

EVALUATION OF HYDRODYNAMIC PARAMETERS OF FLUIDIZED BED ADSORPTION ON PURIFICATION OF NANOBIOPRODUCTS

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The majority of biotechnology processes for producing pharmaceutical or diagnostic products involve the purification of proteins and peptides from a variety of sources. Typically, such purification schemes contain multiple unit operations, including a number of chromatographic steps to ensure the safe removal of critical impurities and contaminants. Each step in the recovery process will affect the overall process economy by increasing operational cost and process time

Nanoparticles are of colloidal size and they are generally sophisticated biological structures composed of one or more types of proteins, lipid and/or nucleic acids and subsequently various problems are associated with their recovery and purification. Nanoparticle sizes are defined from 10 to 1000 nm. Protein nanoparticles generally vary in size from 50-300 nm.

Expanded bed adsorption (EBA) is a novel primary recovery technology allowing the adsorption of target proteins directly from unclarified feedstock without the risk of blocking the bed which innovatively combines solid-liquid separation with an adsorption step in a single unit operation. Expanded bed adsorption is based on controlled stable fluidization, thus combining the hydrodynamic properties of a fluidized bed with the chromatographic properties of a packed bed. Physical parameters are the parameters related to the hydrodynamics and stability of a homogeneous fluidization in the expanded bed. The **Residence Time Distribution (RTD)** analysis was used to acquire hydrodynamic information from changes bed expansion and column diameter, settled bed height to the shape of a tracer pulse as it passed from the expanded bed. The adsorption of **bovine serum albumin (BSA) nanoparticle** on STREAMLINE DEAE, were used to examine how adsorption behaviour was affected by the hydrodynamics of bed by changing bed condition (degree of expansion). In the present work, protein nanoparticles fabricated from bovine serum albumin (BSA) was purified by expanded bed adsorption on the streamline DEAE adsorbent.

- The material was used in this work: BSA, Tween-20, ethanolamine, glutaraldehyde (25% solution), supplied by Sigma Aldrich. Streamline DEAE was provided by Amersham bioscience. Sodium azide from Merck (Germany). all other chemicals were of analytical grade from local sources.
- Preparation of BSA nanoparticle: The bovine serum albumin nanoparticles (BSANPs) were prepared by a coacervation method and chemical cross-linking with glutaraldehyde.
- Glass columns (1.3cm×25 cm, 1.6cm×20cm, 2cm×15cm) were used, which had a top adaptor and a bottom flow distributor. The Liquid from the outlet of the column was transferred through the UV detector. The particle size range for adsorbent is 100-300µm. the approximate mean particle size is 200µm and the approximate mean particle density is 1.2g/ml.
- Estimation of bed voidage and RTD study, bed expansion characteristics and axial dispersion calculation: Tris-Hcl buffer at a 10mM concentration and at pH 7.6 were used as fluidizers. Acetone solution (10%, w/w) as a tracer was injected at the bottom of the column. : Bed voidage(ϵ), number of theoretical plates (N) of the column, (HETP) obtains from following equations:

$$\varepsilon = 1 - \frac{V_p}{V_L} = 1 - \frac{V_p}{A_T \cdot H} = 1 - \frac{m_p}{\rho_p A_T \cdot H} \quad (1), \quad N = \frac{t_r^2}{\sigma_t^2} = \frac{L}{H} \quad (2), \quad \text{HETP} = \frac{t^2}{\sigma^2} \times H \quad (3)$$

It was known that the Richardson–Zaki equation Eq.4 conducted an extensive investigation of the expansion behavior of particles at different velocity. The Bodenstein number (Bo) is a dimensionless term that is often used to relate the convective transport of liquid to dispersion Eq.5. Bo number can be calculated from Eq.6. results show in fig.1.

$$\frac{u}{u_t} = \varepsilon^n \quad (4), \quad B_0 = \frac{UH}{D_{axl} \cdot \varepsilon} \quad (5), \quad \frac{1}{N} = \frac{2}{B_0} + \frac{8}{B_0^2} \quad (6)$$

- The bed expansion characteristics for the streamline DEAE in 1.3cm×25cm column was shown in fig.2. H/H_0 increases linearly at the same flow rate with increasing buffer viscosity. The experimental value of n, 4.77 was obtained fig.3. Results show a good agreement between the experimental and theoretical value of n and u_t .
- The time courses of BSA nanoparticle purification were carried by the EBA on columns with different degree of expansion. Yield of BSA nanoparticle recovery in expanded bed adsorption with different degree of expansion was showed in fig.4.
- The RTD study showed that HEPT, axial dispersion increased with bed height, bed voidage and linear velocity. At 6cm of bed height, it is the best system for BSA nanoparticle recovery by the expanded bed chromatography in streamline DEAE adsorbent. The Best yield of recovery of BSA nanoparticle in optimal condition 80.71% was achieved.

References:

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Figures:

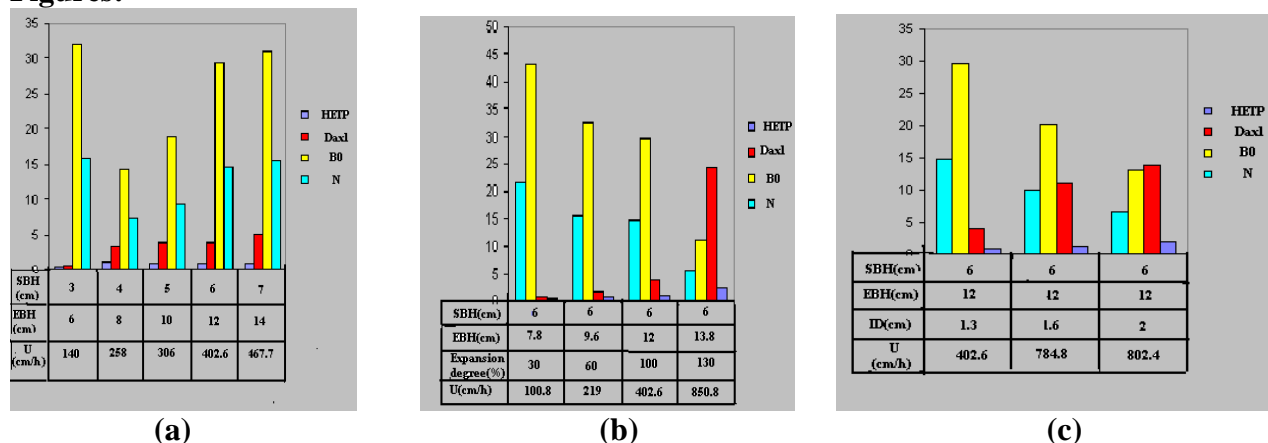


Fig1 .Hydrodynamic parameters of the liquid phase estimated expanded bed operations (a) The effect of the settled bed height (b) The effect of optimum bed expansion (c) The effect of column diameter

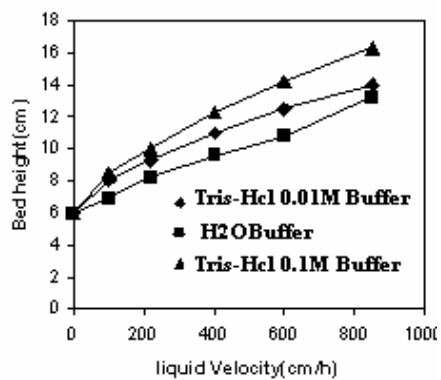


Fig.2.Expanded bed height of streamline DEAE(SBH=6cm) with varying flow rate in a Tris/Hcl buffer system

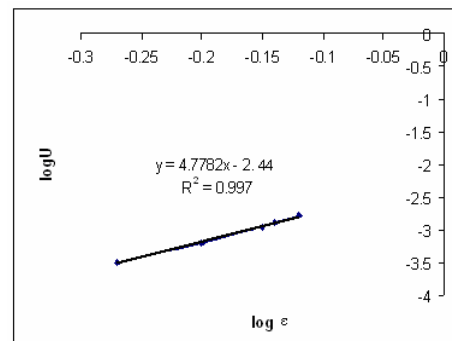


Fig.3.Richardson-Zaki correlation between flow velocity and bed voidage For experimental calculation of n and u

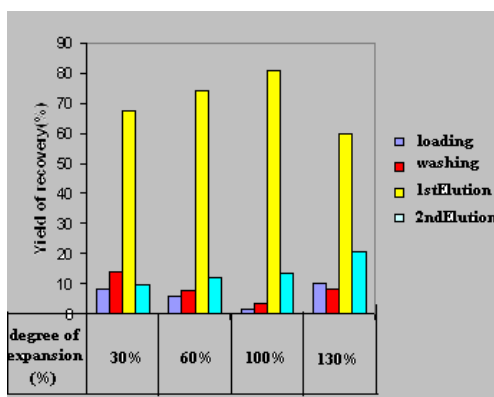


Fig.4. Yield of BSA nanoparticle recovery in expanded bed adsorption