ABSTRACT

The rapid evolution of gene therapy, DNA vaccination and clinical diagnostics has resulted in an increased interest in producing large quantities of pharmaceutical grade plasmid DNA and Immunoglobulin M (IgM). Biological drugs based on Plasmid DNA show high potential in a wide range of areas including preventive and therapeutic vaccines for viral, bacterial and parasitic diseases as well as chronic diseases and cancer. Extensive research in the last two decades has led to the realization of Immunoglobulin M (IgM) as a potential therapeutic and diagnostic agent for autoimmune diseases, infectious diseases, acquired immune deficiency syndrome (AIDS) and cancer vaccine. Growing interest in these two molecules has created a need for accurate, rapid and simple analytical methods to measure plasmid DNA and IgM concentrations during the production (in-process control) in cell culture supernatants as well as in all purification steps within the downstream processing and needs to fulfil the quality requirements of regulatory authorities.

In general, purification process of plasmid DNA consists of several unit operations starting with alkaline lysis, followed by different combinations of filtration, precipitation and several chromatographic steps. One of the key steps in purification of plasmid DNA vaccines for therapeutic use is the separation of supercoiled and open circular isoforms of plasmid DNA. In order to track product quantity and the separation of isoforms throughout the downstream process as well as to verify the separation quality, accurate, reliable and user-friendly analytical methods need to be in place.

In the first part of my doctoral thesis a development of a new monolithic analytical column for an HPLC determination of plasmid DNA quantity and quality is presented. The analytical performance of the column was tested for different samples from various steps of the manufacturing process, starting from fermentation to final product. The performance of the newly developed column was compared to some of the established analytical methods. Differences between these methods are briefly discussed. The applicability of new methods based on analytical monolithic columns for fast and efficient analysis of plasmid DNA based on therapeutics during their production and final quality control is elaborated. In addition, different parameters that influence the behavior of plasmid DNA during the separation process on anion-exchange columns is presented and discussed.

In the second part of my thesis, an immunoaffinity monolithic analytical column with immobilized protein A was developed and applied for the analysis of IgM. Different types of monolithic matrices together with various immobilization strategies were applied. After selecting the best matrix and immobilization conditions, a thorough characterization of the optimal affinity analytical column in terms of its analytical performance was carried out. In particular, chromatographic conditions were selected and optimized in a way that a highly reproducible, robust and efficient method for qualification and quantification of IgM from different process samples was obtained.

Keywords: plasmid DNA, Immunoglobulin M (IgM), monolithic analytical column.