World Academy of Science, Engineering and Technology
International Journal of Biotechnology and Bioengineering
Vol:12, No:04, 2018

Preparation of Pegylated Interferon Alpha-2b with High Antiviral Activity Using Linear 20 KDa Polyethylene Glycol Derivative

Authors : Ehab El-Dabaa, Omnia Ali, Mohamed Abd El-Hady, Ahmed Osman

Abstract : Recombinant human interferon alpha 2 (rhIFN-α2) is FDA approved for treatment of some viral and malignant diseases. Approved pegylated rhIFN-α2 drugs have highly improved pharmacokinetics, pharmacodynamics and therapeutic efficiency compared to native protein. In this work, we studied the pegylation of purified properly refolded rhIFN-α2b using linear 20kDa PEG-NHS (polyethylene glycol- N-hydroxysuccinimidyl ester) to prepare pegylated rhIFN-α2b with high stability and activity. The effect of different parameters like rhIFN-α2b final concentration, pH, rhIFN-α2b/PEG molar ratios and reaction time on the efficiency of pegylation (high percentage of monopegylated rhIFN-α2b) have been studied in small scale (100µl) pegylation reaction trials. Study of the percentages of different components of these reactions (mono, di, polypegylated rhIFN-α2b and unpegylated rhIFN-α2b) indicated that 2h is optimum time to complete the reaction. The pegylation efficiency increased at pH 8 (57.9%) by reducing the protein concentration to 1mg/ml and reducing the rhIFN-α2b/PEG ratio to 1:2. Using larger scale pegylation reaction (65% pegylation efficiency), ion exchange chromatography method has been optimized to prepare and purify the monopegylated rhIFN-α2b with high purity (96%). The prepared monopegylated rhIFN-α2b had apparent Mwt of approximately 65 kDa and high in vitro antiviral activity ($2.1x10^7 \pm 0.8 x10^7$ IU/mg). Although it retained approximately 8.4 % of the antiviral activity of the unpegylated rhIFN-α2b, its activity is high compared to other pegylated rhIFN-α2 developed by using similar approach or higher molecular weight branched PEG.

Keywords : antiviral activity, rhIFN-α2b, pegylation, pegylation efficiency
Conference Title : ICBN 2018 : International Conference on Biotechnology and Nanotechnology
Conference Location : Lisbon, Portugal
Conference Dates : April 16-17, 2018