

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

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**Abstract :** Recombinant human interferon alpha 2 (rhIFN- $\alpha$ 2) is FDA approved for treatment of some viral and malignant diseases. Approved pegylated rhIFN- $\alpha$ 2 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- $\alpha$ 2b using linear 20kDa PEG-NHS (polyethylene glycol- N-hydroxysuccinimidyl ester) to prepare pegylated rhIFN- $\alpha$ 2b with high stability and activity. The effect of different parameters like rhIFN- $\alpha$ 2b final concentration, pH, rhIFN- $\alpha$ 2b/PEG molar ratios and reaction time on the efficiency of pegylation (high percentage of monopegylated rhIFN- $\alpha$ 2b) have been studied in small scale (100 $\mu$ l) pegylation reaction trials. Study of the percentages of different components of these reactions (mono, di, polypegylated rhIFN- $\alpha$ 2b and unpegylated rhIFN- $\alpha$ 2b) 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- $\alpha$ 2b/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- $\alpha$ 2b with high purity (96%). The prepared monopegylated rhIFN- $\alpha$ 2b had apparent Mwt of approximately 65 kDa and high in vitro antiviral activity ( $2.1 \times 10^7 \pm 0.8 \times 10^7$  IU/mg). Although it retained approximately 8.4 % of the antiviral activity of the unpegylated rhIFN- $\alpha$ 2b, its activity is high compared to other pegylated rhIFN- $\alpha$ 2 developed by using similar approach or higher molecular weight branched PEG.

**Keywords :** antiviral activity, rhIFN- $\alpha$ 2b, pegylation, pegylation efficiency

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