



Short communication

Microwave assisted rapid method for hydrolysis of sodium alginate for M/G ratio determination

Mahesh Chhatbar, Ramavatar Meena*, Kamalesh Prasad, A.K. Siddhanta *

Marine Biotechnology and Ecology Discipline, Central Salt and Marine Chemicals Research Institute (Council of Scientific and Industrial Research), G. B. Marg, Bhavnagar 364002, Gujarat, India

ARTICLE INFO

Article history:

Received 24 October 2008

Received in revised form 24 November 2008

Accepted 27 November 2008

Available online 6 December 2008

Keywords:

Sodium alginate

M/G ratio

Microwave irradiation

SEM

¹³C NMR

CD

ABSTRACT

A rapid one-pot method for hydrolysis of sodium alginate for determining M/G ratio has been developed under mild conditions, using microwave irradiation. Poly-mannuronic acid (PMA) and poly-guluronic acid (PGA) ratio was determined (M/G 0.38), which was similar to that (M/G 0.39) obtained by the conventional method, using sodium alginate of Sigma as reference sample for bench marking. For validation of the method PMA and PGA were characterized by density, porosity, viscosity, optical rotation measurements, ¹³C NMR, FT-IR, thermogravimetric analysis (TGA), X-ray diffraction, circular dichroism (CD), molar mass distribution (GPC) and scanning electron microscopy (SEM).

© 2008 Elsevier Ltd. All rights reserved.

1. Introduction

Alginates are mainly derived from marine brown seaweeds and are used in the food industry as texturizing and gelling agents (Rojas-Graü, Tapia, Rodríguez, Carmona, & Martin-Belloso, 2007). Alginate is a linear co-polymer of β-D-mannuronic acid (M) and α-L-guluronic (G) acid, ((Fig. 1) De Silva & Kumar, 1989; Haug, Larsen, & Smidsrd, 1967; Llanes, Ryan, & Marchessault, 2000). The conventional method for determination of M/G ratio under strong acidic conditions for long hours has been reported by Chandía, Matsuhira, and Vásquez (2001). Hydrolysis of starch and gums using microwave irradiation has been reported by Li et al. (2001) and Singh, Sethi, Tewari, Srivastava, and Sanghi (2003). No literature report, however, is available wherein microwave irradiation was used for hydrolyzing alginate.

In this communication we report a rapid and mild method using microwave-assisted hydrolysis of alginate, for determining M/G ratio. The method was validated by carboxyl group assay of the alginic acid as well as by characterizing the hydrolytic products PMA and PGA using FT-IR, ¹³C NMR, SEM, XRD, TGA, CD and GPC.

2. Experimental

Sodium alginate was extracted from *S. wighitii* collected from the Indian waters (22°28'N, 69°05'E) adapting a reported

method (Nishide, Anzai, & Uchida, 1987), and using Sigma (A2158-250G) alginate as a reference sample for benchmarking.

Domestic microwave oven (LG Microwave Appliance, Model No. MS-285SD; made in Korea; Magnetrons were set at a frequency of 2450 MHz), was used in this study. The average bulk temperature at the end of the reaction was measured by inserting thermometer in the reaction mixture.

Alginate (5 g) was dissolved in 125 ml of 0.15 M oxalic acid or 0.25 M H₂SO₄ and 25 ml portions of the alginate solution were exposed to 100% microwave power for five different exposure durations (1–5 min). The experiment was repeated with 0.05, 0.15, 0.25 and 0.5 M oxalic acid/H₂SO₄. After microwave irradiation PMA and PGA were isolated from the hydrolysed samples using pH dependent method (Chandía et al., 2001; Sakugawa, Ikeda, Takemura, & Ono, 2004) Fig. 2.

To check the stability of alginate to microwave irradiation, a control experiment was performed under the same conditions except using acid reagent.

3. Characterizations

Bulk and true densities, pore volume, and porosity of alginate, PGA and PMA were measured as described in our previous study (Meena, Prasad, & Siddhanta, 2007), using Eqs. (1)–(4), respectively.

$$d_0 = (W_2 - W_1)/10 \quad (1)$$

* Corresponding authors. Tel.: +91 278 2567760; fax: +91 278 2567562.

E-mail address: aks@csmcric.org (A.K. Siddhanta).

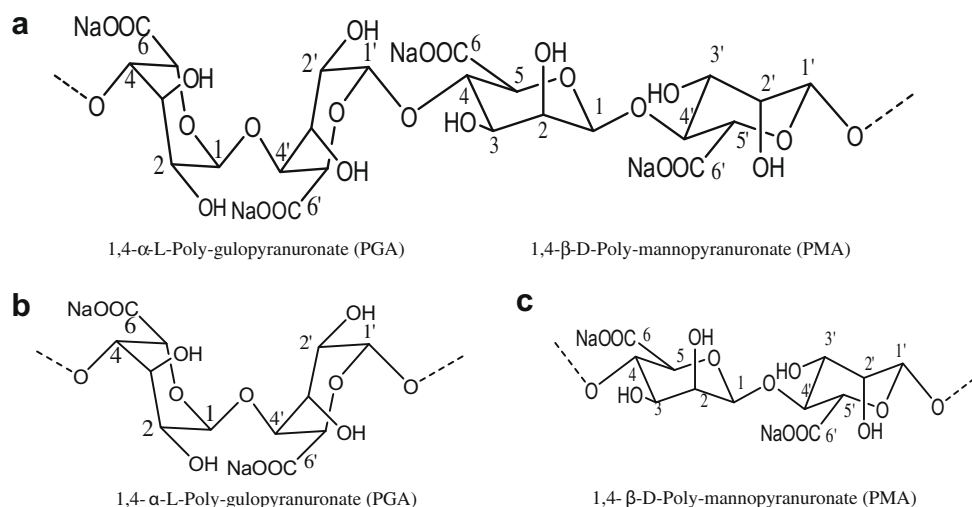


Fig. 1. Chemical structures of (a) repeating uronic acid (PGA and PMA) units in alginate, repeating units of (b) PGA, and (c) PMA.

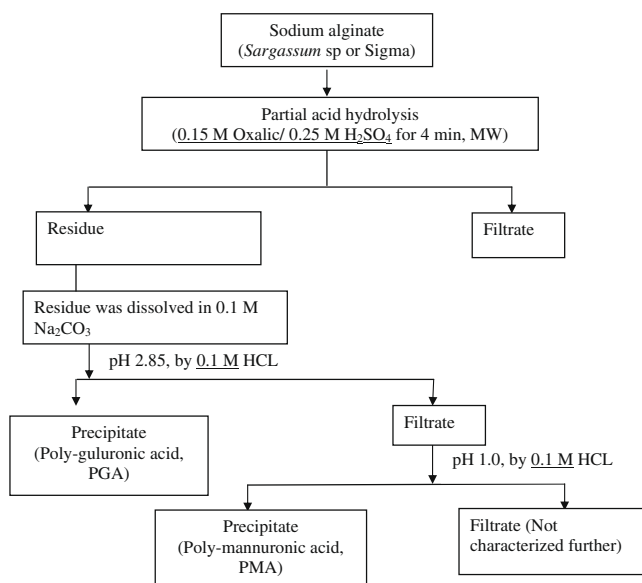


Fig. 2. Procedure for the isolation of poly-mannuronic acid (PMA) and poly-guluronic acid (PGA) from sodium alginate.

where W_2 is the total weight of the polymer and flask, W_1 is the weight of the flask only, and 10 is the volume of flask in ml.

$$d = W_0 / [10 - (W - W_0) / d_c] \quad (2)$$

Table 2

Comparison of M/G ratios of sodium alginate determined by reported methods^a with those of the present study.

Reaction conditions	Alginate of <i>S. wightii</i>			Alginate of Sigma (A2158-250G)		
	PGA ^b (%)	PMA ^b (%)	M/G ratio	PGA ^b (%)	PMA ^b (%)	M/G ratio
Conventional method (80% H ₂ SO ₄ (20 °C, 18 h), and then 2 N H ₂ SO ₄ (100 °C, 6 h)	55.20	21.40	0.387	49.60	25.1	0.51
MW (0.15 M oxalic acid, 4 min)	61.8	22.5	0.364	45.4	22.0	0.484
MW (0.25 M H ₂ SO ₄ , for 4 min)	63.5	22.0	0.346	41.6	19.5	0.468
MW (0.5 M oxalic acid, 4 min)	60.4	22.2	0.367	–	–	–
MW (0.5 M H ₂ SO ₄ , 4 min)	57.9	21.5	0.371	–	–	–

^a Haug and Larsen (1962) and Ji et al. (1981).

^b % of PGA and PMA were calculated by UV-vis spectroscopy as described by Haug and Larsen (1962), and Ji et al. (1981).

Table 1

Optimum hydrolysis condition and M/G ratio measurement for the sodium alginate, developed in the present investigation.

Reaction conditions	Hydrolysis of sodium alginate extracted from <i>S. wightii</i> yield (%)					Hydrolysis of sodium alginate purchased from Sigma (A2158-250G) yield (%)				
	Filtrate	Residue ^a	PGA ^b	PMA ^b	M/G ratio ^c	Filtrate	Residue ^a	PGA ^b	PMA ^b	M/G ratio ^c
Conventional method (80% H ₂ SO ₄ (20 °C, 18 h), and then 1 M H ₂ SO ₄ (100 °C, 6 h)	24.0	76.0	54.5	21.5	0.394	34.6	64.9	38.1	21.6	0.56
M _w (0.05 M Oxalic acid, 4 min)	11.0	89.0	69.5	19.5	0.280	–	–	–	–	–
M _w (0.05 M H ₂ SO ₄ , 4 min)	9.0	91.0	73.0	18.0	0.240	–	–	–	–	–
M _w (0.15 M Oxalic acid, 4 min)	22.0	78.0	56.5	21.5	0.380	–	–	–	–	–
M _w (0.15 M H ₂ SO ₄ , 4 min)	18.5	81.5	60.8	20.7	0.340	–	–	–	–	–
M _w (0.25 M Oxalic acid, 4 min)	20.1	78.9	58.0	21.9	0.377	34.6	64.9	39.4	23.6	0.59
M _w (0.25 M H ₂ SO ₄ , for 4 min)	22.9	77.1	55.6	21.5	0.385	34.6	64.9	37.4	22.8	0.60
M _w (0.5 M Oxalic acid, 4 min)	21.0	79.0	54.8	21.3	0.388	–	–	–	–	–
M _w (0.5 M H ₂ SO ₄ , 4 min)	23.4	76.6	51.9	20.8	0.400	–	–	–	–	–

^a Yield (%) w.r.t. sodium alginate.

^b Yield (%) w.r.t. the residue.

^c M/G ratios were comparable with the 0.15 M, 0.25 M and 0.5 M concentrations of acids after 4 min of M_w irradiation, used in this investigation.

where W is the total weight of sample and solvent; W_0 is the weight of the dry sample; d_c is the density of the solvent ($d_{\text{cyclohexane}} = 0.778 \text{ g/ml}$).

$$\phi = V_p/V_0 \quad (3)$$

where V_p is the pore volume in reactant and product beads; V_0 is the true volume of the same.

$$V_0 = W_0/d \quad (4)$$

where W_0 is the weight of dry reactant and product beads, and d (true density) of reactant and product beads) was determined according to Eq. (2).

The PMA to PGA ratio (M/G) of alginate samples was determined by following the methods of Haug and Larsen (1962) and Ji, Cao, and Han (1981). Noise-decoupled ^{13}C NMR spectra were recorded on a Bruker Avance-II 500 (Ultra shield) Spectrometer, Switzerland, at 500 MHz. Alginate of *Sargassum*, PMA and PGA were dissolved in $d\text{-NaOH}$ (50 mg/ml) and spectra were recorded at 35 °C with 5000–5200 accumulations, pulse duration 5.9 μs , acquisition time 1.2059 s and relaxation delay 6 μs using DMSO as internal standard (ca. δ 39.7). FT-IR spectroscopy was recorded on a Perkin-Elmer Spectrum GX, FT-IR System, USA by taking 2.0 mg of non-hydrolysed and hydrolysed alginate samples in 600 mg of KBr. X-ray diffractions were studied on a Philips analytical X-ray instrument.

Table 3

Comparison of the properties of sodium alginate, PMA and PGA obtained from *S. wightii* and Sigma (A2158–250G).

Properties	<i>S. wightii</i>			Sigma (Product No.A2158–250G)		
	Sodium alginate	PMA	PGA	Sodium alginate	PMA	PGA
Optical rotation $[\alpha]_D$ (c 0.25 in 0.1 M aqueous Na_2CO_3) ^a (°)	−25.67	−112.58	−68.20	−26.98	−109.34	−65.94
Mw	8,77,599	29,205	47,797	7,82,709	38,383	48,221
M_n	2,96,481	18,064	19,829	3,46,834	20,739	22,937
PDI	2.960	1.617	2.410	2.257	1.851	2.102
Mw ratio (M/G)	0.61	NA	NA	0.79	NA	NA
Viscosity (cP s)	110	20	40	200	30	60
Bulk density	0.89	0.82	0.78	0.94	0.88	0.81
True density	1.212	1.189	1.200	1.270	1.210	1.232
Porosity	0.88	0.72	0.69	0.93	0.71	0.67

^a $[\alpha]_D$ (0.3% w/v) −0.098° (10 °C); −0.110° (at 60 °C) (Tako, Yoza, & Tohma, 2000); $[\alpha]_D$ (1.0% w/v) −113° (Bi, Mahmood, Arman, Taj, & Iqbal, 2007).

Circular dichroism (CD) spectra were recorded on Jasco model J-815 CD Spectrometer, using measurement range at 190–250 nm and sample concentration of 0.8 mg/ml (800 ppm).

The ratio of peak height to trough depth was calculated using Eq. (5), described by Morris, Rees, and Thom (1980).

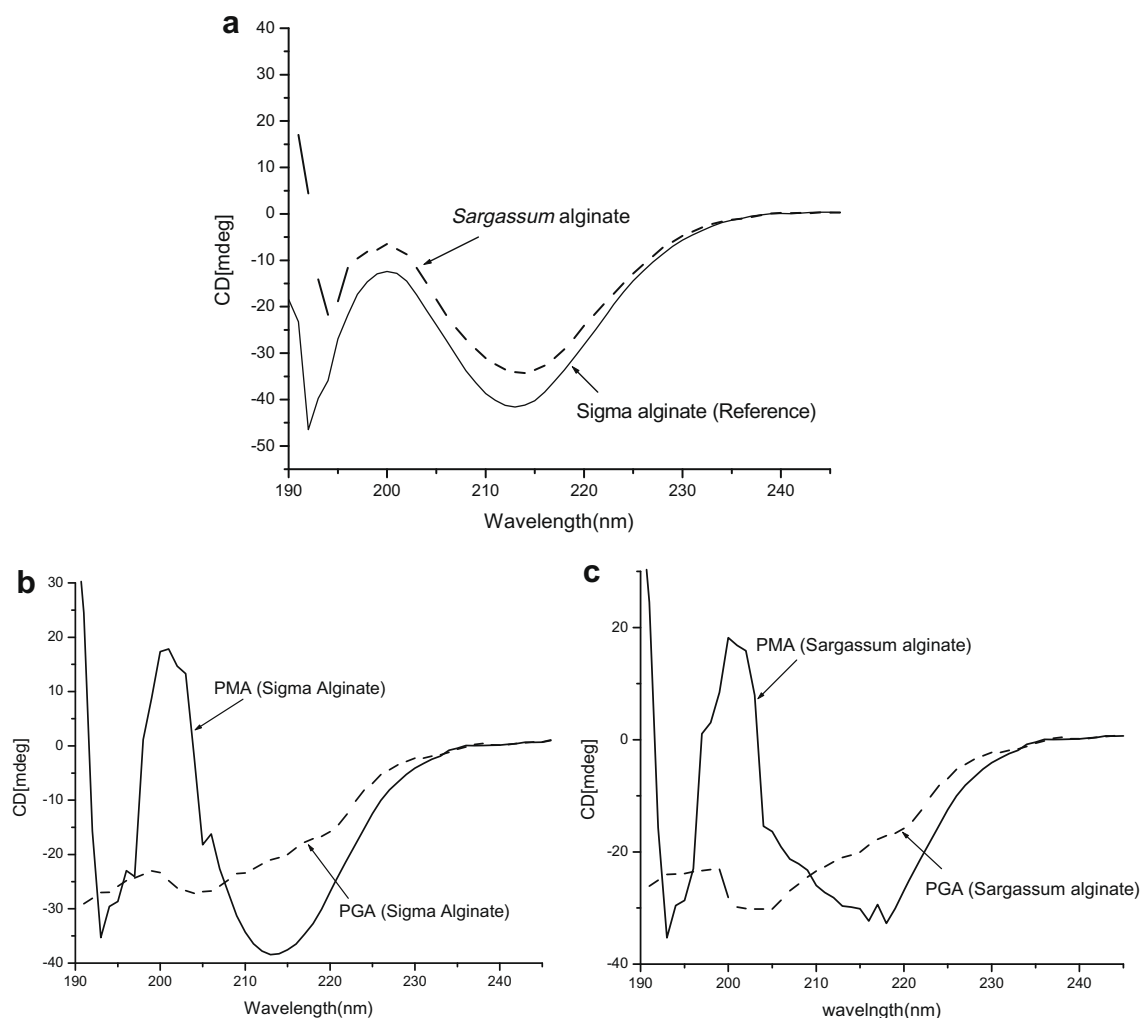


Fig. 3. Circular dichroism (CD) spectra of (a) sodium alginate, (b) PGA, and (c) PMA of Sigma and *S. wightii*.

Table 4¹³C NMR shifts measured in alginic acid, PGA and PMA.

Samples	δ ppm	Assignment of carbon
Sodium alginate	100.69 (98.47) ^a	C-1 of MM
	71.46 (68.78) ^a	C-2 of MM
	72.45	Not assigned
	70.18 (70.06) ^a	C-3 of MM
	76.69 (76.05) ^a	C-4 of MM
	72.49 (74.40) ^a	C-5 of MM
	176.01 (173.99) ^a	C-6 of MM
	100.36 (99.71) ^a	C-1 of GG
	67.35 (67.71) ^a	C-3 of GG
	80.81 (78.99) ^a	C-4 of GG
	72.31 (68.58) ^a	C-5 of GG
	176.38 (174.21) ^a	C-6 of GG
PGA	101.11 (101.54) ^b	C-1 of GG
	99.62 (97.9) ^b	C-1' of GG
	66.61 (64.8) ^b	C-2 of GG
	70.27 (68.95) ^b	C-3 of GG
	71.43	C-3' of GG
	76.75 (80.4) ^b	C-4 of GG
	75.62	C-4' of GG
	67.00 (67.16) ^b	C-5 of GG
	86.10	Not assigned
	175.16 (173.2) ^b	C-6 of GG
	99.77 (100.37) ^b	C-1 of MM
	99.68 (98.9) ^b	C-1' of MM
PMA	69.76 (68.78) ^b	C-2 of MM
	66.96 (66.92) ^b	C-2' of MM
	71.43 (71.2) ^b	C-3 of MM
	70.25	C-3' of MM
	79.09 (78.16) ^b	C-4 of MM
	76.83 (76.21) ^b	C-5 of MM
	75.56	C-5' of MM
	175.24 (174.3) ^b	C-6 of MM

^a Sakugawa et al. (2004).^b Zhang et al. (2004); Sakugawa et al. (2004); Leroux et al. (2004).

$$\text{Peak/trough ratio} = (\theta_{\text{trough}} - \theta_{\text{peak}} / \theta_{\text{trough}}) \quad (5)$$

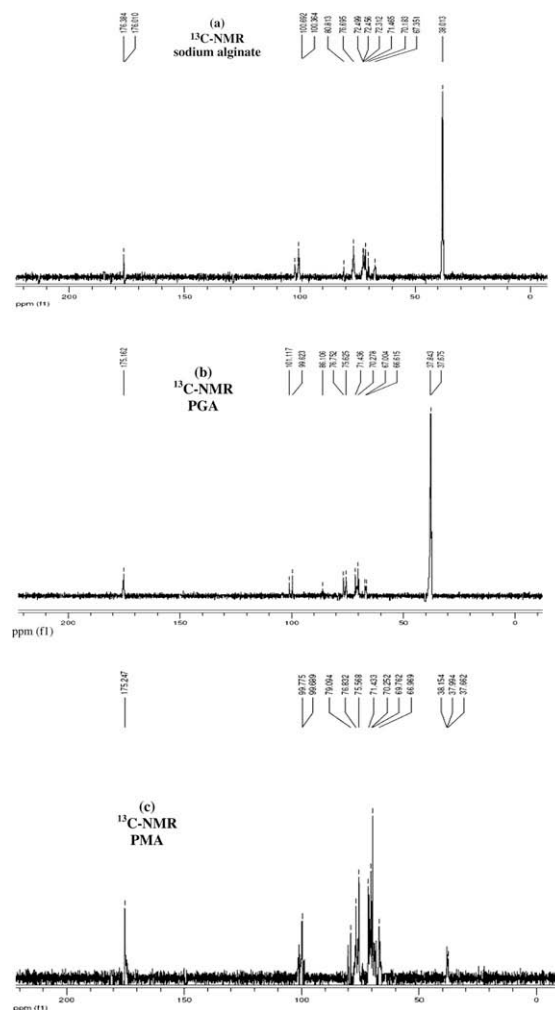
Optical rotation was measured (0.25% (w/v) in 0.1 M Na₂CO₃ solution, at 45 °C), on a Rudolph Digi pol – 781 Polarimeter (Rudolph Instruments Inc., NJ, USA). The samples surface morphology was examined by SEM (Model Carl-Zeiss Leo VP 1430) at an accelerating voltage of 20 kV and 202× magnification. The molar mass (weight average molecular mass, M_w ; number average molecular mass, M_n , and polydispersity) of the alginates and their respective oligomers (500 ppm solution in 0.1 M Na₂CO₃ at 25 °C) were determined by GPC (Alliance, Waters 2695, separation module with Waters 2414 RI Detector, Country).

Validity of the present method was done by assaying the carboxylic group by standard method (cf. British Pharmacopoeia, 1998; Food Chemicals Codex, 1981).

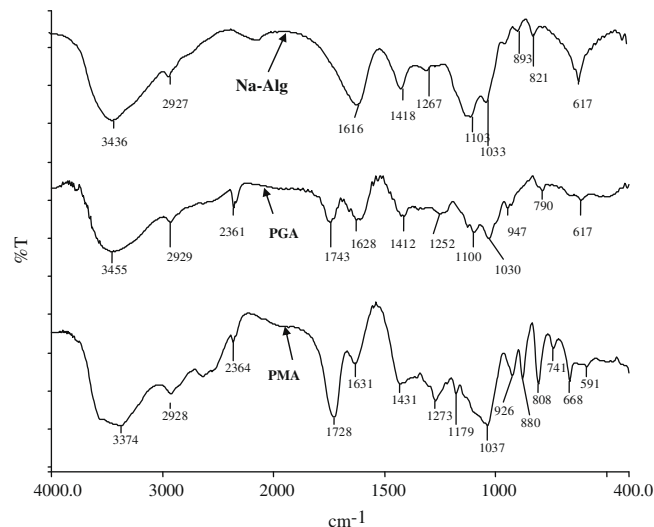
4. Results and discussion

The M/G ratios and % weights of PGA and PMA obtained under optimized microwave (0.15 M Oxalic acid or 0.25 M H₂SO₄, 4 min) conditions in the present method for the alginate of *S. wighitii* and Sigma were comparable to ones obtained by conventional method reported by Haug and Larsen (1962) and Ji et al. (1981) (Tables 1 and 2). M/G ratios obtained with 0.15 M Oxalic acid or 0.25 M H₂SO₄ and 0.5 M Oxalic or 0.5 M H₂SO₄ acids were also comparable (Table 1). This investigation demonstrated that sodium alginate can be hydrolysed under microwave irradiation, using mild hydrolytic conditions, as opposed the reported methods using hydrolytic reaction with high acid concentration requiring longer reaction time.

The CD spectra of PGA and PMA showed entirely negative and strong positive peaks, respectively, while CD spectrum of alginate

**Fig. 4.** ¹³C NMR of (a) sodium alginate, (b) PGA, and (c) PMA of *S. wighitii*.

showed intermediate behavior (Fig. 3). Spectra of alginate show a peak at (ca. 200 nm), and a trough at (ca. 215 nm). The peak/trough ratios were less than one for both the alginate samples, indicates high content of poly-guluronate residue (Morris et al., 1980).

**Fig. 5.** FT-IR of sodium alginate, PGA and PMA of *S. wighitii*.

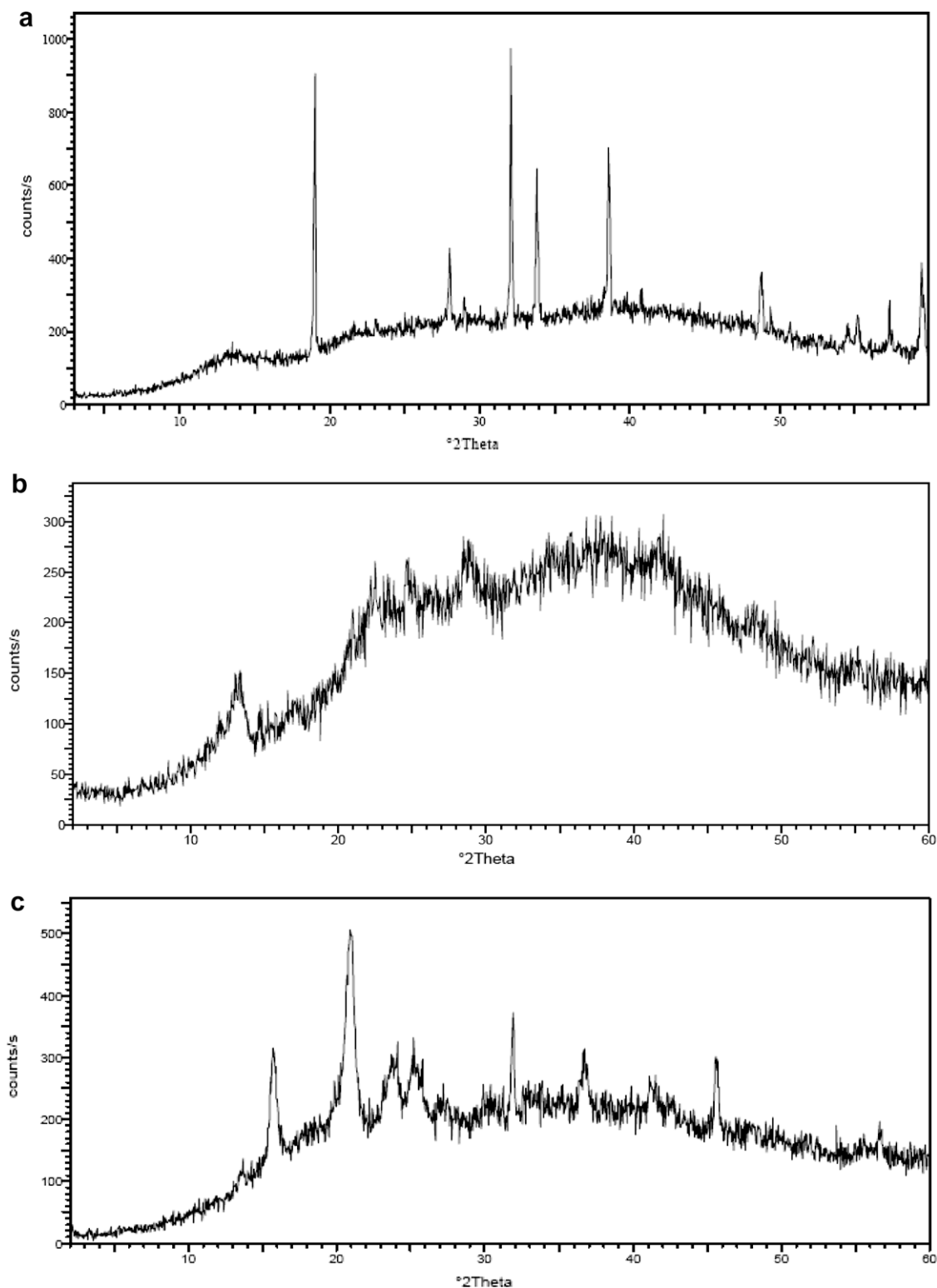


Fig. 6. X-ray diffraction pattern of (a) sodium alginate, (b) PMA, and (c) PGA of *S. wighitii*.

The viscosity, bulk density, true density and porosity of PGA and PMA were 40 cP s; 0.78 g/ml; 1.200 g/ml; 0.69 and 20 cP s; 0.82 g/ml; 1.189 g/ml; 0.72, respectively, which were lower than those of alginate of *Sargassum* sp. 110 cP s; 0.89 g/ml; 1.212 g/ml and 0.88 (Table 3). Similar trend was found in alginate of Sigma (Table 3). The M_w , M_n and PDI of *S. wighitii* and Sigma alginate samples and their respective oligomers are shown in Table 3.

The optical rotations of *S. wighitii* alginate and their oligomers PGA and PMA were $[\alpha]_D^{30} -25.67^\circ$, $[\alpha]_D^{30} -68.20^\circ$ and $[\alpha]_D^{30} -112.58^\circ$, respectively (Table 3), and were comparable with those of Sigma alginate and corresponding PGA and PMA (Table 3).

Chemical shifts for the ^{13}C solution (^{13}C NMR) spectra of the alginate and their respective acids (e.g. PGA and PMA) were shown in Table 4 and Fig. 4, and were comparable with those of reported

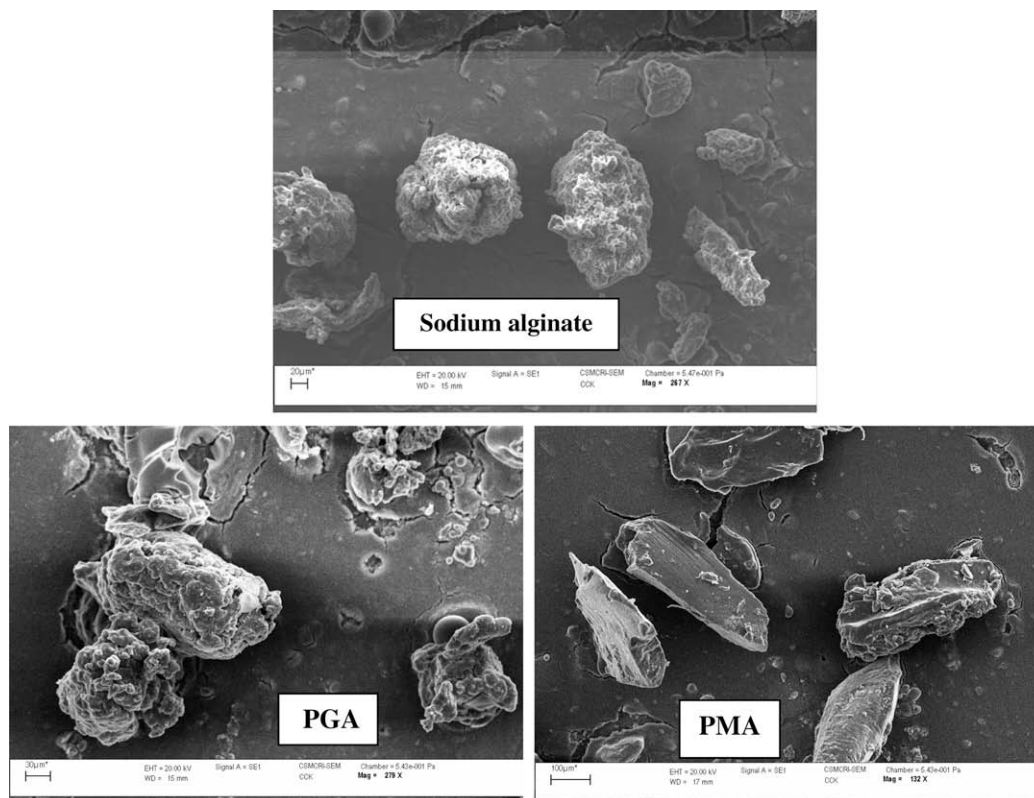


Fig. 7. SEM images of sodium alginate, PGA and PMA of *S. wightii*.

in the literature (Leroux, Gachon, & Besse, 2004; Sakugawa et al., 2004; Zhang et al., 2004).

The FT-IR spectra of the alginate, PGA and PMA showed in the Fig. 5. The spectrum of alginate showed some characterized peaks at 893, 821 and 944 cm^{-1} were related to PMA and PGA acids, but after separation of PMA and PGA showed their characterized peaks at 880, 808, and 947 and 790 cm^{-1} in their IR spectra, respectively, (Fig. 5). The intense peak at 1743 and 1728 cm^{-1} in PGA and PMA appeared may be due to the formation of respective acids during acid hydrolysis. Similar results have been reported in the literature (Chandía et al., 2001).

X-ray diffraction patterns exhibited that the alginate exhibited crystalline in nature, while PGA and PMA appeared in form of amorphous and crystalline, respectively (Fig. 6). X-ray results revealed that PMA content of the alginate was crystalline in nature, may be due to the ordered structure and regular geometric arrangement (Fig. 6). The SEM images of alginate, PGA and PMA have been shown in Fig. 7. The SEM image of the alginate showed mixture of PGA and PMA, while PGA and PMA SEM images showed different morphologies indicates separations of PGA and PMA (Fig. 7). The $-\text{COOH}$ group content of alginic acids obtained by conventional and present methods were 23.71% and 24.01%, respectively, comparable with the reported values in the pharmacopeia (1–25%), which lends credence to the present method.

5. Conclusions

Hydrolysis of alginate was achieved under mild reaction conditions in a rapid method using microwave irradiation in presence of substantially lower concentration of acids, compared to the conventional method reported in the literatures. M/G ratios obtained from the present method were comparable with those of conven-

tional methods. Thus this is a rapid and alternative method for the hydrolysis of alginate.

Acknowledgement

One of the authors (MC) is grateful to the Ministry of Earth Sciences, New Delhi (Sanction No. MoES/9-DS/6/2007-PC-IV), for the award of a fellowship.

References

- Bi, F., Mahmood, S. J., Arman, M., Taj, N., & Iqbal, S. (2007). Physicochemical characterization and ionic studies of sodium alginate from Sargassum terrarium (brown algae). *Physics and Chemistry of Liquids*, 45, 453–461.
- British Pharmacopoeia (1998). Alginic acid. Vol. 1. (pp. 52–53).
- Chandía, N. P., Matsuhira, B., & Vásquez, A. E. (2001). Alginic acid in *Lessonia trabeculata*: Characterisation by formic acid hydrolysis and FT-IR spectroscopy. *Carbohydrate Polymers*, 46, 81–87.
- De Silva, M. S. M., & Kumar, N. S. (1989). Composition and sequence of uronate residues in alginates from three species of brown seaweeds. *Carbohydrate Research*, 191, 167–173.
- Food Chemicals Codex (1981). (3rd ed.). Alginic acid (pp. 13–14).
- Haug, A., & Larsen, B. (1962). Quantitative determination of the uronic acid composition of alginates. *Acta Chemica Scandinavica*, 16, 1908.
- Haug, A., Larsen, B., & Smidsrd, O. (1967). Studies on the sequence of uronic acid residues in alginic acid. *Acta Chemica Scandinavica*, 21, 691–704.
- Ji, M. H., Cao, W. D., & Han, L. J. (1981). In T. Lervig (eds.) Xth International Seaweed Symposium, Walter de Gruyter & Co., Berlin, New York, 1981, p. 425.
- Leroux, F., Gachon, J., & Besse, J.-P. (2004). Biopolymer immobilization during the crystalline growth of layered double hydroxide. *Journal of Solid State Chemistry*, 177, 245–250.
- Li, K., Xia, L., Li, J., Pang, J., Cao, G., & Xi, Z. (2001). Salt-assisted acid hydrolysis of starch to D-glucose under microwave irradiation. *Carbohydrate Research*, 331, 9–12.
- Llanes, F., Ryan, D. H., & Marchessault, R. H. (2000). Magnetic nanostructured composites using alginates of different M/G ratios as polymeric matrix. *International Journal of Biological Macromolecules*, 27, 35–40.

- Meena, R., Prasad, K., & Siddhanta, A. K. (2007). Effect of Genipin, a naturally occurring crosslinker on the properties of kappa-carrageenan. *International Journal of Biological Macromolecules*, 41, 94–101.
- Morris, E. R., Rees, D. A., & Thom, D. (1980). Characterisation of alginate composition and block structure by Circular Dichroism. *Carbohydrate Research*, 81, 305–314.
- Nishide, E., Anzai, H., & Uchida, N. (1987). Extraction of Alginic Acid from a Brazilian brown alga, *Laminaria brasiliensis*. *Hydrobiologia*, 151(152), 551–555.
- Rojas-Graü, M. A., Tapia, M. S., Rodríguez, F. J., Carmona, A. J., & Martín-Belloso, O. (2007). Alginate and gellan-based edible coatings as carriers of antibrowning agents applied on fresh-cut Fuji apples. *Food Hydrocolloids*, 21, 118–127.
- Sakugawa, K., Ikeda, A., Takemura, A., & Ono, H. (2004). Simplified method for estimation of composition of alginates by FTIR. *Journal of Applied Polymer Science*, 93, 1372–1377.
- Singh, V., Sethi, R., Tewari, A., Srivastava, V., & Sanghi, R. (2003). Hydrolysis of plant seed gums by microwave irradiation. *Carbohydrate Polymers*, 54, 523–525.
- Tako, M., Yoza, E., & Tohma, S. (2000). Chemical characterization of acetyl fucoidan and alginate from commercially cultured *Cladosiphon okamuranus*. *Botanica Marina*, 43, 393–398.
- Zhang, Z., Yu, G., Guan, H., Zhao, X., Du, Y., & Jiang, X. (2004). Preparation and structure elucidation of alginate oligosaccharides degraded by alginate lyase from *Vibrio* sp. 510. *Carbohydrate Research*, 339, 1475–1481.