2.2. Methods

2.2.1. Preparation and characterization of low molecular weight chitosan (LMWC)

2.2.1.1. Depolymerization of high molecular weight chitosan (LMWC)

Ten grams of high molecular weight chitosan (the degree of deacetylation is 99%) was dissolved in 830 ml of 0.1M HCl. Then 170 ml of concentrated HCl was added to the solution. The solution was heated (100 °C) for different times under reflux to give different molecular weight. After cooling, 3L of ethanol (96%) was added. The precipitated chitosan was then separated from the liquid and washed thoroughly with ethanol until neutral pH was obtained. The chitosan was then freeze-dried for 48 h, weighted and stored at room temperature for further analysis (Elsayed et al., 2009).

2.2.1.2. Determination of viscosity average molecular weight (M.W.)

The average molecular weight of different chitosan samples was identified by viscometric measurements. The intrinsic viscosity was measured by a viscometer and the viscosity average molecular weights for each chitosan samples were calculated using Mark-Houwink equation.

$$[\eta] = k \cdot M^{a}$$

Where [η] is the intrinsic viscosity, M is the viscosity average molecular weight, while the used k and a values were 0.00058 and 0.69 based on previous study (Kasaai, 2007).