Anders Heebøll-Nielsen - Research outputs - DTU Orbit (25/05/2019)

Critical evaluation and comparison of fluid distribution systems for industrial scale expanded bed adsorption chromatography columns

The hydrodynamic properties of an expanded bed contactor with 30 cm or 150 cm internal diameter, which employs a rotating or oscillating fluid distributor, were compared to prototype columns of 60 cm or 150 cm diameter employing local stirring (fixed wall nozzles plus central bottom mounted stirrer) for fluid distribution. Fluid introduction through a rotating fluid distributor was found to give superior hydrodynamic characteristics in the 30 cm and 150 cm diameter column compared to using the local stirrer in both the 60 cm and 150 cm diameter columns. The shortcomings of the local stirring distributor at large scale were apparent: dead zones were present which could not be removed by increasing rotation rates or flow rates, and such changes led to a deterioration in hydrodynamic properties. In contrast, during fluid introduction through a rotating distributor no dead zones were observed, and residence time distribution tests showed that plate numbers remained constant or increased slightly as flow rate was raised from 200 cm h(-1) to 470 cm h(-1). Under the conditions studied, oscillation of the rotating fluid distributor led to increased mixing and poorer performance than rotary movement. The results imply that further improvement in distributor design is needed and careful attention should be given to the trade off between turbulence and adequate fluid distribution.

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Fractionation of whey proteins with high-capacity superparamagnetic ion-exchangers

In this study we describe the design, preparation and testing of superparamagnetic anion-exchangers, and their use together with cation-exchangers in the fractionation of bovine whey proteins as a model study for high-gradient magnetic fishing. Adsorbents prepared by attachment of trimethyl amine to particles activated in sequential reactions with allyl bromide and N-bromosuccinimide yielded a maximum bovine serum albumin binding capacity of 156 mg g(-1) combined with a dissociation constant of 0.60 muM, whereas ion-exchangers created by linking polyethylene imine through superficial aldehydes bound up to 337 mg g(-1) with a dissociation constant of 0.042 muM. The latter anion-exchanger was selected for studies of whey protein fractionation. In these, crude bovine whey was treated with a superparamagnetic cation-exchanger to adsorb basic protein species, and the supernatant arising from this treatment was then contacted with the anion-exchanger. For both adsorbent classes of ion-exchanger, desorption selectivity was subsequently studied by sequentially increasing the concentration of NaCl in the elution buffer. In the initial cation-exchange step quantitative removal of lactoferrin (LF) and lactoperoxidase (LPO) was achieved with some simultaneous binding of immunoglobulins (1g). The immunoglobulins were separated from the other two proteins by desorbing with a low concentration of NaCl (less than or equal to0.4 M), whereas lactoferrin and lactoperoxidase were co-eluted in significantly purer form, e.g. lactoperoxidase was purified 28-fold over the starting material, when the NaCl concentration was increased to 0.4-1 M. The anion-exchanger adsorbed beta-lactoglobulin (beta-LG) selectively allowing separation from the remaining protein.

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Superparamagnetic adsorbents for high-gradient magnetic fishing of lectins out of legume extracts

This work presents the development, testing, and application in high-gradient magnetic fishing of superparamagnetic supports for adsorption of lectins. Various approaches were examined to produce affinity, mixed mode, and hydrophobic charge induction type adsorbents. In clean monocomponent systems affinity supports created by direct attachment of glucose or maltose to amine-terminated iron oxide particles could bind concanavalin A at levels of up to approximate to 280 mg g\(^{-1}\) support with high affinity (approximate to 1 \(\mu\)M dissociation constants). However, the best performance was delivered by adsorbents featuring coupled tentacular dextran chains displaying a maximum binding capacity of 238 mg g\(^{-1}\) and a dissociation constant of 0.13 \(\mu\)M. Adsorbents derivatized with mixed mode or hydrophobic charge induction ligands likewise demonstrated very high capacities for both concanavalin A and Lens culinaris agglutinin (greater than or equal to 250 mg g\(^{-1}\)) with dissociation constants in the micromolar range, though neither of these systems showed any selectivity for lectins in leguminous extracts. When the affinity supports were applied to carbohydrate containing legume extracts only the dextran-linked adsorbents supplied sufficient competition to dissolved sugars to selectively bind concanavalin A in an extract of jack beans. The dextran-linked supports were employed in a high-gradient magnetic fishing experiment, in which concanavalin A was purified to near homogeneity from a crude, unclarified extract of jack beans.

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Superparamagnetic cation-exchange adsorbents for bioproduct recovery from crude process liquors by high-gradient magnetic fishing

Different routes were screened for the preparation of superparamagnetic cation-exchange adsorbents for the capture of proteins using high-gradient magnetic fishing. Starting from a polyglutaraldehyde-coated base particle, the most successful of these involved attachment of sulphite to oligomers of epichlorohydrin formed on the particle surface. The resultant cation-exchanger had a maximum lysozyme binding capacity of 272 mg g\(^{-1}\) and a dissociation constant of 0.73 \(\mu\)M. Using lysozyme as a model protein in small-scale studies, appropriate conditions were then selected for the capture of lactoperoxidase from sweet bovine whey. Subsequently, a high-gradient magnetic fishing process was constructed for the fractionation of whey, in which lactoperoxidase was purified 36-fold and concentrated 4.7-fold.

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Efficient inclusion body processing using chemical extraction and high gradient magnetic fishing

In this study we introduce a radical new approach for the recovery of proteins expressed in the form of inclusion bodies, involving (W) chemical extraction from the host cells, (ii) adsorptive capture of the target protein onto small magnetic adsorbents, and (iii) subsequent rapid collection of the product-loaded supports with the aid of high gradient magnetic fields. The manufacture and testing of two types of micron-sized nonporous superparamagnetic metal chelator particles derivatized with iminodiacetic acid is described. In small-scale adsorption studies conducted with a hexahistidine tagged form of the L1 coat protein of human papillomavirus type 16 dissolved in 8 M urea-phosphate buffer, the best binding performance (Q(max) = 58 mg g(-1) and K-d similar to 0.08 muM) was exhibited by Cu2+-charged type II support materials. Equilibrium adsorption of L1 to these nonporous supports was achieved very rapidly (100 mM imidazole in the equilibration buffer. The influence of feedstock complexity on L1 adsorption to the Cu2+-charged type II magnetic chelators was studied using various dilutions of four crude chemical E. coli cell extracts containing denatured L1 protein. Undiminished L1 adsorption to these adsorbents (relative to the 8 M urea-phosphate buffer case) was observed with the least complex of these feed materials, i.e., a partially clarified (12 g dry weight L-1) and spermine-treated chemical cell extract (feedstock B). Efficient recovery of L1 from feed B was demonstrated at a 60-fold increased scale using the high gradient magnetic fishing (HGMF) system to collect loaded Cu2+-chelator particles following batch adsorption of L1. Over 70% of the initial L1 present was recovered within the HGMF rig in a highly clarified form in two batch elution cycles with an overall purification factor of similar to10.
A new fluid distribution system for scale-flexible expanded bed adsorption

A new fluid distribution system designed for expanded bed adsorption was introduced and studied in a 150-cm diameter column. Based on fluid application through a rotating distributor, it eradicates the need for perforated plates, meshes, or local mixers. The effect of rotation rate on column performance was examined by fluidizing a 30-cm high bed of supports with tap water and introducing pulses of dye or acetone tracer. Linear bed expansion was seen as the superficial fluid velocity raised from 170 cm$^\text{h}^{-1}$ to 450 cm$^\text{h}^{-1}$ (3000 L$^\text{h}^{-1}$ to 8000 L$^\text{h}^{-1}$), and there was little change in expansion characteristics as distributor rotation rate was increased from 2.5 to 10 rpm. The distributor was observed to generate a flow pattern suitable for expanded bed adsorption when the supports were fluidized at a superficial fluid velocity of 283 cm$^\text{h}^{-1}$ and dye pulses introduced. At a rotation rate of 2.5 rpm, no significant dead zones were observed, and a discrete band was formed that moved up through the bed. Furthermore, the pattern of dye movement could be used to calculate interstitial linear fluid velocities of 460 cm$^\text{h}^{-1}$ and 572 cm$^\text{h}^{-1}$ at the column wall and center, respectively, indicating a parabolic flow profile. The distributor rotation rate giving the best operating conditions was found to be 2.5 rpm when the bed was fluidized at a flow velocity of 283 cm$^\text{h}^{-1}$ and the residence time distribution of acetone tracer examined. Under these conditions, the coefficient of axial dispersion was 6.1 x 10$^{-6}$ m$^2\text{s}^{-1}$ and 29 theoretical plates were measured. When the rotation rate was raised to 10 rpm, the coefficient of axial dispersion increased to 8.08 x 10$^{-6}$ m$^2\text{s}^{-1}$ and the number of theoretical plates decreased to 22.