The viscometer underwent two-point calibration using purified water and 7.434 mPas standard (Viscosity Standard, Poulten Selfe and Lee, UK) at 25°C. For each molecular weight determination, 2.5 gm of the sample was dissolved in 50mL of water and series of dilution were prepared to give final concentrations l%, 2%, 3%, 5%, and 7% w/v. The viscosity of the solvent and the samples were measured at 25°C using the Sine-wave Vibro viscometer (Model: SV-10/SV-100). Each sample was measured duplicate and an average reading was used for the subsequent calculations. The relative and reduced viscosities were calculated and used in determining the viscosity average molecular weight (Qinna et al., 2015).

2.2.1.3. FT-IR Spectroscopy

Measurements have been performed in the transmission mode, with chitosan contained in potassium bromide (KBr) discs. Potassium bromide was mixed with chitosan in mass ratio 100:1 (200 mg KBr and 2 mg chitosan). KBr was placed in an oven at 300°C for 24 h before mixing. Substances were mixed in agate mortar and pressed to disc form using IR hydraulic press at a pressure of l0 tons for 20 seconds. Discs were dried for 24 hours at 50 °C in order to remove moisture. For every kind of chitosan, three discs were produced. The discs were placed in the diffused reflectance cell and the spectrum were recorded over a wave number that ranges from 400-4000 cm^{-1} at room temperature with accumulation of at least 10 seconds and OPD velocity of 2 cm^{-1} using Fourier transform infrared (FTIR) spectroscopy connected to Omnic software. Duplicate lR measurements were made for each sample (Qinna et al., 2015).