335412 Monolith Technology In Isolation Of Human Immunoglobulin G Subclasses

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Human immunoglobulin G (hIgG) constitutes an important therapeutic antibody for a number of diseases. There is an increasing demand for highly purified hIgG and its subclasses due to the wide application in *in vitro* diagnostics and immunotherapy. (1) hIgG consists of four subclasses (IgG1, IgG2, IgG3, and IgG4) that show small differences in some of their physicochemical characteristics and biological properties. Quantitatively, the relative serum concentrations of the human IgG subclasses are as follows: IgG1 > IgG2 > IgG3 ≈ IgG4. The four subclasses show more than 95% homology in the amino acid sequences of the constant domains of the γ -heavy chains. (2) Protein A affinity chromatography is a common method for antibody purification due to its well-characterized structure and high affinity to IgG. (3) To our knowledge, until now Protein A **monolith** column has not been used for the isolation of individual hIgG subclasses. Monolithic columns have attracted significant attention for the purification of large biomolecules due to its flow independent binding capacity that enables rapid separations at high flow rates. (4)

In the present study, a rapid separation method was developed to obtain pure hIgG subclass fractions with monolith technology. pH elution was applied using Protein A monolith column in a stepwise method and conditions were optimized. Since IgG3 has very low affinity to Protein A, elution was occurred immediately upon injection of the sample. IgG1 and IgG4 were eluted simultaneously under the applied conditions. Furthermore, IgG2 was separated with significant purity. Results indicated that the separation between IgG2 and IgG1- 4 could be achieved within 20 min run using a Protein A monolith column. The separation process time could be reduced more by increasing the flow rate without any loss in selectivity. Furthermore optimization of the mobile phase composition is under investigation for the efficient separation of IgG1 and IgG4. According to the present results, monolithic Protein A column could be proposed as a time efficient separation media for the enrichment of IgG2 which is the most often directed antibody against bacterial pathogens. (5)

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