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Evaluation of polymer matrices for an adsorptive approach to plasma detoxification

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Acute liver failure arises when potentially toxic metabolites accumulate in the bloodstream because of a breakdown in liver function. New extracorporeal systems combining membrane and adsorbent technologies are being developed to replace critical liver detoxification functions between diagnosis and transplantation. This study addresses the adsorption of representative plasma components on four different hydrophobic, polymeric adsorbents for possible use in an extracorporeal hemodialysis device. The adsorbents considered span a range of pore sizes and include both strongly hydrophobic divinylbenzene (DVB) matrices as well as a less hydrophobic acrylate matrix. Adsorption equilibrium and rate measurements were made for these matrices using human serum albumin (HSA), polyclonal human immunoglobulin G (IgG), and bilirubin (BR), as representative plasma components. Pore size was found to contribute significantly to selectivity. Results demonstrated that strongly hydrophobic materials with pore sizes that allow free access to protein-bound BR are most effective for BR removal whether they are initially clean or pre-saturated with HSA.

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1. Introduction

Multi-organ failure and sepsis often develop from acute liver failure (ALF) when a variety of potentially toxic metabolites including ammonia, bilirubin, bile acids, and various cytokines accumulate in the bloodstream [1–3]. Many of these components bind strongly to plasma proteins, most notably HSA. As a result their removal by conventional dialysis techniques is inefficient since the protein–toxin complexes do not cross typical dialysis membranes. Extracorporeal systems where protein-bound toxins are removed by adsorption have thus been suggested to sustain patients between diagnosis and treatment procedures [4].

Two current devices in which both water-soluble toxins and protein-bound toxins are removed are the Molecular Adsorbents Recirculating System (MARS) [5–8] and the Prometheus (PROM) System [1,7,9,10]. Two types of adsorption columns are used in these systems, one containing activated carbon and the other an anion exchange resin. The activated carbon is efficient in binding bile acids, long chain fatty acids, phenols and other compounds with low aqueous solubility, while the anion exchange resin is employed in principle to remove bilirubin which is negatively charged at a physiological pH [11]. However, natural anticoagulants, such as heparin and protein C, also possess a large negative charge density at physiological conditions and have been observed to bind to anion exchangers causing coagulation problems within the system [12,13]. Thus, alternative adsorbents are desirable.

This work considers polymeric adsorbents with a range of hydrophobic character and pore size. The structure of these materials is characterized and adsorption equilibrium and rate measurements are made for representative plasma proteins. The relative ability of these materials to selectively remove HSA-bound bilirubin is then investigated.

2. Materials and methods

2.1. Materials

Four polymeric adsorbents were obtained from Dow Water & Process Solutions, formerly Rohm and Haas, (Philadelphia, PA, USA) for use in this study. Adsorbents A, B, and C are DVB-based, strongly hydrophobic materials, while adsorbent D is acrylate-based and less hydrophobic. All adsorbents were received in a 20 percent ethanol solution, except C which was dry. This sample was thus pretreated by first soaking in pure methanol for 1 h followed by washing with deionized, distilled water. Particle sizes were determined from microphotographs and were 85 ± 19, 83 ± 17, and 85 ± 15 μm for adsorbent A, B, and D, respectively. Microphotographs were not taken of adsorbent C, but the manufacturer estimated the particle size to be approximately 500 μm. Finely ground adsorbent C particles were also received dry from the manufacturer and hydrated using the same procedure as for the larger particles. Before use, all materials were washed with a PBS buffer containing 10 mM Na_2HPO_4, 150 mM NaCl buffer adjusted to a pH of 7.4 with phosphoric acid.

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Nomenclature

A_p	pore surface area (m^2/cm^3)
C	protein concentration in the bulk fluid (mg/cm^3)
C_o	initial protein concentration in the fluid phase (mg/cm^3)
C_{BR}	bilirubin concentration in fluid phase (mg/cm^3)
C_{HSA}	HSA concentration in fluid phase (mg/cm^3)
d_p	particle diameter (μm)
D_e	effective pore diffusivity (cm^2/s)
f_j	volume fraction of particles with radius r_{pj}
K_f	external film mass transfer coefficient (cm/s)
K	Langmuir isotherm equilibrium constant (cm^3/mg)
K_D	distribution coefficient for iSEC
q	adsorbed protein concentration (mg/cm^3)
$\bar{q}_j$	adsorbed protein concentration in particle j (mg/cm^3)
$\acute{q}$	average adsorbed protein concentration over all particles (mg/cm^3)
q_m	Langmuir isotherm adsorption capacity (mg/cm^3)
q_{BR}	total adsorbed bilirubin concentration (mg/cm^3)
q_{HSA}	total adsorbed HSA concentration (mg/cm^3)
r_p	particle radius (cm)
$\bar{r}_p$	volume-average particle radius (cm)
r_{pore}	average pore radius (nm)
r_{sj}	radial position of adsorption front in particle (cm)
R_s	viscosity radius of solute in iSEC (nm)
t	time (s)
V_A	volume of adsorbent particles (cm^3)
V_C	bed column volume (cm^3)
V_R	peak retention volume (cm^3)
V_S	volume of solution (cm^3)

Greek symbols

ε_b	extraparticle porosity
ε_p	intraparticle porosity
λ	capacity factor
ϕ_j	dimensionless particle radius
ρ_{sj}	dimensionless position of adsorption front
τ	dimensionless time

HSA (Cat. No. A8763), IgG (Cat. No. G4386), and BR (Cat. No. B4126) were obtained from Sigma Chemical Co (St. Louis, MO, USA) and were used as received. All chemicals and salts used in buffer preparation (sodium phosphate, sodium chloride, tris (hydroxymethyl) aminoethane, phosphoric acid, hydrochloric acid, isopropanol) were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Glucose and dextran probes used in size exclusion experiments were obtained from Sigma and GE Healthcare (Piscataway, NJ, USA), respectively.

2.2. Adsorbent characterization

The structural features and accessible pore size of each adsorbent were determined by transmission electron microscopy (TEM) and inverse size exclusion chromatography (iSEC) [14,15]. TEM images were obtained by dehydrating the adsorbent particles with a series of ethanol–water washes starting with pure water and ending with pure ethanol followed by embedding the particles in a Spurr's resin mixture as outlined in Martin et al. [16]. The resin mixture was prepared by mixing 10 g of vinyl cyclohexene dioxide, 4 g of diglycidyl polypropylene glycol ether, 26 g of nonenyl succinic anhydride, and 0.3 g of dimethyl aminoethanol. After saturating the dehydrated particles with this mixture and curing overnight at 70 °C, the samples were sectioned with a microtome into 80 nm slices, stained with uranyl acetate/lead acetate, and imaged by TEM (Model JEOL 1230) using a Model SIA12-C camera from Scientific Instruments and Applications, Inc. (Atlanta, GA, USA).

Inverse size exclusion chromatography (iSEC) was used to determine the accessible pore size and intraparticle porosity of each adsorbent from the retention of glucose and dextran standards with molecular masses between 10 and 2000 kDa. For this purpose, each adsorbent was flow packed in 0.5 × 20 cm Tricorn columns from GE Healthcare (Piscataway, NJ, USA). Mobile phases used in these experiments were dependent on the type of resin material used. For adsorbent D, the measurements were made in PBS buffer; however, for the strongly hydrophobic materials (A and B) 15% (vol/vol) isopropyl alcohol was added to the PBS buffer to prevent dextran adsorption. A Waters HPLC System (Milford, USA) with a Waters Model R401 refractive index detector was used to determine the retention volume of individual dextrans for 50 μL injections of 5 mg/cm^3 solutions at 1.0 cm^3/min. Retention volumes were calculated from the statistical first moments of the eluted standards. The extra-column volume of the system was estimated by running blank samples through an empty column with the inlet and outlet frits pushed together, and the actual retention volume of each probe was then corrected by subtracting the extra-column volume. A distribution coefficient, K_D, was calculated based on the following equation [17].

$$K_D = \frac{V_R/V_C - \varepsilon_b}{1 - \varepsilon_b} \quad (1)$$

where V_R is the retention volume, V_C is the bed volume, and ε_b is the extraparticle porosity. Since dextran 2000 is completely excluded from the pores of each adsorbent, the retention volume of this standard was used to estimate ε_b.

2.3. Adsorption studies

Equilibrium adsorption studies were conducted in solutions containing either of the two most prevalent plasma proteins, HSA and IgG, and also in solutions containing both proteins at a physiological ratio of 8:3 (mg:mg) [18]. Equilibrium adsorption isotherms were obtained by equilibrating a known mass of hydrated particles with solutions of varying initial protein concentration in sealed 1.5 cm^3 microcentrifuge tubes slowly rotated for 24 h. Prior to starting the experiment, the resin samples were centrifuged at 5000 rpm for 10 min to remove any interstitial buffer from the particles. For the smaller pore materials (adsorbents A and C) the particles were crushed and sieved to less than 38 μm to ensure equilibrium occurred in 24 h. Once equilibrium was achieved, the supernatant was analyzed either using a SpectraMax Plus[384] 96-well microplate spectrophotometer from Molecular Devices (Sunnyvale, CA USA) at 280 nm or by HPLC as described below. The amount of protein adsorbed was then determined by material balance. The hydrated particle density was also determined pycnometrically for each material and used to convert the amount of protein adsorbed from a mass basis to a volume basis in units of mg of protein per cm^3 of hydrated particle volume. Analysis of HSA/IgG mixtures was done on a Waters HPLC system using a 0.5 × 5 cm Tricorn column packed with Source 30Q anion exchange resin (GE Healthcare) with a 16 min, 0–0.45 M NaCl gradient. The final protein concentration was estimated by constructing a calibration curve of peak area versus protein concentration.

Bilirubin adsorption experiments were conducted for solutions containing HSA and BR in a molar ratio of 4:1. Previous studies showed that practically no BR exists free in solution at this ratio and the absorbance at 457 nm is independent of further addition of HSA [19,20]. Thus, in this case, the residual total HSA and total BR concentrations were determined spectrophotometrically. Furthermore, it was assumed that BR binds to HSA in a 1:1 ratio since HSA was found to have one primary binding site with a much lower dissociation constant than alternate, secondary binding sites [21–24].

The protein adsorption kinetics was also determined for each adsorbent using the apparatus described in Carta et al. [25]. For this purpose, samples of the adsorbents were suspended in 20 cm^3 of 2 mg/cm^3 protein solution in a beaker agitated at approximately 300 rpm by a 1.0 cm diameter blade immersion mixer. The supernatant concentration was then monitored by circulating the liquid with a Cole-Palmer peristaltic pump (Chicago, IL, USA) through a Model UV-1 detector (GE Healthcare, Piscataway, NJ, USA) at 280 nm. Absorbance values were converted to protein concentrations by calibration curves, and used to determine the adsorbed protein concentration by mass balance. The effective pore diffusivity, D_e, for each adsorbent was estimated by comparing experimental uptake curves with a pore diffusion model that assumes a rectangular isotherm [14]. All adsorption experiments were carried out at room temperature and using a PBS buffer adjusted to a physiological pH of 7.4 with phosphoric acid.

3. Results and discussion

3.1. Adsorbent characterization

Representative TEM images for adsorbents A, B, and D are shown in Fig. 1. In these images the lighter gray is the embedding resin, while the darker areas are the stained polymeric adsorbent matrix. All these materials exhibit a microgranular structure, which

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