Arabian Journal of Chemistry (2017) **10**, S3707–S3714

King Saud University

Arabian Journal of Chemistry

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ORIGINAL ARTICLE

Extraction and characterization of sodium alginate from Moroccan *Laminaria digitata* brown seaweed

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Received 11 September 2013; accepted 17 May 2014
Available online 24 May 2014

KEYWORDS

Laminaria digitata, Brown seaweed;
NMR spectroscopy;
IR spectroscopy;
Intrinsic viscosity

Abstract Sodium alginate from Moroccan *Laminaria digitata* brown seaweed were extracted under different conditions. The extraction yield was affected by the extraction temperature and the samples' size. Alginates were purified by re-precipitation with ethanol and characterized by ^{1}H NMR, fluorescence spectroscopy and infrared spectroscopy, in order to determine their structural and physicochemical properties.

Viscosimetric measurements gave an intrinsic viscosity of 2.542 dL/g which permits to calculate the average molar mass value (1.14×10^5 g/mol). By analyzing ^{1}H NMR spectra, Moroccan *L. digitata* alginates showed a high quantity of both homopolymeric mannoronic and guluronic blocks ($F_{MM} = 0.47$) and ($F_{GG} = 0.41$) respectively, while the alternating block fractions ($F_{MG} = 0.06$ and $F_{GM} = 0.06$) showed low values than those previously described in the literature. The M/G ratio value is 1.12 allowing the preparation of alginates suitable to form soft and elastic gels more than brittle ones. The characteristics obtained for Moroccan *Laminaria digitata* may be useful to obtain polyelectrolyte complexes for the production of drug delivery micro- and nanoparticles. In some cases, a charged polysaccharide with low viscosity is needed.
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1. Introduction

Alginates are naturally present in brown seaweed cell walls (Kloareg and Quatrano, 1988). These natural polysaccharides are widely used in various industries like textile, agri-food, paper, cosmetic, biomedical and pharmaceutical because of their rheological properties such as gelling, viscosifying and stabilization of dispersions (Draget et al., 2006). Alginates

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.arabjc.2014.05.003
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are preferentially extracted in their sodium form because of their solubility in cold water (Pérez, 1970).

At the molecular level, alginates are linear binary copolymers of (1-4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) monomers, constituting M-, G-, and MG-sequential block structures as shown in Fig. 1 (Moe et al., 1995).

The monomer sequence (M and G) can differ among algal species and also in different tissues of the same species. (Donati et al., 2003). The *M*/*G* ratio and the block structure have an important effect on the physicochemical properties of alginates.

The alginates of *Laminaria digitata* are known for their ability to remove adsorbed heavy metals such as Cd, Cr and Cu. Papageorgiou et al. (2006) reported that coating metal beads with sodium alginate extracted from the brown algae *L. digitata* were found to be a promising material for heavy metal sequestration, in particular copper and cadmium ions in single and binary metal solutions. Also, in this regard, Dittert et al. investigated the role of the *Laminaria* seaweed in its protonated form as an effective biosorbent for aqueous chromium (III) Dittert et al., 2012.

Pharmacological experiments have revealed that *Laminarians* are one of the most important marine medicinal foodstuffs. It was reported that it has a promising ability to prevent obesity and diabetes by enquiring the anti-hyperglycemic and the inhibitory actions on triglyceride absorption (Shirosaki and Koyama, 2011).

Among the recent applications of alginates, we can mention their use as a matrix to encapsulate and/or release cells (Orive et al., 2002) and also for the release of medicine (Chen et al., 2005; Finotelli et al., 2010).

The Moroccan coast extends over 3500 km, 2900 km of Atlantic coastline and 500 km of Mediterranean coastline. It contains important quantity of algal species. Among them, *L. digitata* was not investigated in the literature for its chemical and physicochemical characteristics with regard to their promising potential applications. In consequence, we have studied the extraction conditions of alginates from *L. digitata* and determined their physicochemical properties. We intend to use these alginates in other studies as a matrix for drug encapsulation and as a paper film in packaging.

2. Experimental

2.1. Alginate extraction

Brown seaweeds *L. digitata* were collected in winter from the Atlantic coast of Western Morocco, more precisely from the coast of the city of El Jadida. The biomass was washed with tap water, shade-dried and then cut into pieces of 0.1–0.5 cm in length. The sample was then divided into two parts; small size, less than 1 mm (noted SS), and large size, between 1 and 5 mm (noted BS), to see the effect of sample size on the extraction yield.

Extraction was performed in accordance with Calumpong et al. (1999) with some small modifications. Samples were dried to constant weight at 60 °C in an oven, soaked for one night in a 2% formaldehyde solution to eliminate pigments, then washed with distilled water and added to a 0.2 M HCl solution before being set aside for 24 h.

After this period, the samples were washed once again with distilled water before being extracted under agitation for 5 h with a 2% sodium carbonate solution (referring to the work of Torres et al. (2007).

To determine the effect of the temperature on the extraction yield, we chose three different temperatures (25, 40, and 60 °C). The supernatants collected after different extraction periods were eliminated by centrifugation. Sodium alginate was precipitated with ethanol. At the end, sodium alginate was purified twice with ethanol, then with methanol and acetone before been dried at room temperature.

2.2. Purification monitoring by fluorescence spectroscopy

To follow the purification procedure, fluorescence spectroscopy was used. Alginates are strongly fluorescent due to small amounts of polyphenolic residues. This is a routine technique to measure these contaminants in a wide range of alginates. The spectra were obtained with an Ocean Optics USB2000 spectrofluorimeter following the method described by Klock et al. (1997). The excitation wavelength was 370 nm, and the emission signals were observed in the 400–900 nm range.

2.3. Chemical characterization

^{1}H NMR spectra were acquired on 0.1% w/v solutions of sodium alginate in D_2O with a Fourier-transform Bruker 250 BioSpin supplied with an inverse multinuclear gradient probe-head with z-shielded gradient coils, and with a Silicon Graphics Workstation, at different temperatures.

Infrared spectra of solid samples were recorded on a Perkin-Elmer Paragon 1000 FT-IR spectrophotometer. A total of 32 scans were taken with a resolution of 4 cm^{-1}. Sodium alginate samples were dried for 8 h at 56 °C under vacuum. Spectra of the samples in KBr pellets (10% w/w) were recorded in the 4000–450 cm^{-1} range.

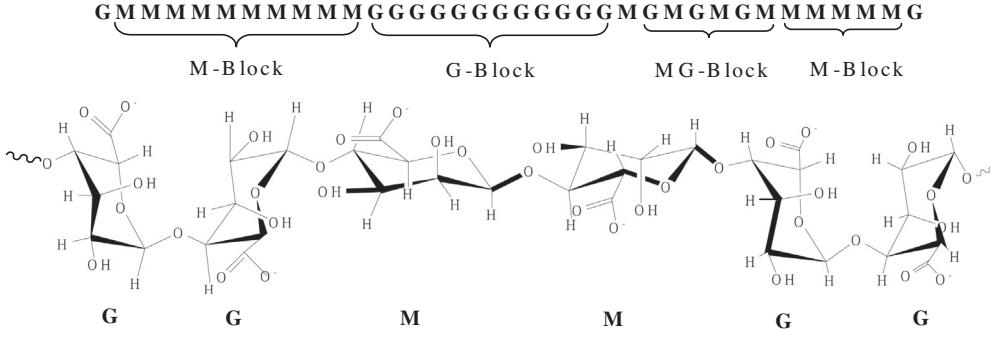

Figure 1 Block distribution and chain conformation of alginates.

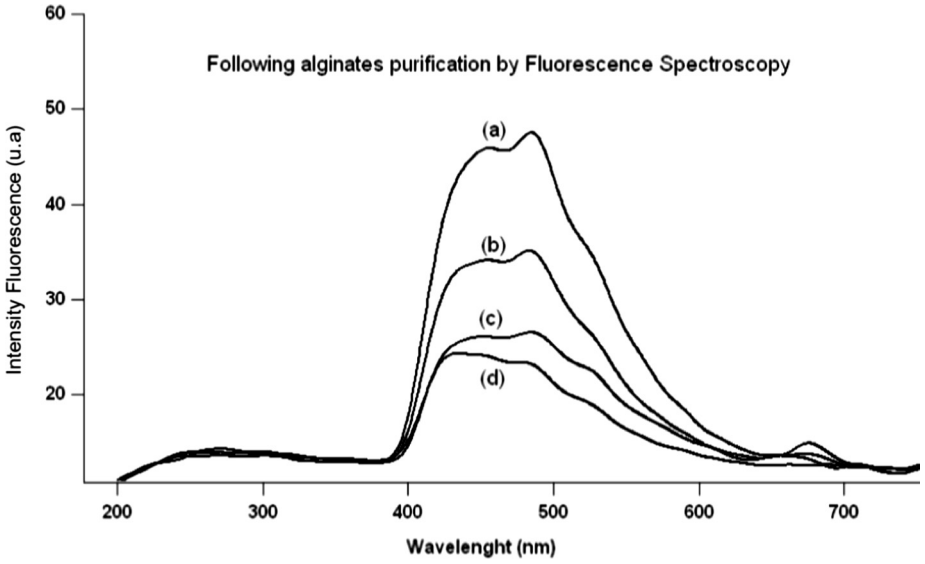

Figure 2 The impurity behavior by fluorescence. (a) Emission curve for the first purification (b) Emission curve for the second purification (c) Emission curve for the third purification (d) Emission curve for the fourth purification.

An Ubbelohde viscometer with a 0.5-mm capillary diameter, and with a solvent flow time (0.1 M NaCl) of 290 mL/s at 25 °C was used to measure specific viscosities. A stock solution in a concentration of 30 mg/10 mL was prepared by stirring for 5 h at room temperature (25 °C). Measurements were done over a range of polysaccharide concentrations from 0.05 to 0.3 g/dL.

3. Results and discussion

3.1. Extraction yield

As we can see from Table 1, extraction yields obtained for SS samples are higher than those obtained for BS samples. This can be explained by the more important surface area in SS samples compared to BS samples. On the other hand, the temperature seems to be an important parameter affecting the extraction yield. Even the high temperature is profit in favor of raising the yield of extraction. Reproducible results obtained in our study showed that the temperature of 40° is appropriate to have a high and pure alginate (51.8% for SS and 44.01% for BS) which can be explained by the degradation that occurred on the natural macromolecule chains when we increased the extraction temperature.

3.2. Impurity monitoring by fluorescence spectroscopy

Alginates are widely used in drug delivery and tissue engineering. So purification is crucial for alginate applications in the biomedical field, since this natural polymer is known to be largely contaminated. Impure alginates can lead to the development of fibrotic cell overgrowth around alginate microcapsules and be consequently responsible for side effects on humans. The principal alginate contaminants are polyphenols, endotoxins, and proteins (Dusseault et al., 2006).

Table 1 Alginate extraction yield for different sample sizes and temperatures.

	Size < 1 mm = SS			1 mm < size < 5 mm = BS		
T (°C)	25	**40**	60	25	**40**	60
Yield (%)	38.33	**51.8**	43.2	35.28	**44.01**	40.2

The purification degree of alginates was checked by fluorescence spectroscopy. Fig. 2 shows the impurity profile of alginates after four operations of purification. For our samples, the analysis shows the presence of residues emitting at 450 and 484 nm. After 4 consecutive repurifications with ethanol, until the intensity profile was constant, the fluorescence intensity was decreased by 51.17% for contaminants emitting at 484 nm and by 52.96% for those emitting at 450 nm. These results are consistent with the values reported by Orive et al. (2002) for *Laminaria hyperborean* sodium alginate (63%) and Torres et al. (2007) for *Sargassum vulgare* a brown algae for which the intensity was reduced by 52.7%. Klock et al. (1994) noted that the remaining contaminants detected in the fluorescence spectra of alginates from *Durvillaea potatorum* (brown algae) could not be identified. The *in vitro* and *in vivo* biocompatibility tests showed that these impurities did not initiate a foreign body reaction (Klock et al., 1997).

3.3. Characterization of sodium alginate by ^1H NMR

The ^{1}H NMR spectra of sodium alginate sample at different temperatures are shown in Fig. 3. These spectra illustrate the importance of choosing the appropriate temperature for ^{1}H NMR analysis. All characteristic signals of sodium alginate appeared just with the acquisition of a ^{1}H NMR spectrum at 343 K; the low field spectral region, assigned to the anomeric protons, can be recorded with a good resolution. Consequently the polymer composition can be determined by analyzing spectrum in this good resolution. We can conclude that the analysis temperature positively affects the viscosity by reducing the peak broadness and moving the DOH resonance far from the more diagnostic signals.

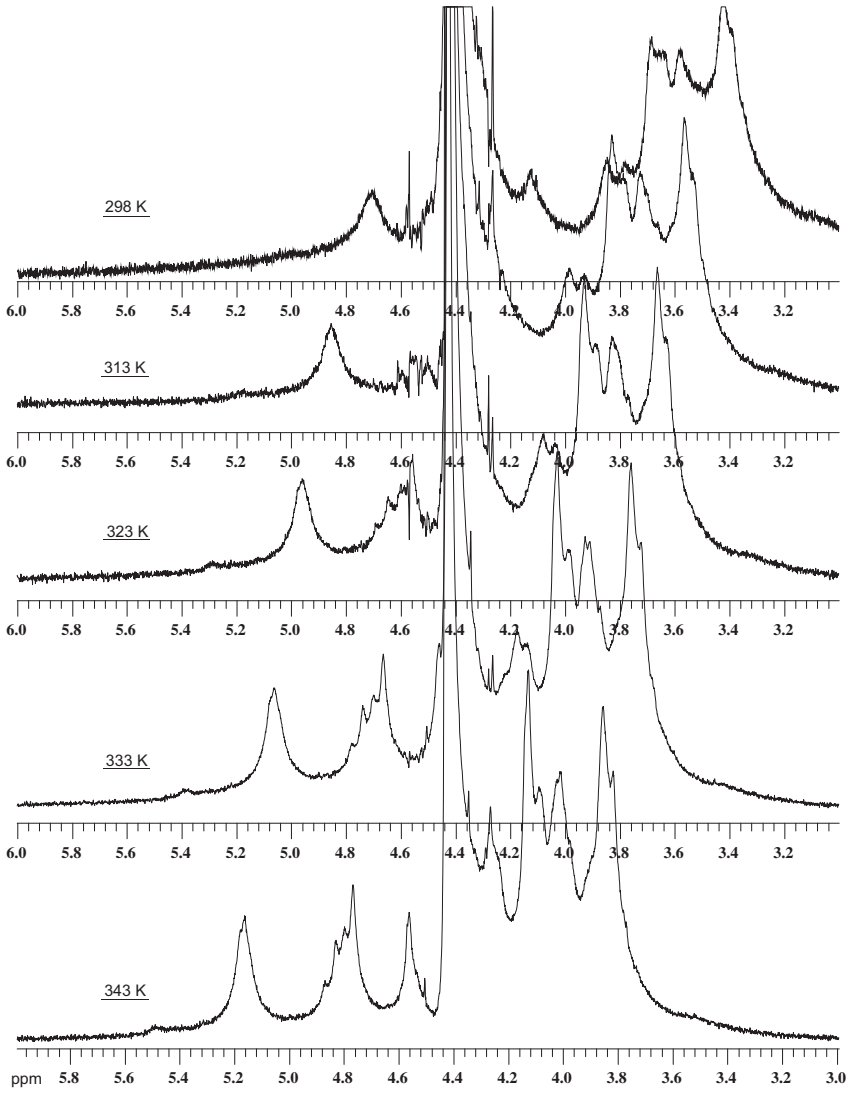

Figure 3 Effect of the temperature on the ^{1}H-NMR signals.

The peaks of the components in sodium alginate of *L. digitata* samples (Fig. 3) were observed and assigned on the basis of the data previously reported in the literature. The integrals are in agreement with those reported for this kind of sodium alginate (Penman and Sanderson, 1972; Dora et al., 2007; Grasdalen et al., 1979).

The anomeric regions of the ^{1}H NMR spectra of sodium alginate samples (Fig. 4), show specific peaks of the guluronic acid anomeric proton (G-1) at 5.17 ppm (peak I); guluronic acid H-5 (G-5) at 4.56 ppm (peak III); and mannuronic acid anomeric proton (M-1) at 4.76 ppm (peak II) and also the C-5 of alternating blocks (GM-5) at 4.82 ppm imbricated with peak III.

This anomeric region can also give information about the linkage between G-blocks and M-blocks as it was previously reported. (Haug et al., 1974; Grasdalen et al., 1979) M-1M and M-1G represent the anomeric proton of an M residue neighboring another M residue or a G residue, respectively. MG-5M, GG-5M, and MG-5G refer to the H-5 proton of the central G residue in an MGM, GGM, or MGG triad, respectively. G-1 refers to the anomeric proton of G residues and GG-5G refers to the anomeric proton of G residues in G-blocks.

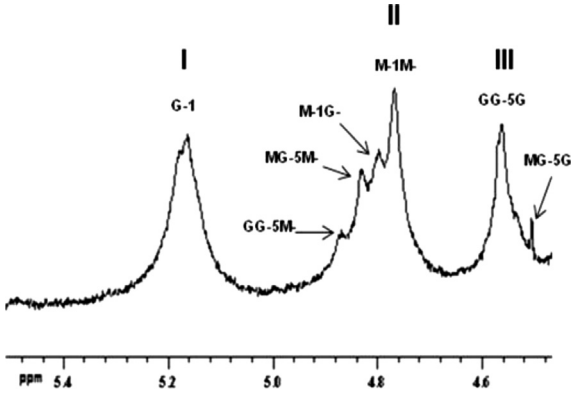

Figure 4 Assignment of the H1 and H5 signals for M and G residues.

Composition and also the block structures of alginate molecules are often determined by ^{1}H NMR spectroscopy (Panikkar and Brasch, 1996; Larsen et al., 2003). The method

proposed by Grasdalen (1983) and Grasdalen et al. (1979) makes it possible to calculate the M/G ratio and to determine the block structure. The signals and the relative area of anomeric protons can be used for the quantitative analysis of the individual guluronic acid (F_G) and the doublet G–G (F_{GG}) fractions.

The area under the peak (I–III) can be used to calculate F_G and F_{GG} using the following equations:

$$F_G = A_I/(A_{II} + A_{III}) \quad (1)$$

$$F_{GG} = A_{III}/(A_{II} + A_{III}) \quad (2)$$

The fraction M is deduced from:

$$F_M = 1 - F_G \quad (3)$$

And the M/G ratio is calculated explicitly by

$$M/G = (1 - F_G)/F_G \quad (4)$$

The physical properties of alginates are largely determined by the relative amount of the three types of blocks present in the copolymer. Therefore, the M/G ratio is an important value for the nature of gel formed from alginates (Penman and Sanderson, 1972). This ability became more important in industrial applications with the use of alginates as bio-adsorbents for heavy metals. This ability depends also on the occurrence of GG block diads (Davis et al., 2003, 2004). Divalent cations preferentially bind toward the G-block rather than the M-block, and the "egg-box" has been considered as a general model to describe the gel formation (Grant et al., 1973; Braccini and Perez, 2001).

F_{GG} and F_{MM} fractions can be calculated from the following relationships (Larsen et al., 2003):

$$F_G = F_{GG} + F_{GM} \quad (5)$$

$$F_M = F_{MM} + F_{MG} \quad (6)$$

The comparison of the M/G ratio and other data for Moroccan *L. digitata* alginates with similar data for alginates from other foreign *Laminarian* species is given in Table 2.

The M/G ratio value of Moroccan *L. digitata* was 1.12. The results obtained for our samples are similar to those observed for those collected from Norway and they are a bit higher compared to other *Laminarian* species (Table 2).

From these results, we can conclude that our alginate samples are suitable to form soft and elastic gels more than brittle ones as mentioned in the work of Penman and Sanderson (1972). They report that brittle gels are obtained from alginates with a low M/G ratio, while elastic gels are formed from alginates with a high M/G ratio. It can be noted from the literature that the M/G ratio varies according to the location from which algae were collected and also according to the extraction procedure as it was reported in the work of nichide et al. (1987) Moreover, there are some studies in which we can find a dissimilarity in M/G ratio values for the same species of algae, in the same location and for different periods of the year (Craigie et al., 1984; Mairh, 1982; Indergaard and Skjak-Braek, 1987).

Even the M/G ratio is considered as a characteristic parameter allowing us to have an idea about the gelation behavior of alginates, but we cannot avoid the importance of the presence of homopolymeric block structures, that is, mannuronic acid blocks (F_{MM}), guluronic acid blocks (F_{GG}), and alternating blocks (F_{MG}).

Most applications of alginates are based on its gel-forming ability through cation binding: for example, the transition from water-soluble sodium alginate to water-insoluble calcium alginates.

Moroccan *L. digitata* alginates possess a high quantity of both homopolymeric mannoronic and guluronic blocks (F_{MM}) and (F_{GG}), respectively while the alternating block fractions (F_{MG} and F_{GM}) showed lower values than those previously described in the literature. *L. digitata* from Norway has a high value of alternating block fraction but with a low homopolymeric fraction F_{GG}. We can conclude the same thing for *Laminaria Japonica*.

The fraction of alternating blocks (F_{MG}, F_{GM}) is lower in the Moroccan *L. digitata* than in other species. The gel formation will then be affected positively because of the non contribution of mixed segments M-G-M-G in this process.

3.4. Infrared measurements

The FT-IR spectrum of sodium alginate from *L. digitata* is presented in Fig. 5. Results of infrared measurements are in remarkably good agreement with the results of Leal et al. for sodium alginate of *Desmarestia ligulata* (Leal et al., 2008). Table 3 summarizes characteristic bands in 3600–1600 cm^{-1} range.

Weak bands at 1316.79, 1125.53 and 1094.66 cm^{-1} may be assigned to C–C–H and O–C–H deformation, C–O stretching, and C–O and C–C stretching vibrations of pyranose rings, respectively; the band at 1035.6 cm^{-1} may be also due to C–O stretching vibrations. The anomeric, region (950–750 cm^{-1}) is the most discussed in carbohydrates (Mathlouthi and Koenig, 1986; Silverstein et al., 1991; Tulchinsky et al., 1976). The spectrum shows a band at 948.2 cm^{-1}, which was assigned to the C–O stretching vibration of uronic acid residues, and one at 902.83 cm^{-1} assigned to the C1–H deformation vibration of β-mannuronic

Table 2 Compositional data of alginates extracted from *Laminarian* species.

Species	Origin	F_G	F_M	M/G	F_{MM}	F_{GG}	F_{GM}	F_{MG}	Ref.
Laminaria digitata	Norway	0.41	0.59	1.44	0.43	0.25	0.16	0.16	Smidsrød and Draget (1996).
Laminaria japonica	China	0.35	0.65	1.86	0.48	0.18	0.17	0.17	Nai-yu et al. (1994)
Macrocystis pyrifera	Argentina	–	–	1.17	–	–	–	–	Gomez et al. (2009)
Laminaria hyperborea (stipe)	Norway	0.71	0.29	0.41	0.17	0.59	0.12	0.12	Grasdalen (1983)
Laminaria hyperborea (leaf)	Norway	0.51	0.49	0.96	0.34	0.36	0.15	0.15	Grasdalen (1983)
Laminaria digitata	France Atlantic ocean	0.40	0.60	1.5	–	–	–	–	Papageorgiou et al. (2006)
Laminaria digitata	Morocco	0.47	0.53	1.12	0.47	0.41	0.06	0.06	This study

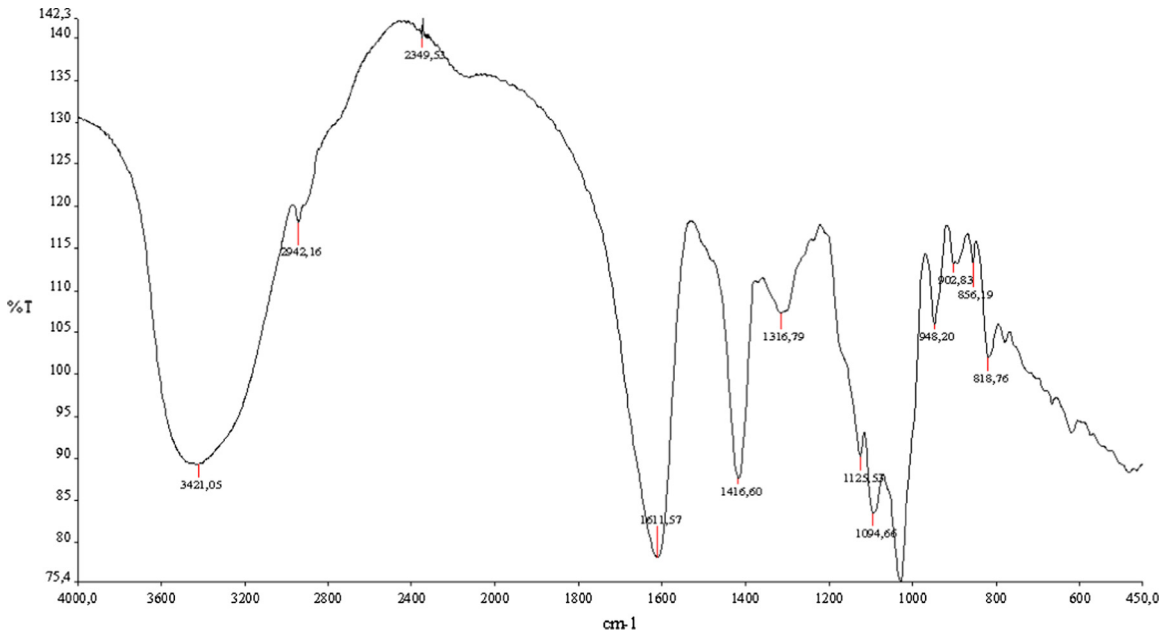

Figure 5 FT-IR spectrum of sodium alginate from Moroccan *Laminaria digitata*.

Table 3 Characteristic bands of sodium alginate found in the FT-IR spectrum.

Bands	Assignments
• Broad band centered at 3421.05 cm^{-1}	Hydrogen bonded O–H stretching vibrations
• Weak signal at 2942.16 cm^{-1}	C–H stretching vibrations
• Intense band at 1611.57 cm^{-1}	Asymmetric stretching of carboxylate O–C–O vibration
• Intense band at 1416.00 cm^{-1}	Symmetric stretching vibration of the carboxylate group
	Mathlouthi and Koenig (1986) and Silverstein et al. (1991)

acid residues. The band at 818.76 cm^{-1} seems to be characteristic of mannuronic acid residues.

3.5. Intrinsic viscosity

The intrinsic viscosity is defined by the Huggins equation (Huggins, 1942) as: $[\eta] = \lim_{c\to 0} \frac{\eta_{sp}}{c}$ where η_{sp} and c are the specific viscosity and the concentration of the solution, respectively. At infinite dilution, this equation represents the measurement of the hydrodynamic volume occupied by the macromolecule. A classical procedure for its determination, based on Eq. (8), consists of the determination of viscosities of solutions with various concentrations, followed by extrapolation of η_{sp}/c to zero concentration. In a range of moderate concentrations, the dependence is linear and can be written as the Huggins:

$$\eta_{sp}/c = [\eta] + kH[\eta]^2 c \quad (8)$$

where kH is the Huggins constant.

Thus, $[\eta]$ can be obtained as the intercept in a linear least-squares fit. The plot of reduced viscosity versus alginate concentration is shown in Fig. 6.

The intrinsic viscosity $[\eta]$ in 0.1 M NaCl at 25 °C for Moroccan *L. digitata* alginates is 2.431 dL/g (Table 4). This value was obtained by a graphical extrapolation based on the Huggins equation (Fig. 6).

The intrinsic viscosity is well dependant on the method of extraction used. Davis et al. (2003) mentioned that there is a dramatic difference in intrinsic viscosity values between two species of alginates extracted by the standard neutral technique (11.6 and 10.0 dL/g) and the alkaline method performed at 80 °C (0.57 and 0.84 dL/g). We can obviously explain the low viscosity obtained for Moroccan *L. digitata* alginates, as our extraction conditions are comparable to the conditions of Davis et al. The low viscosity of our samples has a clear advantage related to the elimination of the hydrolysis step prior to NMR acquisition. On the other hand, we can explain the low viscosity by the high homopolymeric fraction MM which is more flexible than the GG fraction.

The characteristics obtained for Moroccan *L. digitata* may be useful for obtaining polyelectrolyte complexes for the production of drug delivery micro- and nanoparticles. In some cases, a charged polysaccharide with a low viscosity is needed.

3.6. Molecular weight determination

The viscosity average molecular weight M_v was calculated from the Mark–Houwink equation:

$$[\eta] = kM_v^a \quad (7)$$

where $[\eta]$ is the intrinsic viscosity, and the constants a and k are empirical parameters which are depending on the system (polymer, solvent, and temperature).

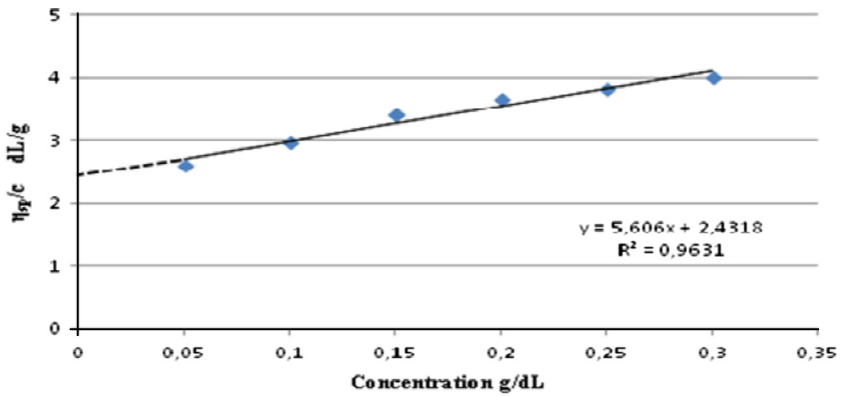

Figure 6 Graphical determination of the specific viscosity of *Laminaria digitata* sodium alginate samples in 0.1 M NaCl.

Table 4 Intrinsic viscosity and average molar masses of alginates from different sources.

Alginate source	$[\eta]$ dL/g	$M_w \times 10^{-5}$ (g/mol)	Ref.
L. hyperborea	6.4	3.05	Clementi et al. (1998)
F. vesiculosus	2.5	1.17	Fourest and Volesky (1997)
A. nodosum	2.8	1.32	Fourest and Volesky (1997)
L. japonica	15.4	7.44	Fourest and Volesky (1997)
S. dentifolium	12.6	6.06	Larsen et al. (2003)
S. asperifolium	15.2	7.34	Larsen et al. (2003)
S. latifolium	8.7	4.16	Larsen et al. (2003)
L. digitata	2.431	1.14	This study

Clementi et al. (1998) proposed empirical relations for $[\eta]$ and the weight-average molar mass (M_w).

$$[\eta] = 0.023 M_w^{0.0984} \quad (8)$$

where $[\eta]$ is given in dL/g and M in kDaltons.

This relationship permits to estimate the M_w values for alginates from *L. digitata* and from other algal sources from intrinsic viscosity data (Table 4). All values obtained have a magnitude of 10^5 g/mol. *L. digitata* has a low molecular weight (1.14×10^5 g/mol) in comparison with other species but it has a similar molecular weight than *Fucus vesiculosus* (1.17×10^5 g/mol) and *Ascophyllum nodosum* (1.32×10^5 g/mol).

4. Conclusions

In this paper, were extracted, purified and characterized Moroccan *L. digitata* alginates. First, we tried to establish the relation between extraction conditions and the extraction yield. We found that a temperature of 40 °C and a sample size less than 1 mm are the appropriate conditions to obtain a high extraction yield. Second, the M/G ratio of Moroccan *L. digitata* alginates was 1.12 which makes it possible to form soft and elastic gels. Third, Moroccan *L. digitata* may be used as polyelectrolyte complexes for the production of drug delivery micro- and nanoparticles.

Acknowledgements

The authors wish to acknowledge the Institut für Organische Chemie und Chemische Biologie of Goethe University-Frankfurt for ^{1}H NMR analysis and the Laboratory of Atomic Molecular Spectroscopy and applications of the University El Manar of Tunis for their contribution on the fluorescence analysis.

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