412b Utilizing Gibbs Free Energy to Optimize the pH and Nacl Operating Conditions of Immobilized Metal Affinity Chromatography for Protein C Purification

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Protein C (PC) is an important anticoagulant, antithrombotic, and anti-inflammatory in blood plasma. Clinical potential for PC as a therapeutic include patients with PC deficiency, advanced sepsis, or patients with major surgery or trauma. PC does not have any known clinical side effects, such as bleeding and skin necrosis that have been observed with patients using coumadin and heparin as an anticoagulant treatment. However, the purification of PC from plasma is difficult due to its homology to other plasma proteins, most of which are coagulants. Immunoaffinity chromatography (IAC) is the current method used for purifying PC, but it is very expensive. Immobilized metal affinity chromatography (IMAC) has demonstrated the specificity to PC at a particular operating condition that provided separation from prothrombin, a protein structurally homologous to PC but a coagulant. Cohn Fraction IV-1, a source known to have a large amount of PC, has usually been a discarded side-stream from the blood plasma fractionation process collecting human serum albumin and several other blood factors. IMAC has shown a high PC yield from Cohn Fraction IV-1. A thermodynamic approach has been taken to optimize the IMAC process for PC purification from Cohn Fraction IV-1. Gibbs free energy (ΔG^o) is the measure of energy in a reaction system toward equilibrium, and negative ΔG^o values indicate reactions favorable. ΔG^o can be used to characterize the adsorption reaction of proteins during an IMAC process. The pH affects the electron-donor and acceptor properties of the protein and metal ion, and the NaCl concentration controls the hydrophobic interactions of the protein to the IMAC gel matrix. Isothermal titration calorimetry (ITC) measurements were used to obtain ΔG^os for the adsorption of PC and other homologous proteins at various IMAC operating conditions of pH and NaCl concentrations.