

FTIR and Rheology study of Alginate samples: Effect of Radiation

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Abstract

Two well characterised alginate samples with high and low M/G ratio were subjected to radiation decontamination dose at 15 kGy. Subsequently, a number of techniques were used to determine the effect of radiation on the gelling properties. These techniques include: (GPC-MALLS) gel permeation chromatography linked to a multi-angle laser light scattering and refractive index detector for the determination of molecular weight and distribution. To observe the mechanics, the deformation with flow of α - β alginates rheological measurements in the dynamic oscillation modes, and distinguish the functional group of the samples along with identifying the M/G ratio for quantitative determination of mannuronic and guluronic acid, the FTIR techniques were performed respectively. The results showed clear reduction in the molecular weight following irradiation which was also accompanied by a loss of the gelling properties as well as clear reduction intensity of FTIR peak at 1080 -1400 cm^{-1} for carboxylic group, OH-bonding obtained at 1030 cm^{-1} (1026 and 1028 cm^{-1}) while the weak signal and skeletal region is attributed at >2800 and <700 cm^{-1} respectively. A sample (n=1; H120L) is homopolyguluronic enriched (820 cm^{-1} in fingerprint region) with small amount of mannuronic acid fraction. Sample (n=2; LFR-5/60) is heteropolyguluronic acid enriched in presence of tiny amount guluronic acid (814 cm^{-1} peak) i.e. comparatively high G to M in this fraction but %G is lower than n=1. The rheological parameter storage modulus (G') and loss modulus (G'') were noticed higher with the constant frequency mode, a solid-like nature gels was obtained with increasing the frequency in a certain time of relaxation. The gelling properties of the samples calculated is $M/G < 1$ which able to make hard and rigid gel. Thus, radiation effects for rheology and FTIR in case of functional group distribution was significant.

Keywords: FTIR, Spectroscopy, gelling properties, alginate, chemical composition, M/G ratio, Rheology

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INTRODUCTION

Alginates are structural polysaccharide originated from brown seaweeds consisting a linear copolymer of α -L-guluronic acid (G) (1-4)-linked and of β -D-mannuronic acid (M). It is a group of homopolymeric (M or G) blocks i.e. poly (β -D-mannuronic acid/ MM) and poly (α -L-guluronic) acid/ GGorheteropolymeric /MG while the linkage of G is dominant over the linkage of M (Leal et al. 2008; Ammar et al., 2018; Ghorbani Gorji et al., 2018, Jiao et al., 2019; Feng et al., 2017). Alginates have been used in pharmaceutical, health sectors and food industries due to its unique gelling properties (Bashir and Rajendran, 2018; Song et al., 2018). Alginate can form a gel ("egg-box") by acting with a bond of polyguluronic acid and divalent ions (Szekalska et al., 2016; Urbanova et al., 2019; Abasalizadeh et al., 2020). Hence, the estimation of the M/G ration is vital to use alginate with their types of forming gel (Urtuvia et al., 2017). In general, polymannuronate is analogous to cellulose to form β -1-4-linkage, whose indication is linear flexible a ribbon like chain. However, indication of polyguluronate is that of rigid folded like a hard polymer. It is proven that the structural composition of the alginate touches the gelling properties. Accordingly the index, the ration of M and G, the component in the alginate is specifically key in pilot scale use (Vara Prasad et al., 2020, Jung II et al., 2020). The sequential arrangement of the mannuronic acid and guluronic acid residues representing in a block like decoration of G-residues and M-residues interpreted along with MG interchanging blocks (Larsen et al., 2015; Urtuvia et al., 2017). As a consequent, topographical and types of deviations transpire in the quantities of mannuronate and guluronate (M/G ratio) and this has a reflective effect on the polymeric somatic properties, and hence its industrial application (Annison G., 1983; Vara Prasad et al., 2016, Sehgal et al., 2019). For the diversification of the M & G content accent is given in this research in gelling properties and desire to estimate the M/G ratio. Radiation processing method is encouraging to investigate the degradation. Among them, ionizing radiation is keenly used in different fields to produce viscose, flesh, pharmaceutical, food and bioactive agents. The natural polymeric derivatives and polysaccharides are sensitive to ionized radiation which enhance the sustainable development of natural biopolymers (Tamada and Kudo, 2018; Ashfaq et al., 2020, MZI Mollah et al. 2022).

Profoundly, infrared (IR) spectroscopy is a useful technique to identify polysaccharide structures on the basis of absorption and transmittance peaks at definite wave numbers (cm^{-1}). In an alga sample, carrageenans and polysaccharide were rapidly identified by FTIR-spectroscopy straight on few milligrams of dries milled algal material eluding time expanding sample preparation processes (Gomez, 2011). The mode of second derivative FTIR spectra is simplified method for distinguishing agar-producing and carrageenan-producing seaweeds (Pereira et al. 2003). As the separation of polymannuronic acid and polyguluronic acid by hydrolysis process following the ^{13}C -NMR spectra method is time consuming, scientists are searching the quick methods to estimate the M/G proportion

Mollah, M.Z.I., Faruque, M.R.I., Bradley, D.A., Khandaker, M.U., Al-Assaf, Saphwan. FTIR and rheology study of alginate samples: Effect of radiation. [Radiation Physics and Chemistry Volume 202](https://www.sciencedirect.com/science/article/pii/S0969806X22005369), January 2023, 110500. <https://www.sciencedirect.com/science/article/pii/S0969806X22005369>

by FTIR which is a modified method (Sakugawa et al. 2004). It is a simplified process to measure the quantitative identification of alginate, carrageenan and agar in milled and dried samples from red and brown seaweeds. The increasing demands for seaweed polysaccharide by the entrepreneurs of industrial application of alginate sought to determine the M/G ratio and the composition which directly affect the gelling properties of alginate. In this research we applied the combination method as described by the several scientists and followed their instruction for the wave length occurred in specific absorbance strength of frequency region (fingerprint) of the samples.

Rheology is studied the process of mechanics, the deformation with flow of samples especially liquid or soft like gel consisting complex structure, suspension, polymers, foods, fluids and other natural materials. A lot of polymers are used but alginate is the most proposed applications in different delivery systems, biosensors, food technology and biotechnology [Francesca Cuomo et al.2019]. In food technology, alginate is used as additives to keep ingredients in foods as a thickener or stabilizer. The alginate particles are designed for the use of emulsion technology as an internal gelation process [Reis et al. 2006]. Nanoemulsion based hydrogel having Polyphenon 60 has been developed for urinary treatment delivering via an intravaginal way [Kaur et al. 2017]. Encapsulated nobiletin as a nanoemulsion droplets of alginate based hydrogel has been proposed [Lei et al. 2017]. A flavonoid, nobiletin is secluded from citrus peels having superb bioactivities, such as anti-inflammatory, anti-cancer, and anti-dementia. Thus, in vitro digestion studies and in vitro release, the experimental findings were concluded that nano-emulsion-filled hydrogels can succeed the constant discharge and immersion of nobiletin and preclude its drizzle in the gastrointestinal tract. Therefore, the scope of hydrogel, indulgent of the rheological properties, such as gelation behaviour, gelled structures comprehending emulsifying or alleviating agents come to be necessary for enlightening the systems' properties or rheological characteristics. Finally, aims of the study is to obtain more knowledge on FTIR and rheological characteristics of specific alginate samples which will help further research on alginate based materials.

MATERIALS AND METHODS

The alginate two samples (n=1; H120L and n=2; LFR 5/60) with known molecular weight ($M_w = 404$ kDa and 35 kDa respectively) were obtained from FMC, Norway, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and D-glucono- δ -lactone (GDL) were purchased from Fisher Scientific UK.

Radiation Processing and Molecular weight measuring

The ionized radiation source Co-60 gamma is applied on samples, the dose was measured to be 15 kGy by Harwell amber perspex dosimeter (Batch V, type: 3042, range 1-30). Three ambers were placed in appropriate position for dosimetry measurement, the average thickness was 0.298 cm of the amber and absorbance was 0.977 detected in 651 nm. The specific absorbance (3.278 cm^{-1}) was calculated

following the $\left(\frac{\text{abs. amber}}{\text{object thickness}} = \text{specific abs.}\right)$ equation, then fitted in the Harwell calibration chart, finalized the absorbed dose. The sample was characterized and estimated the molecular weight both of the sample n=1 and n=2 by gel permeation chromatography (GPC-MALLS) methods. The molecular weight was calculated followed by $[\eta]=KMa$, where K and a are the Mark-Houwink's parameter constant ($a= 0.91$ to 0.94 , $K=7.254 \times 10^{-5}$ to 8.78×10^{-5}), M is molecular weight and η is the intrinsic viscosity [Mollah MZI et al. 2021]. The estimated molecular weight was recorded in both samples while as ten times or more decreased in irradiated samples.

FTIR-Spectroscopy and M/G ratio

The spectrum of the alginic acid samples were performed by 64 scans in absorbance with a 4 cm^{-1} resolution using FTIR –RXI spectrometer. It was blended and triturated by an agate mortar. The mixture of 2 mg alginate along with 200 mg dried KBr was compressed by using IR-hydraulic press in a pressure of approximate 8 tons, kept in 10 min. The spectra of samples were noted with a Wave number of $4000\text{-}400 \text{ cm}^{-1}$ in the forms of KBr pellets. The spectra were obtained at room temperature then processed by FTIR-spectrum 5 software, version 5.3.1. The air background spectrum scanned was performed before each series of spectrum measurement under similar instrumental conditions. The sample ran thrice, taken the mean of average spectrum, finally suitable bands was considered for the determination of M & G content and M/G ratio of the samples. The percent mannuronic (M) and golluronic (G) content is determined using simple calculating methods followed $(M/100-M)= Ra$ or $(100-G/G)=Ra$; where, M and G is mannuronic and guluronic acid content respectively, 'Ra' is the ratio of the two unit M & G acid in the alginate samples.

Determination of Rheology

The sample was prepared by adding salt (CaCl_2) and protein "D-glucono- δ -lactone" (GDL). Rheological properties of alginate aqueous solution 0.5% (w/w) in 0.02 M CaCl_2 solvent were observed in isothermally oscillation shear mode at 25°C with parallel plate and double concentrated cylindrical geometry. The dynamic storage modulus (G') in a constant time dependence and mechanical loss tangent ($\tan\delta$) distinct by G''/G' (G'' ; dynamic loss modulus) was perceived. The gelation mechanism was monitored with a TA-AR advanced Rheometer, strain controlled (TA, AR 550, Advanced rheometer, USA) settled with controlled-temperature water bath. The small amount of aqueous solution was placed between parallel-plate & geometry/double concentrated cylinder and the system geometry head was placed on solution. After putting the geometry on samples carefully cleaned and surface of the specimen was covered with silicon oil by putting drop wise to prevent evaporation during measurement. To determine the stress sweep, the frequency fixed at 6.283 rad/sec (1Hz) and oscillation

stress fixed at 0.07958 to 3978 Pa, applied strain was controlled automatically with a linear viscoelastic region throughout the measurements. Subsequently, completion of the strain sweep test, the frequency sweeps were carried out to confirm the test in the linear viscoelastic series. Whereas, dynamic frequency sweep tests were performed by the 0.10 to 10 rad/sec on log mode at 25°C and controlled variable oscillation stress 0.49 Pa fixed from the sweep tests for sample H120L using GDL and using CaCl₂ for the sample H120L & LFR-5/60 are fixed at 398 Pa. The cylindrical geometry used for LFR-5/60 using GDL and oscillation stress fixed at 0.0089 Pa for frequency test and for stress sweep oscillation stress fixed at 2.976×10⁻³ to 148.7 Pa.

RESULTS AND DISCUSSION

Different FTIR spectra of alginate (H120L)

The FTIR spectra of H120L represented in the Fig. 1 (a, b) indicating different wave number occur for the absorbance strength of the different content of M and G residues. There are several number of bands were observed 800 cm⁻¹ to 1800 cm⁻¹ which are directly affected by the sample composition and there second derivatives. In the fingerprint region (anomeric) 820 cm⁻¹ wave spectra is for the mannopyranuronic acid residues and 946 cm⁻¹ is for the α-1-4-linkage. The normal spectrum 818 cm⁻¹ while 822 cm⁻¹ is the second derivative associated to small amount of mannuronic acid in the enriched fraction of homopolyguluronic respectively [Chandia N.P. et al 2001]. On the other hand the band at around 948 cm⁻¹ and 903 cm⁻¹ was attributed to α-1-4-linkage and the α-L-gulopyranuronic asymmetric ring vibration (Mathlouthi and Koenig 1986) respectively. The spectrum 1800-700 cm⁻¹ region for the alginic acids can provide useful qualitative information [Mackie W., 1971]. There is a particular differences of these spectra in the 1800-700 cm⁻¹ region, where he mentioned the bands of particular interest occurred at 808 and 787 cm⁻¹ in represented polymannuronic acid and polyguluronic acid respectively. In the protalan H120L samples respect type of band 787 cm⁻¹ and 808 cm⁻¹ is absent which is occurred for the presence of guluronic and manuronic acid but the second derivative is clearly attributed. The band at 903 cm⁻¹ or similar was not attributed in that of H120L sample which is indicated to the α-L-gulopyranuronic asymmetric ring.

The spectrum band of 1028 cm⁻¹ is assigned for the OH bending. The spectrum bands are 1104 cm⁻¹ and 1302 cm⁻¹ is occurred for the C=O group stretching of M and G residues and their interpretation of stretching vibration. It has been reported that [Sakugawa K. et al. 2004] the concentrated vibration peak causes shifting band peak; which is responsible to OH bending at 1030 cm⁻¹ and is similar to respect of protalan H120L (1028 cm⁻¹). The absorbance at 1080 cm⁻¹ has a slight change with the vibration of M/G ratio. Moreover, this absorbance is for the six-membered ring C-O-C stretching vibration of both mannuronate and guluronate. From the findings, it can be concluded that the exact 1080 cm⁻¹ spectrum is absent and the stretching is occurred in the wave of 1104 cm⁻¹ and 1302 cm⁻¹ for the M and G content

respectively. For the sample H120L the local symmetry is occurred at 1418 cm^{-1} and 1618 cm^{-1} for concentration variation of M and G content. The normal spectrum for the H120L is showed in Fig. 2 (control and irradiated) while in the irradiated sample as light change observed for the FTIR intensity.

In the polysaccharides structural analysis, it is well established five frequency regions (Mathlouthi and Koenig 1987) that can be notable in the standard spectra ($4000\text{-}650\text{ cm}^{-1}$): (i) district of OH- and CH stretching vibration at $3600\text{-}2800\text{ cm}^{-1}$; (ii) local symmetry at $1500\text{-}1200\text{ cm}^{-1}$; (iii) C=O stretching vibration region at $1200\text{-}950\text{ cm}^{-1}$; (iv) anomeric fingerprint region at $950\text{-}700\text{ cm}^{-1}$ and (v) $\geq 700\text{ cm}^{-1}$ is skeletal region. A centred 3260 cm^{-1} broad band assigned to hydrogen bonded (O-H stretching) and a weak signal at 2926 cm^{-1} due to C-H stretching [Gomez and Ruperez, 2011]. Finally, $1200\text{-}970\text{ cm}^{-1}$ (medium to strong IR absorption) bands are mainly due to C-C and C-O stretching in pyranoid ring and to C-O-C stretching of glycosidic bonds. The fundamental absorption peak region is a common phenomenon for all polysaccharides.

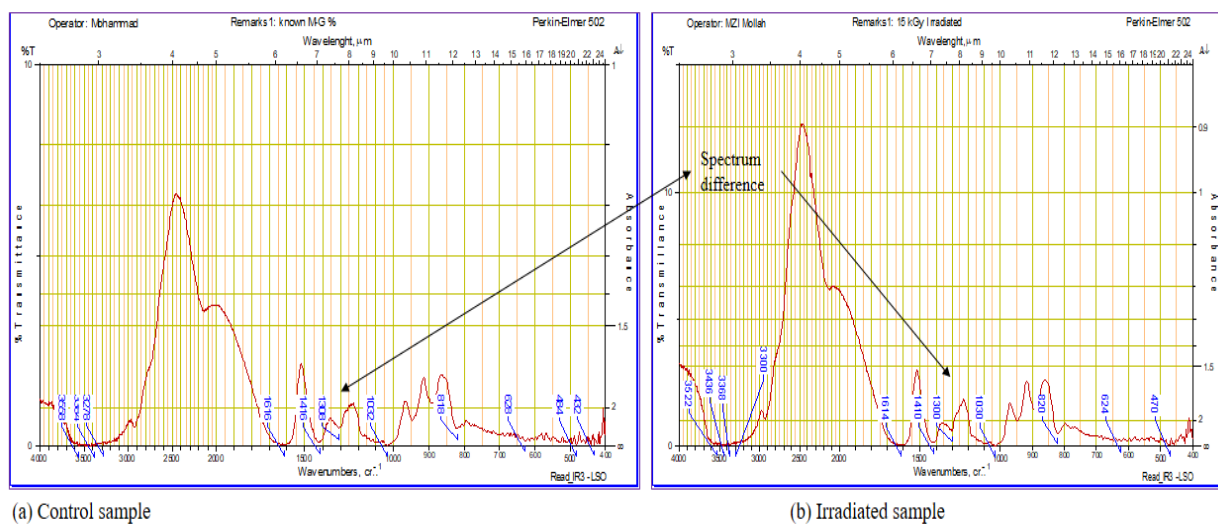


Figure 1: IR spectra of alginate sample n=1; H120L (a= Control, b= irradiated sample)

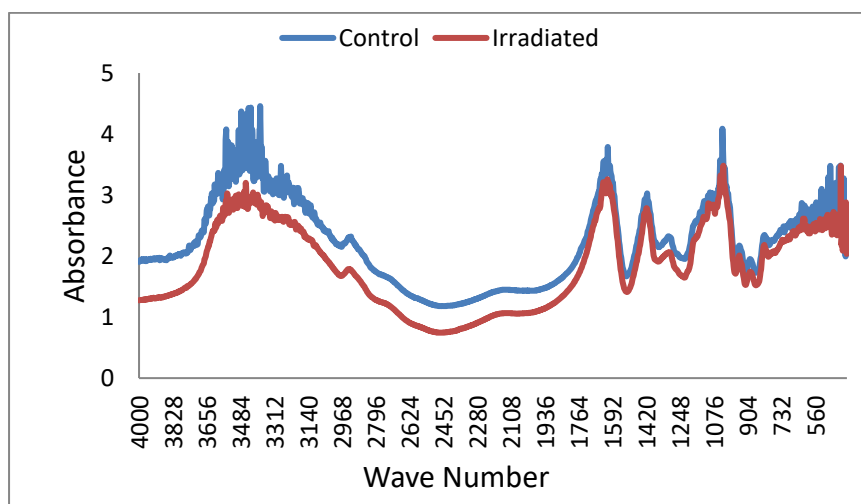


Fig 2: The normal spectroscopy of n=1(control and irradiated) represented for the M & G distribution

Different FTIR spectra of alginate (n=2; LFR 5/60)

The FTIR spectra of LFR 5/60 represented in the Fig. 3 (a, b) indicating different wave number occur for the absorbance strength of the different content of M and G residues. There are several number of bands were observed 700 cm^{-1} to 1700 cm^{-1} which are directly affected by the sample composition and there second derivatives. In the fingerprint region 814 cm^{-1} wave spectra is for polyguluronate enriched acid residues while the α -1-4-linkage wave spectrum is absolutely absent. The findings noted that around wave at 814 cm^{-1} is attributed for enriched polyguluronic but polymannuronic enriched wave fraction is absent [Chandia *et al.* 2001].The exact result (814 cm^{-1}) indicated the second derivative spectrum is well resolved to guluronic acid residues indicating the presence of small amount guluronate in the enriched fraction of homopolyguluronate.

In contrast, 948 cm^{-1} band was pointed to α -1-4-linkage, and the band at 903 cm^{-1} was ascribed to the α -L-gulopyranuronic asymmetric ring (Mathlouthi & Koenig 1986) which is un appeared in LFR-5/60. The spectrum $1800\text{-}700\text{ cm}^{-1}$ region, IR spectra of alginic acids can provide useful qualitative information with their composition [Mackie W., 1971]. There are numerous differences on these two spectra in the $1800\text{-}700\text{ cm}^{-1}$, but a particular interest occur at 808 and 787 cm^{-1} for the spectra of polymannuronate acid and polyguluronate acid respectively. In LFR 5/60 samples respect this type of band 787 cm^{-1} and 808 cm^{-1} is not demonstrated for the presence of guluronic and manuronic acid but the second derivative of G residues (814 cm^{-1}) is clearly attributed. The band at 903 cm^{-1} or similar was not showed in that of LFR 5/60 which is not also indicated to α -L-gulopyranuronate asymmetric ring shaking.

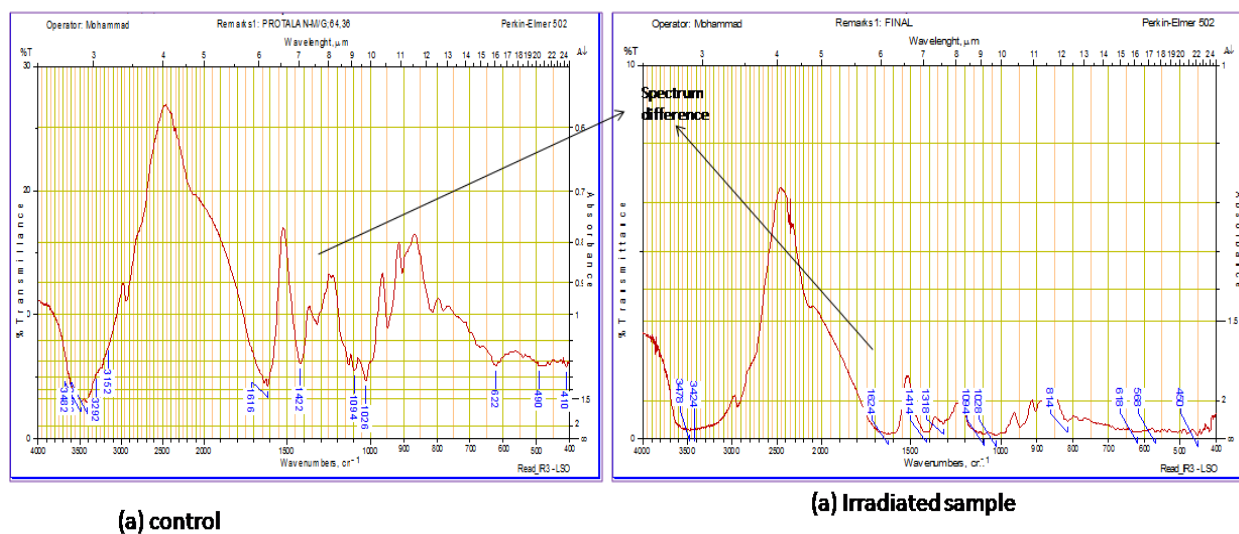


Fig 3: IR spectra of LFR 5/60 (n=2), a= control and b= irradiated sample, showing the difference spectrum

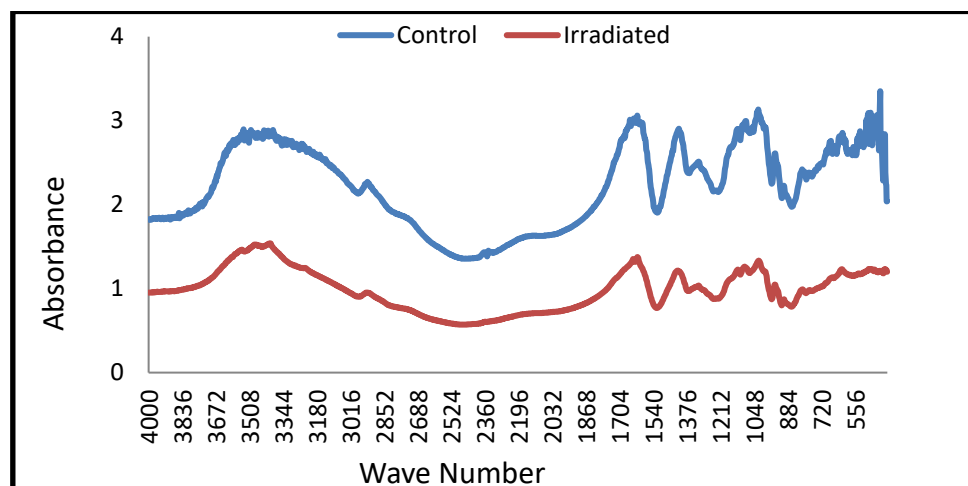


Fig 4: The wave number and absorbance, normal spectrum of n=2 (control & irradiated) represented for the M and G content

The spectrum band of 1028 cm^{-1} is assigned for the OH bending. The spectrum bands are 1094 cm^{-1} and 1318 cm^{-1} is observed for the stretching of C=O group of M and G residues and their stretching vibration. The vibration causes shifting of the peak [Sakugawa et al. 2004]; which is assignable to OH bending at 1030 cm^{-1} absorbance peak and same is represented in LFR 5/60 (1028 cm^{-1}). The absorbance peak (1080 cm^{-1}) has a slight change of the vibration of M/G ratio in this specific sample. In addition, C-O-C stretching of the six-membered ring variation occurred for both of mannuronic and guluronic acid. Thus, from this experiment it is concluded that the exact 1080 cm^{-1} spectrum is absent and the stretching is occurred in the wave of 1094 cm^{-1} and 1318 cm^{-1} for the M and G content stretching vibration respectively. For the sample LFR 5/60 the local symmetry is plotted at 1416 cm^{-1} and 1624 cm^{-1} for concentration variation of M and G content, reported findings are described [Mathlouthi et al. 1987], while the same nature is found in protalan sample.. The normal spectrum for the LFR 5/60 is presented in Fig. 4 (control and irradiated).

Determination of M/G ratios

In this experiment specific absorbance strength combination is applied to estimate the M/G ratio of the samples. As the concentration increases, the absorbance decreases and the specific absorbance peak is occurred for the concentration variation of the mannuronate and guluronate content in the alginate samples. The actual spectrum in the local symmetric region was demanded for the estimation and avoided the second derivatives peak for M and G residues. This type of spectra 1418 cm^{-1} & 1618 cm^{-1} and 1414 cm^{-1} & 1624 cm^{-1} was recorded from H120L and LFR 5/60 samples respectively for M and G composition variation. The M/G was found 0.856 and 0.943 for H120L and LFR 5/60 respectively.

A one-pot rapid method for hydrolysis of sodium alginate to estimate M/G ratio has been developed under mild conditions, by a microwave-assisted irradiation hydrolysis [Chattbar et al. 2009]. This method was confirmed by carboxyl group assay along with characterizing the hydrolytic PMA and PGA

products of alginate by using FTIR. Moreover, poly-mannuronic acid (PMA) and poly-guluronic acid (PGA) ratio was estimated ($M/G = 0.38$). The same results ($M/G = 0.39$) obtained from the convention process, using sodium alginate as a benchmarking reference sample. Also alginate samples demonstrated several characteristic bands in the IR spectra for the mannuronic and guluronic acid respectively at 808 and 787 cm^{-1} (Mackie, 1971, Gomez E. 2011) and at 1030 and 1080 cm^{-1} (Sakugawa et al., 2004). They suggested that it is possible to gain a fair estimation of the M/G ratio of alginate quotient by these intensity of bands. The method to determine transmittance in a specific wave number in the average IR spectra was applied (Rochas *et al.* 1986) with the base line peak. Usually, scientists determine the M/G ratio by calculating quotient of the absorbance in corresponding wave numbers followed by equation: $A = \log T_b/T_p$; where transmittance (T), absorbance (A) of base line (b) and peak (p).

M/G for gelling properties

The quotient of intensities for M & G in FTIR is roughly 0.856 and 0.943 which were similarly obtained to standard alginate samples [Gomez E. 2011]. When the alginate samples show $M/G > 1$, means mannuronic (M) \geq guluronic (G). The M/G ratio varied in brown seaweed alginates from 0.5 to 2.5 , caused by heterogeneity of the chain length and distribution of G and M blocks (Miller, 1996). Furthermore, the ratio of mannuronic to guluronic acid is an index of the nature of gels produced. The M/G ratio difference means that alginates obtained from different seaweeds having dissimilar physico-chemical properties. Overall, a low M/G ratio ($M/G < 1$) with a large fraction of guluronic acid blocks form a strong and rigid gel (Draget *et al.* 2006). In contrast, alginate with a low number of guluronic acid blocks, i.e. $M/G > 1$ produces a lenient and flexible gels. The ratio of M - and G -units is varied either diverse species or also some extent with the age or part of the plant and collecting season (Gomez E. 2011). This heterogeneity of alginates can play a vital role for food and other industrial applications. The supplied protalan H120L and LFR 5/60 samples both are able to produce a hard and rigid gel and they are G enrich samples.

M & G content in H120L & LFR 5/60

The M & G was calculated followed the proposed method and found that M 46% & 48% and G 54% & 52% for H120L and LFR-5/60 samples respectively. Both the samples are G enriched but comparatively the LFR-5/60 sample contained higher M content than that of H120L sample. The H120L and LFR-5/60 samples are same nature of gelling properties. Gelling properties also studied by acidifying with GDL and adding CaCl_2 salts for the mechanical strength of supplied samples.

Rheological Parameters

The storage modulus $G'(\omega)$; contribution of the resistance to deformation of the elasticity and loss modulus $G''(\omega)$; influence of the resistance to deformation of the inelastic are plotted in Fig 5 (H120L) and Fig 6 (LFR-5/60) at a concentration of 0.5% (w/w) in 0.02M CaCl_2 . A gel point, it is easy to monitor the gelation process of a viscoelastic fluid. In the rheology method, the viscoelasticity modifies noticeably at the gel point. The gelation temperature of the solution increased with increasing the concentration of the cations of the gellan liquids. The frequency "storage (elastic) G' and loss (viscous) moduli which are same as (cross-over) represents that the starting of the rubbery section [Lin Dai et al 2010] with the behaviour as acting by CaCl_2 . It is observed that G' and G'' gradually increased with increasing frequency. Subsequently, storage modulus is higher than the loss modulus in the viscoelastic range of linear frequency (i.e. $G' > G''$), the elastic behaviour predominates over viscous behaviour of the samples while swollen performance exhibits mechanical rigidity. As well, in a low relaxation time, the sample flexibility is reduced and the swollen sample converts progressively rigid. In this Fig 5&6, the same process was recurrent twice to crisscross the reproducibility of the scheme. The mechanical properties of gel strength also measured with GDL for both of the samples. For the LFR-5/60 was determined by double concentrated cylindrical geometry and H120L was determined by 40 mm parallel plate. The mechanical gel strength (G' and G'') and cross over frequency were presented in Table 1. The high G' and G'' values proved that the mechanical properties i.e. rigidity of gels are absolutely caused by the presence of higher guluronic acid content or homopolyguluronic unit blocks.

Table 1: G' and G'' modulus at different frequency and cross-over frequency of H120L and LFR-5/60 alginate samples respectively

Sample ID	Acid/Salt	G' at 0.1 Hz Pa	G'' at 0.1Hz Pa	G' at 1 Hz Pa	G'' at 1Hz Pa	G' at 9.99Hz Pa	G'' at 9.99Hz Pa	Cross over Freq. (Hz)
LFR-5/60	CaCl_2	#N/A	466.7	345.7	3095	3558	12380	~3.55
H120L		#N/A	#N/A	11040	10760	17220	15760	~4.95
LFR-5/60	GDL	0.3724	0.05232	0.4092	0.09732	0.3415	0.4547	7.94
H120L		0.3961	0.2905	0.8642	0.4123	16.19	27.32	9.99

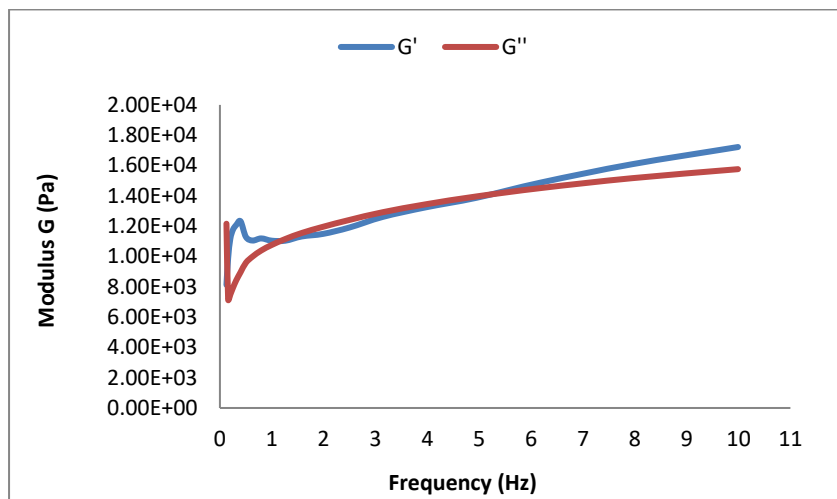


Fig 5: Effect of CaCl₂ on frequency sweep and modulus of H120L sample

The samples binding with cationic salt (CaCl₂) and created certain shape like spherical or cylindrical, it has been ensured that single isolated samples with a salt formed swollen hydrogel [Ramazani-Harandi et al. 2006].

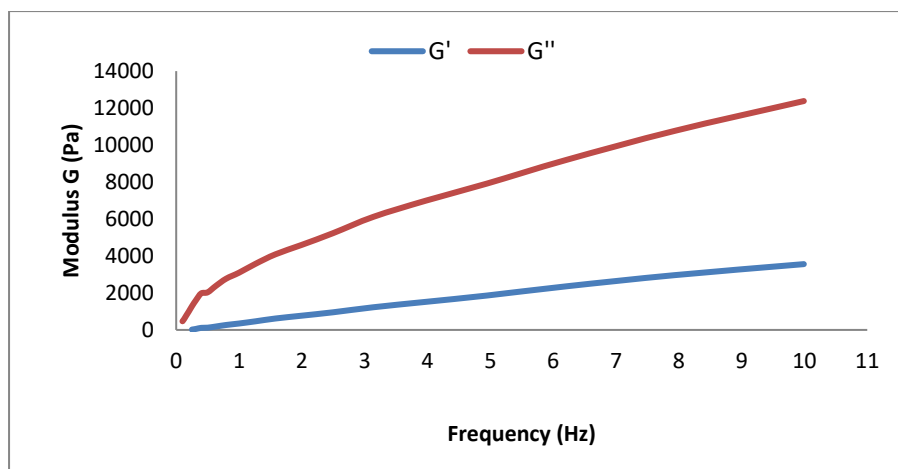


Fig 6: Effect of CaCl₂ on frequency sweep and modulus of LFR-5/60 sample in same concentration

The sample along with salt formed a type of hydrogels, rheological properties were assumed dependant on the structure. The solution reported as a viscous with low frequencies which tending to well fit the scaling laws: $G' \sim \omega^2$ and $G'' \sim \omega$ while elasticity dominates ($G' > G''$) by high frequencies. This findings can be corresponded to Maxwell-type phenomena by a single relaxation time increasing proportional to concentration which are determined from the crossover point (Gulrez et al.2011; Xinxing et al. 2003; Winter H.H. and Chambon, 1986). The crossover point is determined from the rheological data at 0.1hz and 9.99hz respectively for both of the samples adding CaCl₂ and D-glucono- δ -lactone (GDL). The sample LFR-5/60 showed crossover point (3.55) while sample H120L presented 4.94hz crossover point

in case of acting with calcium salt. On the other hand, both samples noticed high crossover frequency whereas the sample H120L > LFR-5/60. The findings are supported with the literature, samples with a high content of guluronic acid blocks produced considerably higher strength gels than mannuronate rich alginate. Homopolymeric enriched blocks seemed to favour the formation of junction zones compared to cationic alginates gels [Draget et al. 1994].

CONCLUSIONS

In the studied samples, the second-derivative mode is detected from the FTIR spectroscopy which denoted that alginates are polymannuronic acid enriched given intensity peaks at ~ 820 & 946 cm^{-1} , whereas only one peak 814 cm^{-1} is for the polyguluronic acid enriched fraction. The spectroscopy data indicated that H120L is guluronic plentiful sample and LFR-5/60 is polyguluronic enrich in residues not highly content of guluronic acid. In the fingerprint region for the sample H120L is responsible for the particular bands 946 and 820 cm^{-1} for M and G content respectively. Alternatively, the LFR-5/60 is responsible for only guluronic residues at 814 cm^{-1} where the band for mannuronic is absent. The C=O stretching vibration peaks are occurred at 1080 - 1400 cm^{-1} in both samples and OH bending is obtained in between 1030 cm^{-1} (1026 cm^{-1} and 1028 cm^{-1}). The local symmetry is found clearly at 1400 cm^{-1} – 1700 cm^{-1} where the weak signal and skeletal region is attributed at $>2800\text{ cm}^{-1}$ and $<700\text{ cm}^{-1}$ respectively in both samples. The gelling properties and their ratio $M/G < 1$ which mean both the samples are G enriched and they make hard and rigid gel. But comparatively LFR-5/60 is enriched in M in case of H120L protalan samples. The rheological properties showed high G' and G'' values in constant frequency. The mechanical strength i.e. G' and G'' were increased with increasing the frequency at a certain time of relaxation. The sample $n=1$ and irradiated sample $n=2$ is significantly evaluated, the rheological and FTIR data was measured smoothly but the salt and protein based rheology was completely different respectively which is proven by the crossover point in 0.1 Hz and 9.9 Hz . The rheological parameter storage modulus (G') and loss modulus (G'') were noticed higher with the constant frequency mode, a solid-like nature gels was obtained with increasing the frequency in a certain time of relaxation.

ACKNOWLEDGEMENT

MM is thankful to SA-A, a host Professor in the United Kingdom who allowed us to do research in his laboratory under the scholarship awarded by the IAEA (TC-BGD/8/021). The authors would like to extend their sincere thanks to the Research Universiti Grant, Universiti Kebangsaan Malaysia, Dana Impak Perdana (DIP), Code: DIP-2020-018 for supporting APC of journal and conference expenses.

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