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Enhanced refolding efficacy of interferon alpha 2b in a pilot study using low cost method

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Background and Aims: Interferon alpha 2b (IFN-2b) was employed for treatment of viral diseases and cancer as a recombinant therapeutic protein. Such other E.coli expressed therapeutic proteins; the major problem of IFN-2b production is refolding step in pharmaceutical biotechnology. Usually interferon refolding has low yield due to high aggregation in downstream processing. To resolve this problem and enhance refolding efficacy, an increasing pH method was employed.
Methods: Inclusion bodies of IFN-2b were dissolved in 6 M Guanidine HCl. Then, the different pH Refolding buffers (pH 7, 8 and 8.5) containing Cu(SO)4 was slowly added under stirring condition for 18 hr. and the process continued with centrifugation at 4˚C. The protein content of supernatants was determined by Lowry method. The refolded proteins were purified in two steps affinity chromatography (Chelating Sepharose) and gel filtration. Purified proteins were subjected to Circular Dichroism (CD) spectroscopy and biological activity assay. Finally, the results were compared to the current method in Production and Research Complex (Karaj, Institute Pasteur of Iran) which used pH 7 for IFN-2b refolding.
Results: Studying of pH effect on protein refolding showed that with increasing of pH, from pH 7 to 8.5, the refolding efficacy was increased linearly relative to the pH 7. However, the biological activity of interferon increased with pH but phenomenon did not continue above pH 8 and low biological activity was observed at pH 8.5. The CD spectrums demonstrated that when pH increased to 8.5, protein secondary structure altered dramatically (relative to the pH 7).
Conclusion: Increasing of pH until 8 was improved refolding efficacy whereas serves biological activity. The low biological activity, observed at pH 8.5, can be explained to significant alteration of secondary protein structure. In conclusion, we suggests a low costing way to be employed in downstream processing of IFN-2b for higher refolding yield.

Keywords: Interferon alpha 2b; Refolding; pH