CHAPTER 1

INTRODUCTION

1.1 Background of the Study

IBs are an aggregated protein that has been found in cytoplasm or periplasm of expression host which occur during high level of expression. Besides that, it is also a pure and insoluble protein. IBs form when the high concentration of polypeptide chain emerging from ribosome and thus lead to formation of partially folded or misfolded protein that occur in cytoplasm (Ventura, 2005). These intermediate proteins have the surface exposed to hydrophobic patches which will bring the protein to assemble together and form the IBs. Protein will functioning very well, if native secondary structure is maintained. When this aggregated protein is not properly folded, the native structure is disrupted. The IBs have its own advantage and disadvantage. The IBs become a nuisance factor for biotechnology and pharmaceutical industries. Abnormal protein aggregation can cause more than 20 different diseases in human being (Stefani and Dobson, 2008). Heterologous protein overexpression in *Escherichia coli* (*E.coli*) lead to protein accumulating in dense water insoluble aggregates. One of the example is expression of EGFP in *E.coli* contain only small amount of soluble protein whereas most of the protein is in insoluble particles (Tsumoto *et al.*, 2003). EGFP has a very useful application in order to monitor folding on protein over expression. Over expression can be easily measured using fluorescent spectrometry (Tsumoto *et al.*, 2003).
1.2 Motivation

The disadvantages of IBs have been monopolied by the recent studies. Nevertheless, this IBs can be viewed as a positive side in large scope. First of all it can be considered an advantage for basic research as for protein production. It is also play crucial role in biomedicine field and use as an alternative method to produce low cost proteins. In biomedicine field it can be used as naturally immobilized enzymes or as nanomaterials based on its specification as a pure recombinant protein (Garcia-Fruitos et al., 2009). IBs are very useful in biocatalysis process and provide innovative stage in industrial catalysis market (Roessl et al., 2010). Besides that, by understanding protein aggregation that occur in inclusion body we can discover strategies to control this process. IBs will be used as model to study insoluble protein deposits that lead to some complex human disease (Ramon et al., 2014).

IBs as a source of almost pure protein (Ramon et al., 2014). In order to obtain the native folded and active protein, solubilisation and refolding are the most crucial steps (Burgess, 2009). The effectiveness of solubilisation process will affect the refolding efficiency. Mild solubilisation is one of the method for recovery of bioactive proteins. Mild solubilisation method can preserve the existing native-like secondary structure during refolding and allow for higher recovery of bioactive form (Singh et al., 2014). This is because it will help the protein to fold properly by preventing the hydrophobic interactions and inhibit the molecules aggregation during refolding.
1.3 Problem Statement

Freeze and thaw method combined with low concentration of urea has been extensively studied to increase the efficiency of the solubilisation process (Strambini and Gabellieri, 1996). Freeze and thaw affect the protein stability in two different categories which are physical and chemical degradation. For physical degradation, freezing is a condition in which physical stress is applied by the formation of ice crystal hence applied several stresses for denaturing IBs. In terms of chemical degradation, freezing affect the environment of the buffer solutes which will result the change in buffer solution pH. Thus, it is very important to study the factors that affect the freeze and thaw method. The factors are pH buffer, rate of freezing and thawing, number of cycle and incubation period. pH change will affect the performance of the process (Cao et al., 2003) because it does affect the instability of the protein. Number of cycle does affect the protein stability in terms of the occurrence of protein degradation probability and thus indirectly determine the recovery efficiency of the functional protein. Incubation period affect the process by applying different amount of stress needed for certain protein to undergone denaturing. The amount of stress may irreversibly denature complex macromolecular structure, and could alter the protein stability. If too much of stress applied, the protein will degraded, if less of stress applied than the required stress needed, the protein will not be denatured. From this two condition the recovery of bioactive protein cannot be achieve because it does directly affect the solubilisation process, where the unfolding and refolding of the protein could not be established.