those rich in o-mannuronate are weaker but more flexible [31, 32]. Rapid swelling and dissolution of gel beads made from alginates with a low G/M ratio occurred at neutral pH; in contrast, beads made from a high G/M ratio alginate did not disintegrate under identical conditions [33–34].

2.3.2 XRD, FTIR, and NMR Spectroscopy for Alginate Structure Analysis

The structure of alginates has been investigated using X-ray diffraction and infrared spectroscopy. Alginic acid fibers give sharp diffraction peaks, which indicate a high crystallinity and a more perfect ordering. Comparatively, calcium alginate fibers give broad diffraction peaks, pointing to defective crystals and low crystallinity [32]. According to Atkins's assignment, the Miller indexes for the two main diffraction peaks of alginate acid (110) and (002) are at 2θ of ~13.4° and ~22.9°, which correspond to *d*-spacing of 0.69 and 0.39 nm, respectively, while the shoulder at the 2θ of about 10.6° corresponds to the (001) position [33].

In FTIR spectra, -C-H str of mannuronic acid functional group is assigned at wavenumber 884 cm^{-1} and the uronic acid at wavenumber 939 cm^{-1}, while the asymmetric stretching of carboxylate -O-C=O str is at 1603 cm^{-1} and C–OH deformation vibration with contribution of O–C–O symmetric stretching vibration of carboxylate group is present at 1410 cm^{-1} (occurrence of carboxyl and carbonyl groups, typical to alginate); -OH functional group has peak at wavenumber 3200–3400 cm^{-1}, and -CH_2 stretching bands are present at wavenumber 2928 cm^{-1} [22]. FTIR spectroscopy has also been used as a tool to determine chemical composition of the M/G ratio in the alginates [35–36]. Separation of M-block and G-block from sodium alginate as highly purified polymannuronic acid and polyguluronic acid was possible through acid hydrolysis using oxalic acid, followed by isolation using the difference of solubility with pH value. In the FTIR spectra, the ratio of specific absorbance combinations (A_{1030}/A_{1080}) and (A_{1010}/A_{1025}) in the spectra of Ca and Mn salts of alginic acid (presence of cations enhances the specific peaks) indicates the M/G ratio of the sample.

Historically, the determination of monomer content began with total acid hydrolysis of the glycosidic bonds followed by separation techniques such as paper chromatography, thin layer chromatography, anion-exchange liquid chromatography, and gas–liquid chromatography [37]. Nowadays, the most common method used for the structural analysis of sodium alginates is ^{1}H and ^{13}C solution-state nuclear magnetic resonance (NMR) spectroscopy. From solution-state NMR data, the G/M ratio can