped after 3 minutes by the addition of 100 yl of 100 p1 of 3 N sulfuric acid to each well. Bound chromophore was detected at 490 nm using a Bio-Tek microplate ELISA reader.

Determination of Mab by ELISA

The concentrations of Mab in various chromatographic fractions were determined by the ELISA procedure outlined elsewhere [241.

Labeling of T-gel Beads with Fluorescein Thiocyanate

The distribution of active sites in cellulose beads activated with T-gel at various densities was determined by irnmunofluorescent microscopy. hIgG labelecl with fluorescence thiocyanate (FITC) was bound to cellulose ds prepared by nlethod A and B from a solution concentration of ***0.0,*** 1.0 and 5.0 mg Mablml. FITC labeled beads were rotated at 0 - 4°C in the dark. The beads were analyzed by horizontal scanning (section scanning) fluorescent light in a confocal microscope attached to a Nikon Diaphot inverted microscope (BioRad Labs., Hercules, CA) ecluipped with a 15 nlW Krypton / Argon Excitation filters allowing 488 nm, 568 lim, 647 11m or combination of all three-laser lines were used. The samples were viewed at **1** Ox magnification. Digital images were collected on a Compaq ProSignia model 300 personal computer using Laser sharp version 3.1 software (BioRad Labs., Hercules, CA). Publication quality prints were made utilizing Adobe PhotoShop version 5.0 and a Tektronix Phaser 340 printer (Tektronix, Wilsonville, OR 97070)) or a Fujix Pictography 3000 digital image printer (Fuji North America, Elmsford, NY 10523). Gel *Electrophoresis* The purity of the recovered IgG was analyzed by SDS-PAGE gel electrophoresis I under non-denaturing conditions [25]. In brief, all chromatographic fractions were diluted to a protein concentration of 0.4 mglml. Samples were mixed with non-reducing buffer at a ratio of 1 : I and were heated to 95OC for 5 min in a water bath. Proteins were analyzed on 8-16 % gradient gel and visualized by silver staining [26]. Stained gels were further analyzed by digital image processing to assess the purity.

RESULTS

DVS Activation and T-Gel Modification

Table I summarizes the results of the DVS activation of cellulose beads. The amount of HC1 used in the titration reaction was used to quantify the active vinyl groups and is shown in column 4. Cellulose beads activated with 10% DVS using method A gave 3.0 to 4.0, pnoles of

vinyl groupslml of gel, respectively. Cellulose beads activated with 5% DVS and 10% DVS using method B gave 5.0 to 6.0, 8.0 to 9.5 pmoles of vinyl groups11111 of gel, respectively. As expected, the amount of reactive vinyl groups increased with an increase in the amount of DVS used in the activation process by methods A and B. A control incubation of the beads at the same pH, but with DVS omitted, showed no titratable vinyl groups. In an attempt to achieve higher pnloles of vinyl groupslml of gel, activation reactions were also carried out in the presence of 20%, 30% and 40% DVS (data not included). However, in reactions containing 20%, 30% and 40% DVS, the beads acquired a milky/whitish appearance after the activation step. All the beads were examined under a microscope before and after the activation process to record any gross change in morphology. Activation reactions with 20, 30 and 40% DVS resulted in beads with a "rigid doughnut like" appearance. The cellulose beads activated by DVS were further end modified with p-mercaptoethanol to yield thiophilic cellulose beads (TC-beads). The amounts of unreacted vinyl groups were estimated at the end of the thiophilic reaction step. No titratable vinyl groups were found after coupling of mercaptoethanol to DVS activated cellulose beads, indicating complete reaction. A control incubation of the beads at the same pH, but with mercaptoethanol omitted showed titratable vinyl groups. The immobilized ligand (P-mercaptoethanol) density was also determined by elemental sulfur analysis. TC-beads made with method A and method B contained 5.24 + 0.01 % sulfur (%S) and 7.31 A 0.12 %S, respectively. T-gel from Pierce Chemical Company contained 5.66 %S. TABLE I

TABLE I Summary of DVS activation

Support	*Method*	*% DVS*	*μmole vinyl groups/ml of gel*[a]	*Qmax*[b]	*% Sulfur*
Cellulose	A	10	3.0 – 4.0	3.44	5.24 ± 0.01
	B	5	5.0–6.0	1.31	n.a.
	B	10	8.0 – 9.5	40.4 ± 12	7.31 ± 0.12
T-gel				26.97 ± 10.5	5.66

Thiophilic cellulose beads were prepared by method A and method B as described in the methods section. T-gel was purchased from Pierce Chemical Company. Reactive vinyl groups were determined by titration. The ligand binding parameter (Q_{max}) was determined by isotherm analyses and double reciprocal plots. The percent sulfur in the thiophic matrices was determined by the procedure in the methods section.
n.a. not analyzed

a. Range of values obtained from independent determinations is reported
b. An average value from three independent experiments is reported.

Ligand Binding Isotherms

The static binding capacity (Qmax) was determined by plotting the static adsorption isotherm for T-gel obtained from Pierce Chemical Company and the TC-beads made by methods A and B, respectively. A representative set of static adsorption isotherms is shown in Figure 1. T-gel obtained from PCC and TC-beads made by method B in the presence of 10% DVS were not completely saturated with hIgG, as shown by the upward slope of their respective isotherms. TC-beads made, by method A in the presence of 10% DVS, and by method B in the presence of 5% DVS appear to be saturated, as judged by their respective isotherms. An estimate of Q,,, was obtained by double-reciprocal analyses for isotherm data collected from multiple sets and the average values are presented in Table I. The Q,,, of T-gel obtained from Pierce Chemical Company for human IgG was found to be 26.97 A 10.5 mg hIgG /m1 of gel. The Q,,, for cellulose beads activated by method B in the presence of 5% and 10% DVS were found to be 1.31 mg hIgG/ml of gel and 40.4 +- 12 mg hIgGIm1 of gel, respectively. The batch binding capacity of T-gel obtained from cellulose beads activated by method A in the presence of 10% DVS was found to be 3.44 mg hIgG /m1 of gel.

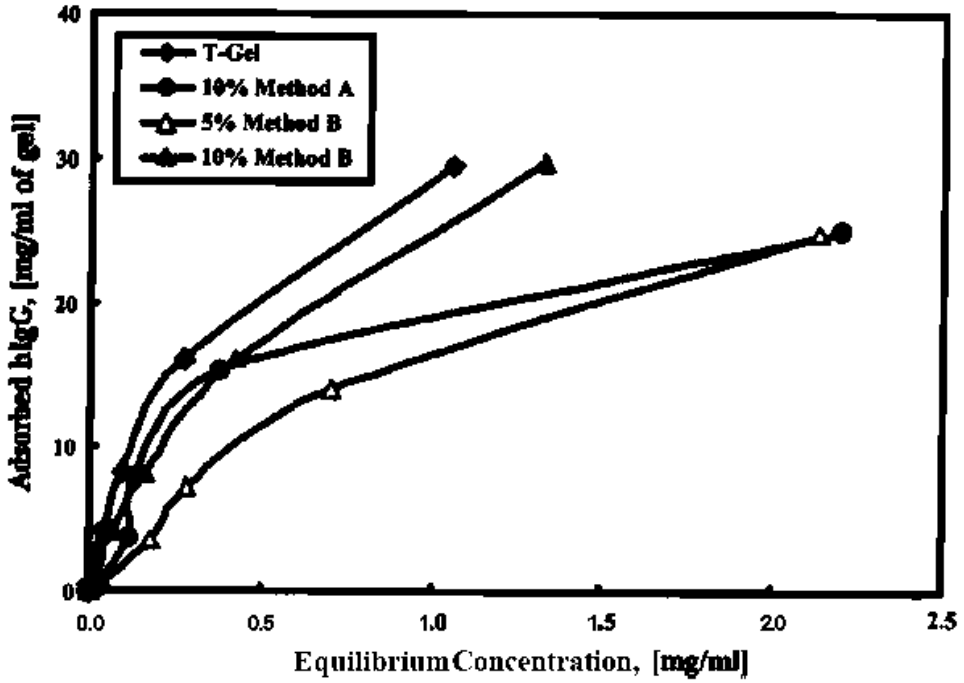

FIGURE 1 Static adsorption isotherms for T-gel and thiophilic cellulose beads. Thiophilic cellulose beads and T-gel beads were contacted with different concentrations for hIgG as described in the methods section. The equilibrium concentrations were measured spectrophotometrically at 280 nm. The amount of hIgG bound was determined by difference. All experiments were carried out at room temperature. A representative set of isotherms is shown

Column Chromatography

Based on the static binding capacity and % sulfur obtained for the thiophilic cellulose (TC) beads made by method B, we selected to evaluate the chromatographic performance of TC-beads made by method B in the presence of 10% DVS. Figure 2shows a typical chromatographic profile for the isolation of human IgG from the serum. Unbound or very weakly retained proteins passed through the column during the first five column volumes (CV). The UV trace at 280 nm returned to baseline by seven CV indicating near complete elution of unbound protein. A step change to the elution buffer was made to elute the bound IgG. A chromatographic peak at 280 nm indicates elution of bound protein, which wc identify as retained IgG. This peak eluted between 2-7 column volumes. Finally, to elute any proteins bound by non-specific interactions, a step increase to l M NaCl at fourteen minutes was employed. Flushing of the column continued for approximately twelve column volumes. To restore the column the next run, it was then flushed with loading buffer for fifteen minutes prior to the next injection.

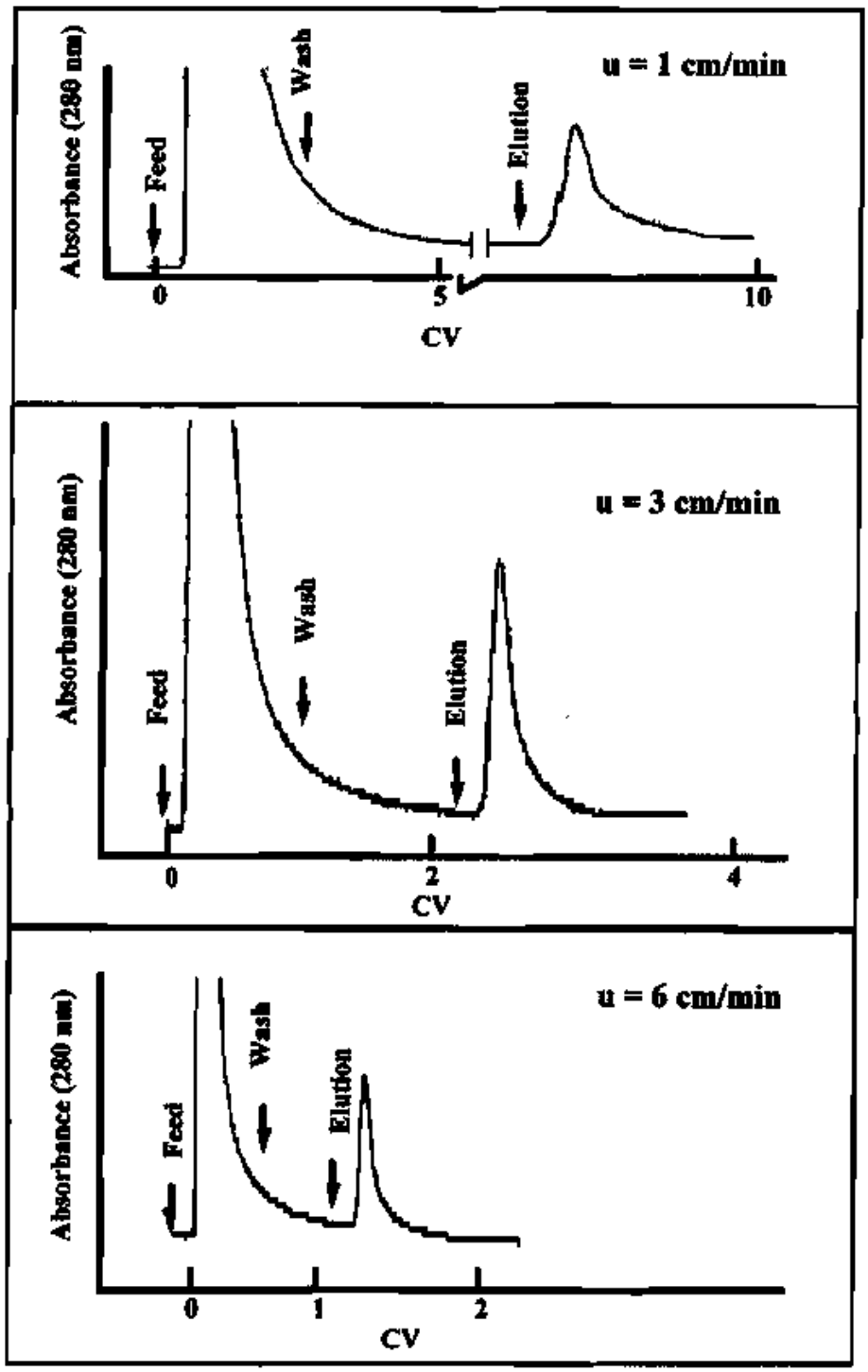

FIGURE 2 Purification of human IgG from a human serum on thiophilic cellulose beads. Beads were packed in 12 cm x 1 cm i.d columns. The linear velocity is shown on each diagram. Sample loading and column wash buffer was 0.5 M K_2SO_4, 50 mM $NaPO_4$ pH = 8.0. Elution of bound IgG was achieved by a step change to 50 mM $NaPO_4$ pH =8.0. All columns were operated at temperature 4 °C; detection was performed at 280 nm. Sample feed was 1.5 ml of human serum

Yield of IgG from Serum

Table I1 sunnnarizes the hIgG and porcine IgG yields in the various chromatographic fractions at different linear velocities when human serum and porcine serum was usecl as feed to the column, respectively. Chromatographic profiles with similar characteristics to one described earlier were obtained for all runs in Table 11. The total protein concentration in different chromatographic fractions in each individual run was estimated by measuring the absorbance at 280 nm. The perceni total recovery of protein was delerinined as a ratio of the total protein in the elllate fraction and fall through to the total protein in the feed sample. In runs using human serum as a feed sample, total recoveries in the range of 82-90% were obtained. In runs using pig serum as a feed sample, total recoveries greater than 998 were obtained. A majority of the protein was recorded in the fall through fraction thus allowing enrichment of IgG in the elution fraction.

The hIgG concentration, in different chromatographic fractions of runs 1.2 and 3, was also estimated by the ELBA protocol and the values are presented in Table IT. The percent yield of hIgG in the eluate fraction was determined as a ratio of total hlgG in the eluate fraction to the total hIgG challenge in the feed. The yield of hIgG in run I was *73%,* the yield of hIgG in run 2 was 50.62%, and the yield of hIgG in run 3 was 5 %. With an increase in linear velocity lower hIgG yields were obtained. However the total protein recovery remained high, greater than 99 percent.

TABLE I]

Feed sample	*Linear velocity, cm/min*	*% Total Recovery*[a]		*% Yield*[b]
		OD 280 nm	*ELISA*	*ELISA*
Human Serum	1 (12.2 min)[c]	89.67	98.61	73.00
	3 (4.07 min)	82.03	98.45	50.62
	6 (2.03 min)	84.69	102.91	5.0
Porcine Serum	1 (12.2 min)	99.23	n.a.	n.a
	3 (4.07 min)	101.96	n.a.	n.a
	6 (2.03 min)	117.13	n.a.	n.a.
Cell Supernatant	1 (12.2 min)	98.68	89.00	42.21

Thiophilic cellulose bends prepared by method B (see Table I) were challenged with human serum, porcine serum, and cell supernatant in 0.5 M K_2SO_4, 50 mM NaH_2PO_4, pH 8.0 at linear velocities of 1, 3, and 6 cm/min, as separate experiments respectively. Bound IgG was eluted with 50 mM NaH_2PO_4, pH 8.0.
n.a. Not assayed by ELISA.

a. Percent total recovery is defined as the ratio of the sum of the total protein in eluate and column fall-through fractions to the total protein present in the feed.
b. Percent yield is defined as the ration of the IgG (human) present in the eluate fraction to the total amount of IgG in the feed.
c. Column residence time (t_R) is shown in parentheses, where t_R = [length of column / linear velocity]

Yield of Mab from Cell Culture Supernatant

Table I1 gives the Mab yield when cell culture supernatant serum was used as feed to the column. Chromatographic profiles with similar characteristics to one described earlier were obtained. The percent total recovery of protein was determined as a ratio of the total protein in the eluate fraction and fall through to the total protein in the feed sample. In runs using cell culture supernatant as a feed sample, total recoveries in the range of 80-99 % were obtained. A majority of the protein was recorded in the fall through fraction. The Mab concentration was also estimated by the ELISA protocol described in the methods section. The percent yield of Mab in the eluate fraction was determined as a ratio of total Mab in the eluate fraction to the total Mab challenge in the feed. Mab yields in the range of 40-50% were obtained.

Gel Electrophoresis

Figure 3 shows a silver-stained, SDS-PAGE gel of the starting human serum (feed) and the chromatographic fractions from a typical separation run on TC-beads. Chromatographic fractions from Runs 1, 2 and 3 corresponding to u = 1, 3, and 6 cndmin were selected for electrophoretic analysis. Lanes 1 shows a molecular weight ladder. Lanes 2 shows an application of pure hIgG a total protein level of 6 pgs. Lanes 3 shows an application of hulnarl serum (feed to the column) at a total protein level of 4 pgs. The serum has two distinct protein bands corresponding to human serum albumin HSA with a molecular weight of 56,000 Dalton and hIgG with a molecular weight of 150,000 Dalton. Some additional minor bands are also observed. Lanes 4, *5,* and 6 shows the elution fractions from runs 1, 2 and 3, respectively, at a total protein level of 4 pgs. Lanes 7, 8, and 9 shows the fall through fractions from runs **1 ,** 2, and 3, respectively, at a total protein level of 3 ygs. The eluate fractions gave a band around 150 kDa similar to the pure hIgG in Lane 3. In addition to the major Mab band at 150 kDa, a minor band at 56 kDa accounting for less than 10% of the area obtained by digital image processing was observed. The fall through fractions shown in lanes 7, 8, 9 gave a band around 56 kDa similar to the pure HSA. The purity of the hIgG in the eluate fraction (Lanes 4, 5 and 6) is estimated to be greater than 90% by digital image processing. Similar electrophoretic pattelms were obtained with the fractions from other runs listed in Table I1 (data not shown).

FITC Labeling

To better understand the installation and distribution of thiophilic adsorption sites within the cross section of cellulose beads (0.8 mm in diameter) FITC labeled hIgG was bound to TC-gel in a batch experiment. hIgG was bound on TC-beads made with method A and method B. In Figure 4 a series of scanning images for the adsorption of hIgG to TC-beads are shown. The cross section of TC-beads is filled with antibody as evidenced by the green fluorescent

stain throughout the cross section. No discernible differences in fluorescent intensities were observed between sections taken from either method A or method B. Thus we were able to install thiophilic sites throughout the cross section of the bead.

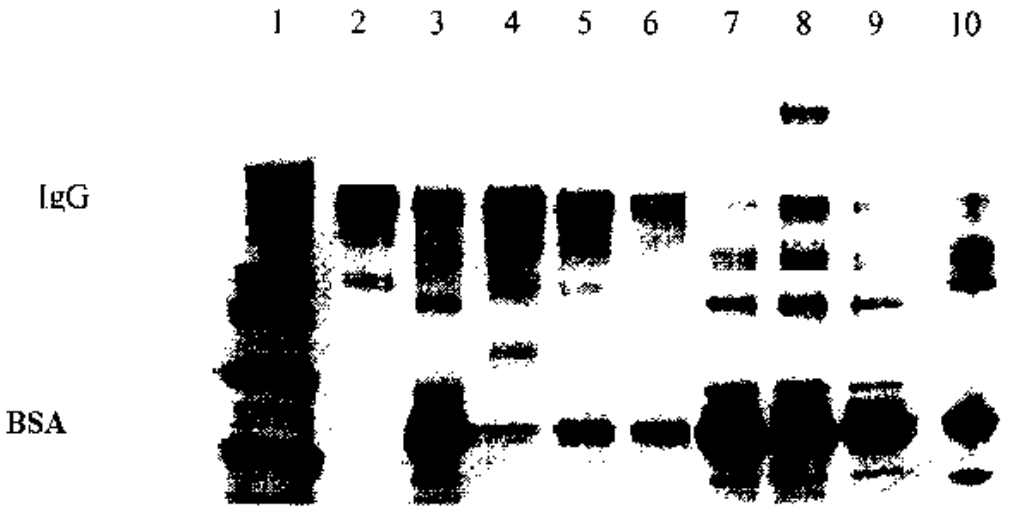

FIGURE 3 Sodium dodecylsulfate (0.1%)-polyacrylamide gel (8–16% gradient) electrophoresis of human IgG that was purified from serum using thiophilic cellulose beads. Lane 1 shows a molecular weight ladders. Lanes 2 and 3 show application of pure human IgG and human serum at a total protein level of 6 ygs, respectively. Lanes 4, 5 and 6 show application of elution fractions obtained from u = 1, 3, and 6 cm/min, respectively at a total protein level of 6 μgs. Lane 7, 8 and 9 shows the fall through fraction obtained from u = 1, 3, and 6 cm/min, respectively at a total protein level of 6 μgs. Lane 10 shows an application of pure BSA at a total protein level of 6 ygs

DISCUSSION

New matrix technology based on naturally occurring polysaccharides for large-scale protein separations have been recently developed with the goal of achieving process savings [20,21]. Recovery of research expenses is a leading thiophilic mode. We believe the true leaps in throughput and productivity in bioprocessing processing will result when the merits of large bead technology will be merged with new and improved activation or ligand immobilization strategies.

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