XXI SIMPÓSIO NACIONAL DE BIOPROCESSOS
XII SIMPÓSIO DE HIDRÓLISE ENZIMÁTICA DE BIOMASSA

3 a 6 de setembro de 2017
Aracaju, Sergipe, Brasil

HUMAN IgG ADSORPTION BY AFFINITY CHROMATOGRAPHY USING FPLC CONTICHROM

Santangela O. Santos[1], Diego R. Gondim[1], Diana C. S. Azevedo[1] and Ivanildo J. Silva Jr[1].

[1] Universidade Federal do Ceará, Departamento de Engenharia Química

E-mail: ivanildo@gpsa.ufc.br

ABSTRACT

Purification of human IgG by dye-ligand affinity chromatography was investigated. Procion Red and Reactive Red 120 dyes were immobilized on epoxide chitosan/alginate. Chromatographic experiments were conducted by using binary solution (IgG and human serum albumin-HSA) and human serum solution. The results showed that IgG was preferentially retained in both chromatographic matrices according to the electrophoresis analyze.

1. INTRODUCTION

Immunoglobulins G (IgG) stand out as the major proteins of the immune system, so they are commonly referred to as antibodies. These proteins are indicated for the treatment of various diseases, such as cancer, infectious diseases and selective antibody deficiencies (Burnouf, 2007). Affinity chromatography is the main technic for protein adsorption and offers a significant advantage for antibody purification (Ayyar *et al*. 2012).

The objective of this work was the IgG purification using immobilized dyes (Reactive red 120 and procion red MX-5B) on epoxide chitosan/alginate. Comparative experiments were performed for IgG purification using two commercial affinity matrices (Protein A HP and Cibacron Blue HP) using FPLC Contichrom system.

2. MATERIALS AND METHODS

2.1. Synthesis of Chitosan /Alginate Epoxide and Dye Immobilization

The synthesis of the epoxide chitosan/alginate (QAE) was made according to Gondim *et al*. (2012). Reactive Red 120 (RV120) and Procion Red MX-5B (PR-MX) dyes were immobilized in composite according to Ruckenstein and Zeng (1998) with some modification (Gondim *et al*. 2012). The composites were identified as QAE-RV120 and QAE-PR-MX.

2.2. Adsorptions experiments in FPLC

Chromatography experiments were performed using QAE-RV120 and QAE-PR-MX and two commercial columns (Hitrap Protein A HP and Hitrap Cibacron Blue HP). Binary solutions of IgG and HSA, and diluted human serum were injected in FPLC Contichrom system. Experiments conditions: injection volume (3.0 mL), flow rate (0.8 mL min^{-1}), adsorption buffer (TRIS-HCl 25 mM, pH 7.2), elution buffer (NaCl 1.0 M add in adsorption buffer), and proteins concentrations (1.0 mg mL^{-1} for binary solutions and 4.0-5.0 mg mL^{-1} for human serum). All samples during chromatography procedure were collected and analyzed by electrophoresis. Concentrations containing IgG and HSA were determined by absorbance in the UV-Vis light at wavelength 280 nm (UV-Vis Biomate 3, ThermoScientific, USA). Human serum samples were quantified according to Bradford's methodology (Bradford, 1976).

3. RESULTS AND DISCUSSIONS

In previous experiments performed in stirred tank systems, a significant IgG adsorption capacity was observed, and both dyes demonstrated acting as medium affinity ligands. Therefore, the continuation of this study was made under the ideal conditions obtained in stirred tank.

Figure 1 show the electrophoresis analysis of the adsorption samples and the elution of the two tests. At pH 7.2 HSA shows a negative apparent charge, whereas IgG is exactly at its pI, thus with apparent net charge. Affinity adsorption is a highly dependent pI technique, since in pI there is a decrease in protein-protein interactions thus facilitating interactions between protein and affinity linker. According to the electrophoresis, it was possible to confirm that the only protein adsorbed was IgG, which proves the potential of these stationary phases for selective IgG adsorption.

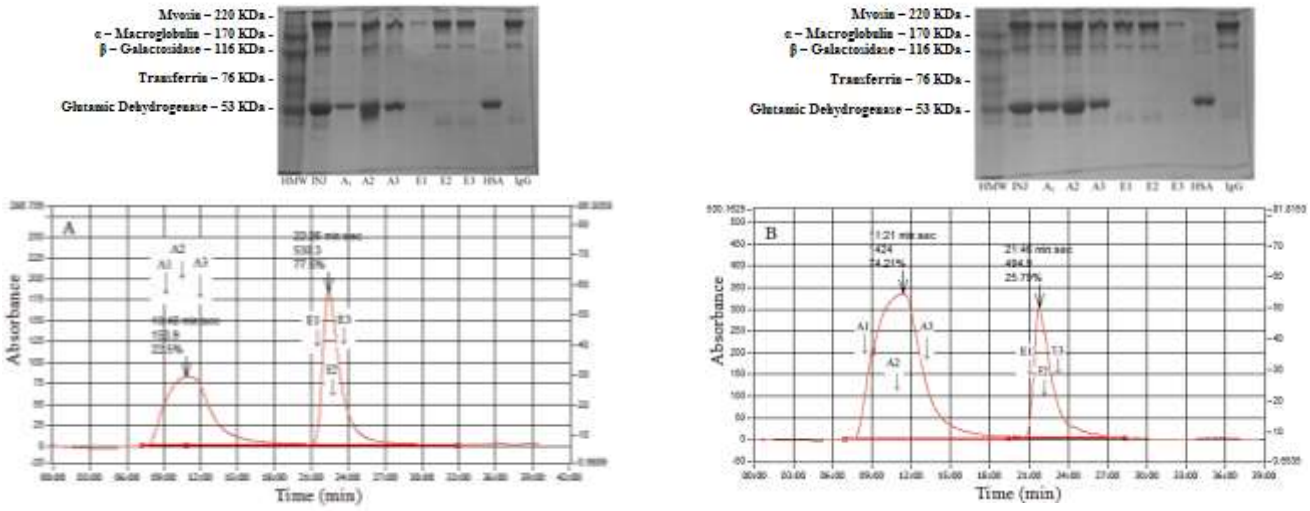
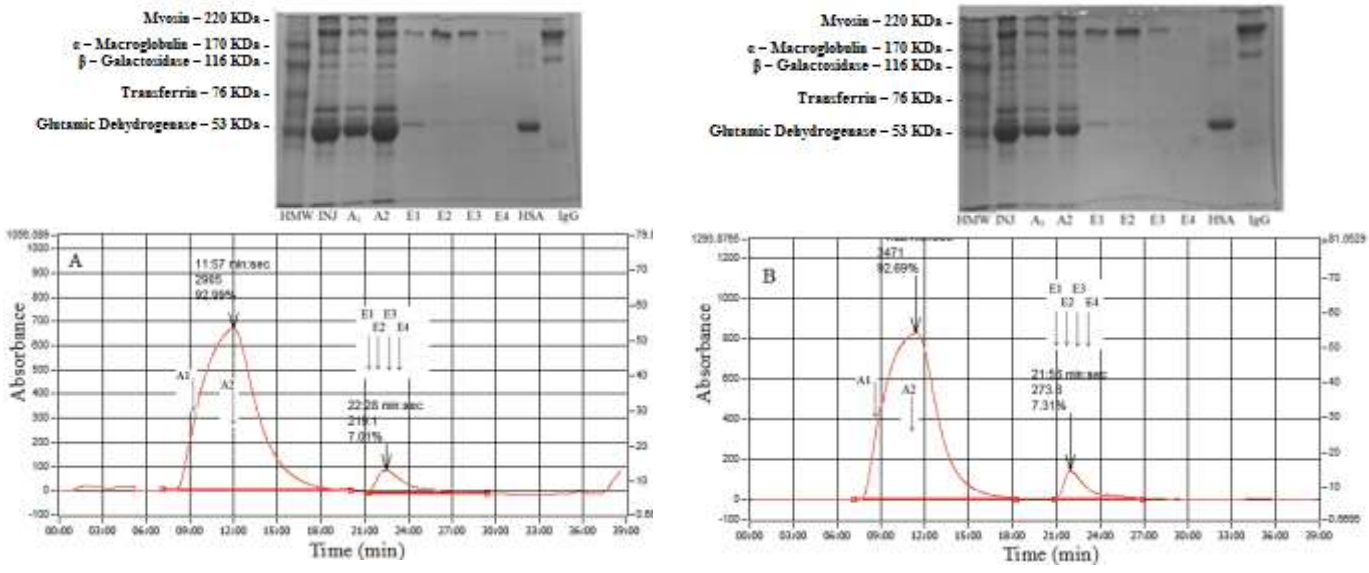


Figure 1. Chromatograms profiles using FPLC Contichrom and injection of a binary mixture solution containing IgG/HSA (1.0 mg ml^{-1}) using QAE-RV120 (A) and QAE-PR-MX (B). Electrophoresis: Molecular weight marker (HMW); Injection (INJ); Adsorption (A1 and A2), Elution (E1, E2, E3 and E4).

Figure 2 shows the chromatographic profiles for a human serum injection using all four affinity matrices. It was possible to note that most of the proteins were not adsorbed on QAE-RV120 and QAE-PR-MX. However in elution fractions, the proteins were essentially composed for IgG. From electrophoresis analyses of the elution fractions shows an great potential for human IgG purification.



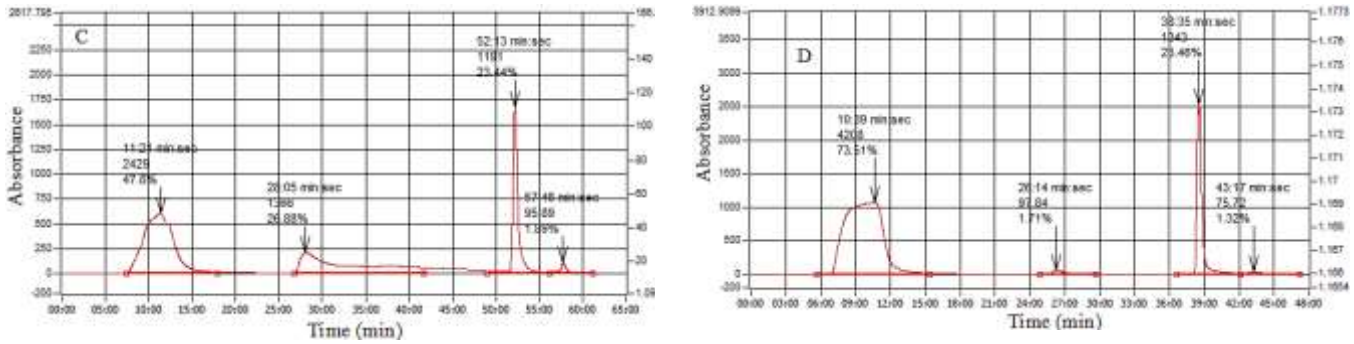


Figure 2. Chromatographic profiles of FPLC Contichrom assays with human serum using affinity matrices: QAE-RV120 (A), QAE-PR-MX (B), HCB (C) and HPA (D). Electrophoresis: Molecular weight marker (HMW); Injection Sample (INJ); Adsorption samples (A1 and A2), Elution samples (E1, E2, E3 and E4).

4. CONCLUSIONS

Efficiency of dye ligand epoxide chitosan/alginate for selective IgG purification were confirmed by electrophoresis analysis of the eluted fractions. When compared to commercial resins, it was possible to verify that even though they did not provide interaction as strong as that of the protein A ligand, these matrices provided IgG fractions with a high apparent degree of purity. Commercial resins proved to be powerful affinity matrices for protein purification. However, its costs and drastic elution conditions make these the main reasons for investing in the use of the QAE-RV120 and QAE-PR-MX matrices synthesized in the present work.

5. REFERENCES

Ayyar, B. V. et al. Affinity chromatography as a tool for antibody purification. Methods, v. 56, n. 2, p. 116-29, Feb 2012. ISSN 1046-2023.

Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem, v. 72, p. 248-54, May 07 1976. ISSN 0003-2697 (Print) 0003-2697.

Burnouf, T. [Plasma fractionation in the world: current status]. Transfus Clin Biol, v. 14, n. 1, p. 41-50, May 2007. ISSN 1246-7820 (Print) 1246-7820.

Gondim, D. et al. Dye Ligand Epoxide Chitosan/Alginate: A Potential New Stationary Phase for Human IgG Purification. Adsorption Science & Technology, v. 30, n. 8, p. 701-712, 09/01/ 2012.

Ruckenstein, E.; Zeng, X. Albumin separation with Cibacron Blue carrying macroporous chitosan and chitin affinity membranes. Journal of Membrane Science, v. 142, n. 1, p. 13-26, 2/2/ 1998. ISSN 0376-7388.