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BIOMECHANICAL DESIGN FACTOR FOR SOFT GEL CHROMATOGRAPHY COLUMNS TO SEPARATE HOMOLOGOUS, HIGH MOLECULAR WEIGHT, THERAPEUTIC PROTEINS

Wendy W. He [1] and Duane F. Bruley [2]

1. INTRODUCTION

Protein C (model molecule) is an anti-coagulant in blood. It also possesses anti-thrombotic and anti-inflammatory characteristics that are very effective in treating blood clotting phenomena found in many disease states. Recombinant production of Protein C and recovery from human plasma via immuno affinity chromatography are both very expensive technologies for the manufacture of a protein C product. Immobilized metal affinity chromatography (IMAC) is being examined as a less expensive process for Protein C purification from blood plasma. The purpose of this investigation is to examine how the mechanical characteristics of chromatographic resins influence the biomechanical design of chromatography columns. It is crucial for preparative chromatography that column scale-up takes into consideration the collapse potential of the gels because of the large cost and inconvenience of such an episode. Also, it is desirable to minimize process cycle-time considering resin mechanical properties to achieve economical preparative scale production of a desired protein. It is also true that a resin can have desirable mechanical characteristics but the surface chemistry for adsorption of the desired product might be inappropriate for the separation and purification of the specific product. Therefore, it is important to consider both the biomechanical and the biochemical characteristics of the resin systems to attain optimal process design and performance for reduced production cost.

This reseach has established theoretical and experimental guidlinelines and relationships for the understanding of column behavior and the scale-up of Immobilized Metal Affinity Chromatography (IMAC) [1] and Immuno Affinity Chromatography for the low cost production of Protein C.

Gel matrices for Protein C separation [2] have been analyzed here to determine the relationship between mechanical characteristics of the gel, flow conditions and column collapse potential have been analyzed here to determine the relationship between mechanical characteristics of the gel, flow conditions and column collapse potential.

[1] Wendy W. He, Assistant Professor, Lake Superior State University;
[2] Duane F. Bruley, Professor, University of Maryland Baltimore County/Synthesizer, Inc.

Using Biot's Consolidation Theory,[3] Darcy's Law and experimental data of flow rate vs. pressure drop, a column design relationship was determined. The design factor provides a first approximation for column scale-up without the occurrence of the catastrophic phenomena of column collapse.

2. MATERIALS AND METHODS

Due to availability, the primary gel tested was Sepharose 2B linked with Protein C monoclonal antibody, donated by the American Red Cross.

Experimental studies were performed to determine the correlation of the flow rate vs. pressure drop for a chromatography column (MT 20 column), packed with Sepharose 2B. The fluid flow rate and pressure drop (ΔP) were examined to determine how they influence column mechanical stability.

Specific MT 20 columns, 15 mm diameter and 130 mm length, were purchased from the Bio-Rad company (Hercules, California). The gel slurry was equilibrated with water until all ethanol was removed. The gel was then placed in a flask under vacuum for thirty minutes. Next, the degassed gel was injected in small quantities into the column, using a pipette, to a height of about 80 mm. The column was allowed to settle until there was no further reducton in gel height. This step was done overnight, before doing a flow rate vs. pressure drop experiment, to allow the gel matrix to achieve most of the expected bulk compression.

A packed column was connected to a triaxial pressure panel and cell. First, measurements were made at pressure increments starting from a minimum gauge pressure drop of zero psi. Using 5 psi increments, the gauge pressure drop reached a maximum (100psi) after which the pressure drop increments were reversed and data were recorded at 5 to 10 psi increments back to zero. The pressure drop across the column was read from a digital pressure gauge on the control panel. After each pressure increment the column was allowed to achieve steady state and then the time for 20ml of water to flow through the column matrix was recorded. The water flowed directly through a tube at the bottom of the chromatography column and into a burette at atmospheric pressure.

3. THEORETICAL MODEL

Biot's Consolidation Theory was reduced to one dimension and time to develop a set of systems equations for the microscopic theoretical analysis of the column. These equations describe the pore pressure distribution and the solid skeleton displacement in the porous media (gel).

Neglecting the initial effective stress and body forces and assuming a one-dimensional, time-dependent process along the axial (z) direction, the governing equations for the solid and fluid respectively are:

$$(\lambda + 2G)\frac{\partial^2 u}{\partial z^2} - \frac{\partial p}{\partial z} = 0 \tag{1}$$

$$\frac{\partial}{\partial z}[\frac{k}{\mu}(\frac{\partial p}{\partial z})] = (1-\phi)C_s\frac{\partial p}{\partial t} + \frac{\partial^2 u}{\partial t \partial z} \tag{2}$$