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Preparation, characterization and benchmarking of agarose from *Gracilaria dura* of Indian waters

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Abstract

Agarose was prepared from a red alga *Gracilaria dura* occurring in the Arabian Sea at the west coast of India. The agarose has been characterized by studying its physicochemical properties as well as by FTIR, ^{13}C NMR and CP-MAS spectra, inductively coupled plasma (ICP) spectrophotometric and rheological measurements. This agarose had gel strength 2200 g cm^{-2}, gelling temperature $\leqslant$ 35 °C, sulphate content $\leqslant$ 0.25%, $[\alpha]_{589}^{45} - 22^{\circ}$ and M_w 1.25×10^5 g mol^{-1}. These properties were benchmarked against those of the commercially available agarose products of Sigma (A0576) and Fluka, and were found to be comparable.

Keywords: *Gracilaria dura*; Agarose; ^{13}C NMR; CP-MAS

1. Introduction

Natural occurrence of the red seaweed *Gracilaria dura* (C. Agardh) J. Agardh has been reported from the west coast of India (Oza & Zaidi, 2001). The low gel strength agars from *G. dura* of Indian waters (Siddhanta, Shanmugam, Ramavat, & Mody, 1997) and from other regions of the world were reported in the literature (Marinho-Soriano & Bourret, 2005; Marinho-Soriano (2001); Murano, Brandolin, Zanetti, Paoletti, & Rizzo, 1990; Murano et al., 1992).

The seaweed polysaccharides agar and agarose (Fig. 1) are one of the most used polysaccharides in biotechnological applications (Meer, 1980; Renn, 1984). Fluka Catalog (2003–2004) mentions the greatest gel strength agarose (Product No. 05071) with gel strength $\geqslant$1800 g cm^{-2} (in 1.5% gel), gelling temperature in the range of 40–43 °C and sulphate content $\leqslant$0.30%. Sigma Catalog of 2004–2005 mentions agarose having gel strength in the range of 100–1800 g cm^{-2} (1.0% gels), gelling temperatures 36–42 °C and sulphate contents 0.10–0.30%. Numerous processes and studies have been done on the agarose preparation from the high quality agars and from the low-grade agarose using complex or multi-step purification processes. They used high quality agar or low quality agarose for the preparation of high quality agarose by further purification e.g. either by chromatographic procedure or by fractionation using organic solvents (Alfred, 1966; Arai & Maeda, 1970; Kirkpatrick, Guiseley, Provonchee, & Nochumson, 1991; Provonchee, 1991). Partially purified agarose was prepared by precipitating of the charged impurities using quaternary base (Craigie & Leigh, 1978). Fractionation of galactans isolated from *G. dura* collected in the Black Sea and the analytical results, which are slightly different from those reported by the present authors, were published earlier (Usov & Ivanova, 1990).

Agarose is an industrially important high value material and is extensively used in biotechnology and molecular biology applications. In a continuing program of value addition of Indian seaweeds in our laboratory, the present study demonstrates that Indian *G. dura*, an agarophyte

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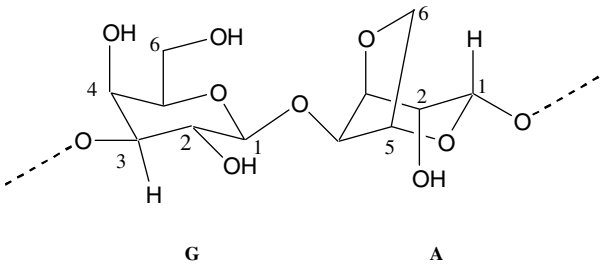

Fig. 1. Basic disaccharide repeating units of agarose, G: 1,3-β-D-galactose and A: 1,4-α-L-3,6-anhydrogalactose.

which has not been reported as a source of good quality agar, can be used for producing agarose in a cost-effective and environment friendly method.

We report herein, for the first time, the preparation of agarose from the agarophyte *G. dura*, using an improved and cost-effective method (Siddhanta et al., 2005), and characterization of the agarose. Comparison of the physicochemical properties of this agarose was done with those of the commercially available products of Sigma and Fluka for benchmarking, which were found to be comparable.

2. Experimental

2.1. Materials

The agarose polymer investigated was obtained from specimens of *G. dura* (C. Agardh) J. Agardh (Gracilariaceae, Rhodophyta), growing in Indian waters. The agarose preparation process corresponded to a patent specification (Siddhanta et al., 2005). Thalli of this species were collected from its natural habitat (during November to July) at the west coast of India (20.54°N 70.22°E). Harvested plants were brought to the laboratory, air-dried and stored in plastic bags. Sample specimen of the seaweed after identification was submitted to the CSMCRI Herbarium. For comparative study, agarose was purchased from Sigma–Aldrich, USA (Cat. No. A0576), because of its low sulphate content and gelling point as well as high gel strength.

2.2. Native agar preparation

Native agar (without alkali pre-treatment), was prepared from *G. dura* (100 g dry) by soaking the seaweed in tap water for 1 h at room temperature and then heated in tap water at 80–90 °C on a water-bath for 1.5 h. The soaked seaweed was autoclaved with demineralised water (1:35 w/v or 350 ml DM water for 10 g of seaweed) for 1.5 h at 120 °C. The extractive was homogenized; the homogenate was boiled and filtered hot through Celite bed under reduced pressure. The filtrate was frozen (at −20 °C for 15 h) and thawed; after removing the thawed liquid the agar was dried in the air followed by drying in the oven at 50 °C for 4 h to get the native agar.

2.3. Agarose preparation

The agarose polymer was prepared in the laboratory and pilot plant scale, using a cost effective, direct, solvent free and simple, an improved method (Siddhanta et al., 2005). Dry *G. dura* samples (100 g–1.5 kg dry each) were soaked in tap water for 1 h at room temperature and alkali treated with 10% aqueous NaOH at 85 °C for 2 h. The excess alkali was removed from the pretreated seaweed which was then autoclaved in water to obtain extractive, treating the extractive with charcoal and Celite, vacuum filtering the hot extractive over a Celite bed, freezing the filtrate and thawed the mass, straining the product to remove thawed liquid and thereafter squeezed to obtain agarose, which was dried and ground.

2.4. Physical properties

The native agar and agarose was powdered and used for various measurements. Native agar and agarose gel samples (1.0% gel) were prepared by dissolving in demineralised water in an autoclave at 120 °C. Gel strength measurements were done on a Gel Tester (Kiya Seisakusho, Ltd., Tokyo, Japan). Gelling and melting temperatures of gel samples were measured following the method described by (Craigie & Leigh, 1978). The gelling temperature of agarose gel samples was also confirmed on the basis of rheological signatures as described by Prasad et al. (Prasad, Siddhanta, Rakshit, Bhattacharya, & Ghosh, 2005). Apparent viscosity was measured on a Brookfield Viscometer (Synchrolectric Viscometer, Stoughton, MASS 02072), using Spindle No. 1 at a speed of 60 rpm. Optical rotation was measured in 0.25% agarose sol at 45 °C, on a Rudolph Digi pol – 781 Polarimeter (Rudolph Instruments Inc, NJ, USA).

2.5. Chemical properties

The 3,6-anhydrogalactose was estimated by improved phenol-resorcinol method using fructose as standard (Yaphe & Arsenault, 1965). Metal ion and sulphate contents analyses (ICP) were carried out on a Perkin-Elmer ICP-OES Optima 2000DV machine following the method described by Wolnik (1988).

2.6. FT-IR spectra

Infrared spectrum was recorded on a Perkin-Elmer Spectrum GX, FT-IR System, USA in KBr (by taking 2.0 mg of agarose in 600 mg of KBr to prepare the pellet) and compared with the IR spectrum of Sigma agarose (A0576) (Christiaen & Bodard, 1983).

2.7. ^{13}C NMR

Noise-decoupled ^{13}C NMR spectra were recorded on a Bruker Advance DPX 200 Spectrometer, Switzerland, at

50 MHz. *G. dura* and Sigma agarose samples were dissolved in D_2O (50 mg/ml) and the spectra were recorded at 70 °C with 5400–5500 accumulations, pulse duration 5.9 μs, acquisition time 1.2059 s and relaxation delay 6 μs using DMSO as internal standard (ca. δ 39.5). The solid state spectroscopy (CP-MAS ^{13}C NMR) used magic angle spinning of 4 KHz and cross-polarization techniques employing contact and repetition times of 16 ms and 5 s, respectively, and 450–550 scans were collected. Samples were used directly and spectra were recorded at ambient temperature. Chemical shifts were referenced to adamantane run as an initial sample and are quoted relative to tetramethylsilane (TMS).

2.8. Weight average molecular weight (M_w)

Intrinsic viscosities [η] were determined at 32 °C using an Ostwald viscometer. Sols of agarose samples were prepared in 1.0 M NaCl at a concentration 0.02–0.12% (Meena, Prasad, & Siddhanta, 2006; Prasad, Mehta, Meena, & Siddhanta, 2006). Weight average molecular weight was calculated from the intrinsic viscosity using the Mark–Houwink equation for agarose as described by Rochas and Lahaye (1989).

$$[\eta] = 0.07\ M^{0.72}$$

where, [η] is intrinsic viscosity in ml/g and, M is the average molecular weight.

2.9. Rheological measurements

Dynamic rheological measurements of sol and gel samples of both agarose samples were carried out on a rheometer (RS1, HAAKE Instruments, Karlsruhe, Germany). The cone/plate (60 mm diameter, 1° rad angle) geometry was selected for dynamic viscosity measurement at 45 °C. The plate/plate (35 mm diameter) geometry was selected for oscillation measurements of agarose gel samples in the controlled deformation mode with a strain value 0.05%, the temperature of gel being maintained at 25 °C using the DC50 water circulator. Measurements of G' and G'' were performed over 60 min. Subsequent measurements were carried out immediately after placing gel sample on the plate. For measurements at all temperatures the exposed part of the samples were covered with silicone oil to minimize losses due to evaporation. All rheological data present were means of three replicate measurements.

2.10. Statistical analyses

Data were analyzed using one way analysis of variance (ANOVA). Results were considered statistically significant when $p < 0.05$. Calculations were performed using Origin Software, Version 6 (Microcal Software Inc., MA, USA). To carry out the analysis of the variance (ANOVA) four replications ($n = 4$) of each parameter in three groups were made.

3. Results

3.1. Yield (%)

Yields were calculated on the basis of as received dry seaweed containing nil moisture (Table 1). The yield of native agar was 27 ± 0.81% for different naturally occurring *G. dura* samples collected from the west coast. The yields of agarose samples, which were obtained with the 10% NaOH alkali pre-treatment, were 23 ± 0.45% for all the seaweed samples investigated in this study (Table 1).

3.2. Physical properties

The optical rotation of *G. dura* agarose was $[\alpha]_{589}^{45} - 22°$ (c0.25, H_2O), and that of Sigma agarose (A0576) was −21° (c0.25, H_2O). Apparent viscosities of the native agar and agarose were 32 ± 0.5 and 44 ± 0.81 cP in 1.0% sol at 80 °C. The gel strength of native agar was 250 ± 8.16 g cm^{-2} and those of agarose samples of *G. dura* and of Sigma (A0576), were 2200 ± 25 and >1800 g cm^{-2}, respectively (Table 2). The analysis of variance revealed that the gel strength of *G. dura* agarose was significantly greater than those of Sigma and Fluka agarose gel samples ($p < 0.05$). The variations in the gel strengths from 500 to 2200 g cm^{-2} with alkali concentrations are shown in Table 1. The gelling

Table 1
Properties of agarose extracted from *Gracilaria dura*[a] under different alkali pretreatment conditions

Alkali conc. (%NaOH)	Yield[b] (%) ±SD	Gel strength[c] (g cm^{-2}) ±SD	M_w (g mol^{-1}); ±SD	3,6-Anhydro-galactose (%); ±SD	Ash (%); ±SD	Sulphate (%); ±SD
0	27 ± 0.81	250 ± 8.16	$(3.15 \pm 0.07) \times 10^5$	15 ± 0.95	8.16 ± 0.12	3.32 ± 0.057
1.5	25 ± 0.57	280 ± 9.57	$(3.00 \pm 0.06) \times 10^5$	24 ± 0.57	5.28 ± 0.12	2.12 ± 0.057
3	25 ± 0.50	700 ± 15.0	$(2.98 \pm 0.04) \times 10^5$	32 ± 0.57	3.43 ± 0.076	1.84 ± 0.11
5	24 ± 0.57	1600 ± 19.13	$(1.5 \pm 0.05) \times 10^5$	37 ± 0.5	2.02 ± 0.019	0.50 ± 0.028
7	23 ± 0.57	1875 ± 11.08	$(1.25 \pm 0.04) \times 10^5$	39 ± 0.57	1.58 ± 0.04	0.30 ± 0.024
10	23 ± 0.45	2200 ± 25	$(1.23 \pm 0.079) \times 10^5$	42 ± 0.84	0.90 ± 0.033	0.25 ± 0.006
15	22 ± 0.95	2200 ± 25	$(1.02 \pm 0.09) \times 10^5$	42 ± 0.5	0.88 ± 0.024	0.25 ± 0.006

[a] All samples of *Gracilaria dura* were collected during November to July from the natural stock at the west coast of India.
[b] Yields were calculated on the basis of as received dry seaweed containing nil moisture, the moisture of the seaweed being in the range from 10% to 15%.
[c] Gel strength of all samples were measured in 1.0% gel at 20 °C.

Table 2
Comparison of native agar and agarose of *Gracilaria dura* with Sigma and Fluka agaroses

Agar/agarose source	Gel strength[a] (in g cm^{-2} at 20 °C); ±SD	Gelling temperature (°C); ±SD	Sulphate content (%); ±SD	Ash content (%); ±SD
Gracilaria dura (native agar)	270 ± 10.84	34 ± 0.57	3.32 ± 0.057	8.5 ± 0.054
Gracilaria dura (agarose)	2200[b] ± 25	35 ± 0.5	0.25 ± 0.006	0.9 ± 0.08
Sigma[c] (A0576)	>1800[b]	36 ± 1.5	⩽0.12	⩽0.25
Sigma[c] (A9918)	>1000[b] >2000 (1.5%)	36 ± 1.5	<0.25	⩽0.5
Sigma[c] (A9793)	>750[b] >1000 (1.5%)	36 ± 1.5	<0.25	⩽1.1
Sigma[c] (A9668)	>700[b] >1100 (1.5%)	36 ± 1.5	<0.30	⩽1.5
Sigma[c] (A3643)	⩾650[b]	36 ± 1.5	⩽0.25	NR[d]
Sigma[c] (A3768)	⩾800[b]	42 ± 1.5	⩽0.30	NR[d]
Fluka[c] (05068)	⩾1500 (1.5%)	34–37	⩽0.60	⩽1.0
Fluka[c] (05070)	1400 (1.5%)	40–43	⩽0.50	⩽1.0
Fluka[c] (05071)	⩾1800 (1.5%)	40–43	⩽0.30	⩽1.0
Fluka[c] (05077)	⩾2000 (1.5%)	40–43	⩽0.30	⩽1.0

[a] Gel strength was measured in 1.5% gel, unless otherwise stated.
[b] In 1% gel.
[c] As mentioned in the Sigma and Fluka catalogue 2004–2005.
[d] NR, not reported.

and melting temperatures of native agar gel are 34 ± 0.57 and 88 ± 0.52 °C, and those of agarose gel were 35 ± 0.55 and 98 ± 0.76 °C, respectively (Tables 1 and 2).

3.3. Weight average molecular weight

The weight average molecular weights of native agar and agaroses were determined and detailed given in Table 1. Molecular weights of agarose polymers decreased with increase in the concentration of alkali used in the alkali pretreatment step (Table 1). The greatest weight average molecular weight, $(3.15 \pm 0.07) \times 10^5$ g mol^{-1}, was observed for native agar sample, and the lowest was $(1.02 \pm 0.01) \times 10^5$ g mol^{-1}, for the agarose polymer obtained by 15% NaOH pretreatment, while agarose prepared with 10% NaOH pretreatment, had M_w $(1.23 \pm 0.079) \times 10^5$ g mol^{-1}.

3.4. Chemical properties

The 3,6-anhydrogalactose content increased from 15 ± 0.95% for native agar (i.e. with no alkali

Table 3
Comparison of metal ion contents in native agar and agarose of *Gracilaria dura* with those of Fluka products[a]

Metal ions	*Gracilaria dura* native agar (ppm)	*Gracilaria dura* agarose (ppm)	Fluka agar (Cat. No. 5038) (ppm)	Fluka agarose (Cat. No. 05068) (ppm)
Ca	⩽3933	⩽680	⩽1000	⩽500
Cd	⩽0.45	ND[b]	⩽5	⩽5
Co	⩽0.45	ND[b]	⩽5	⩽5
Cr	⩽0.89	ND[b]	⩽5	⩽5
Cu	⩽8.48	⩽0.078	⩽5	⩽5
Fe	⩽104	ND[b]	⩽50	⩽20
K	⩽13,495	⩽22.3	⩽1000	⩽50
Mg	⩽3463	⩽200	⩽200	⩽5
Mn	⩽9.8	ND[b]	⩽5	⩽5
Na	⩽7058	⩽233	⩽5000	⩽2000
Ni	⩽5.35	⩽0.15	⩽5	⩽5
Pb	⩽1.34	ND[b]	⩽5	⩽5
B	⩽58.9	⩽1.30	NR[c]	NR[c]
As	ND[b]	ND[b]	NR[c]	NR[c]
Al	⩽141.1	⩽0.76	NR[c]	NR[c]
Zn	⩽266	⩽3.77	⩽10	⩽5

[a] All values are in ppm.
[b] ND, not detected.
[c] NR, not reported.

pre-treatment) to $42 \pm 0.84\%$ which was associated with decrease in the sulphate contents (from 3.32% to 0.25%) for agarose obtained with 10% NaOH pre-treatment (Table 1).

The metal ion analyses using inductively coupled plasma spectrophotometry (ICP) of the native agar and agarose samples were carried out and compared with those of Fluka agar and agarose (Table 3). The metal ion contents of *G. dura* agarose were identical with those of Fluka agarose (Table 3). The native agar of *G. dura* showed higher calcium and magnesium ion contents than that of the Fluka agar. In case of sodium ion, Fluka products showed higher value than those of the native agar and agarose of *G. dura* studied herein (Table 3). The analysis of variance revealed that the sulphate and metal ion contents of *G. dura* native agar was significantly greater than that of agarose sample ($p < 0.05$).

3.5. FT-IR spectra

The FT-IR spectra of the *G. dura* and Sigma agaroses were carried out and depicted in Fig 2. The principal IR bands for *G. dura* and Sigma agaroses were identical and are in good agreement with the previous report (Rosangela, Rosangela, & Marguerite, 2000).

3.6. ^{13}C NMR spectroscopy

^{13}C NMR spectra of the agarose samples as well as their solid state spectra (CP-MAS) are presented in Figs. 3 and 4, respectively. The chemical shifts of the 12 carbon atoms (Fig. 3) of the disaccharide repeating units of agarose (Fig. 1) were comparable with those reported in the literature (Lahaye, Yaphe, Viet, & Rochas, 1989; Truus et al., 2006; Usov, Yarotsky, & Shashkov, 1980) (Table 4). The solid state spectra (CP-MAS) exhibited five peaks at

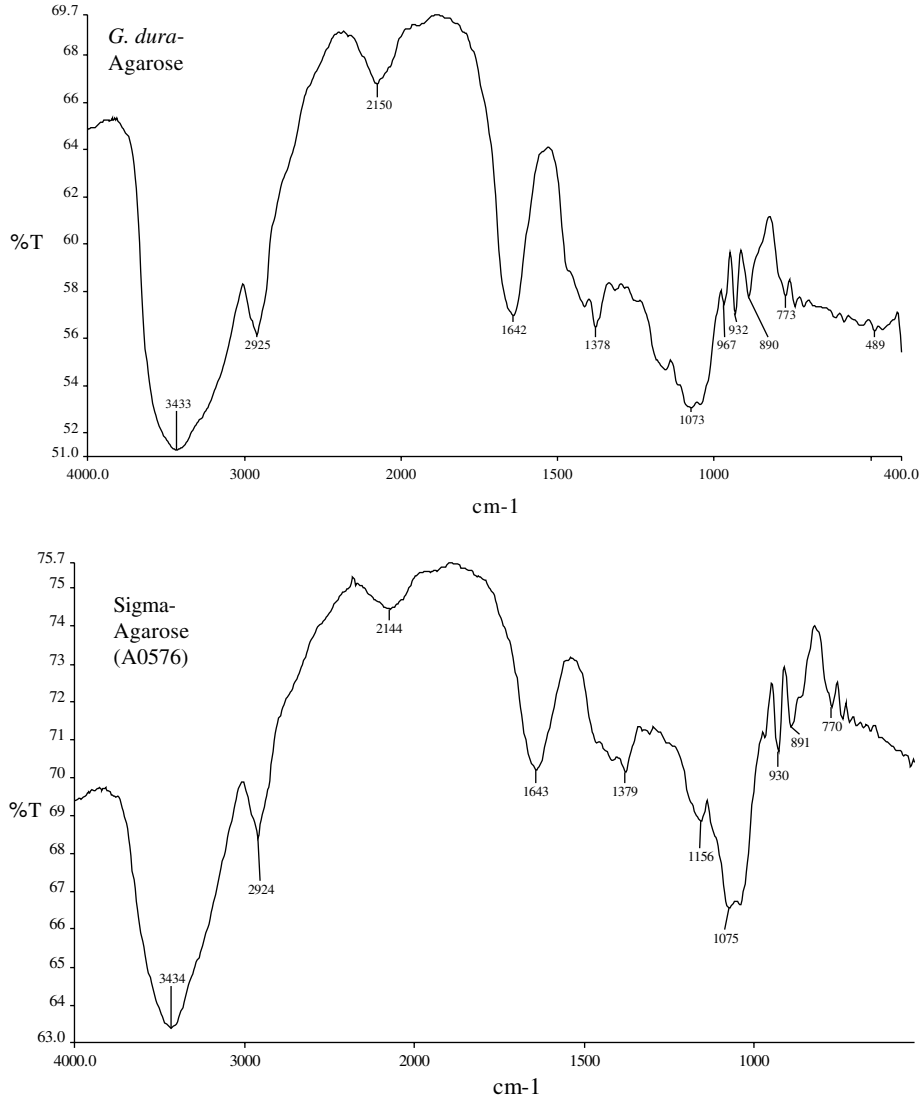

Fig. 2. FT-IR spectra of the *Gracilaria dura* and Sigma (A0576) agaroses.

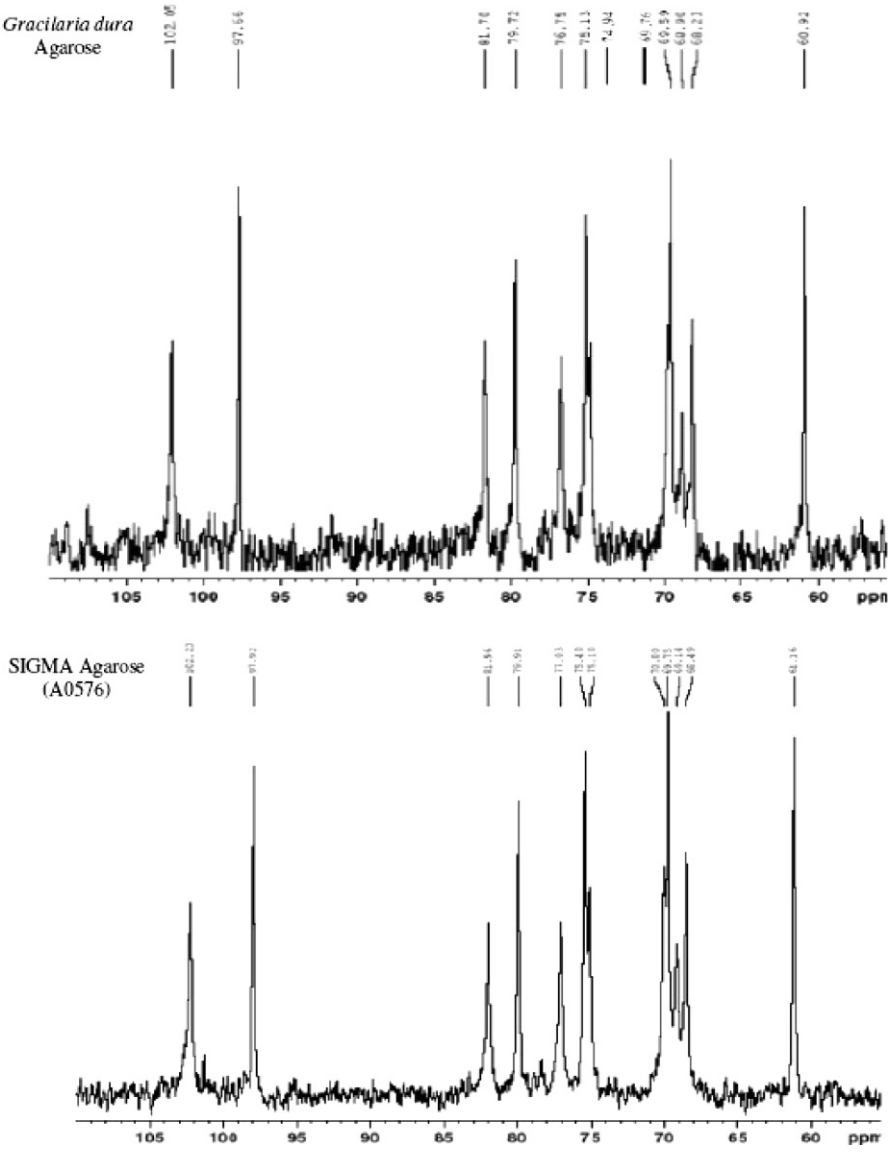

Fig. 3. ^{13}C NMR spectra of the *Gracilaria dura* and Sigma (A0576) agaroses.

62.59, 69.90, 75.67, 79.83 and 99.55 ppm for *G. dura* agarose while the Sigma agarose showed peaks at 62.45, 69.79, 75.38, 79.35 and 100.08 ppm (Fig. 4), which was similar to those reported by Rochas and Lahaye (1989).

3.7. Dynamic viscosity measurement

The variations in dynamic viscosity of gels of *G. dura* and Sigma (A0576) agaroses are shown in Fig. 5. The dynamic viscosity of the both gel samples decreased with increasing shear rate. Non-Newtonian or shear thinning behavior was observed in both the agarose gels (Fig. 5).

3.8. Oscillatory measurements

The temperature dependence of storage (G') and loss (G'') moduli of *G. dura* and Sigma (A0576) agarose gels were studied (Fig. 6). The storage modulus increased with decreasing temperature for both agarose gel samples. Slightly higher values of the G' for *G. dura* agarose indicated more rigidity than that of the Sigma agarose (A0576) gel. The sudden increase in G' value and cross the G'' near the gelling point also confirmed the low gelling point of the gel samples, which was measured by manual method described by Craigie and Leigh, 1978 (Fig. 6). The time dependence of storage and loss moduli were also studied at a constant temperature 25 °C (Fig. 7).

4. Discussion

Superior quality agarose polymer was prepared from *G. dura*, an agarophyte of Indian waters, using an improved method (Siddhanta et al., 2005). To our knowledge, this is the first report of direct, cost-effective and solvent-free

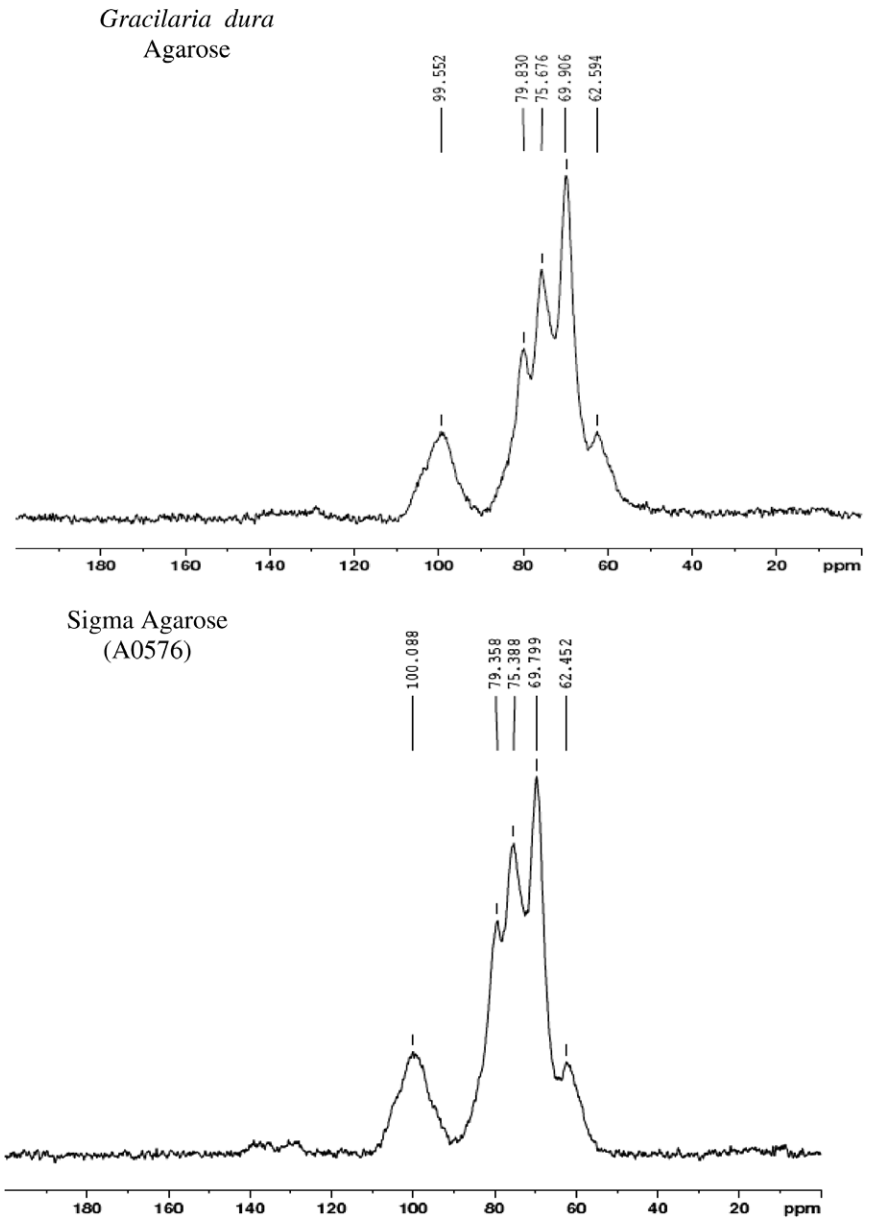

Fig. 4. Solid state ^{13}C NMR (CP-MAS) spectra of the *Gracilaria dura* and Sigma (A0576) agaroses.

process for preparation of agarose from an agarophyte. More particularly, this constitutes the first report of a low-gelling agarose having the greatest gel strength among those reported till date in the public domain. This agarose has the specifications comparable to some commercially available superior quality agaroses (Tables 1 and 2). In this study, the quality of native agar has been significantly improved by the present method. The pre-treatment conditions and concentration of NaOH were optimized and it was found that the agarose obtained with 10% NaOH pre-treatment was superior in yield and quality. The yield of native agar of *G. dura* was greater than that of the agarose obtained from *G. dura* samples with 10% NaOH pre-treatment (Table 1). These results are in agreement with the fact that the yield loss was due to polymer degradation caused by alkaline hydrolysis (Armisen & Galatas, 1987; Nishinari & Watase, 1983; Siddhanta et al., 1997). The alkali mediated desulphation of the native agar has led to a superior quality agarose with the careful control of pH (>7) in the post alkali treatment step ensuring minimum degradation of the acid sensitive galactan polymer.

There was no significant temporal difference in the quality of agarose polymers which were prepared from the different natural samples of *G. dura* as well as from the samples that were cultivated in the sea both in the south-east and west coasts of India. This observation has unfolded the ruggedness of this particular renewable seaweed resource of Indian waters. Our observation is particularly significant *vis a vis* the literature reports on *Gracilaria* (Armisen, 1995; Critchley, 1993) describing that the species

Table 4
Chemical shift assignments for ^{13}C NMR spectra of *Gracilaria dura* agarose[a]

Unit	^{13}C chemical shifts (ppm)						References
	C1	C2	C3	C4	C5	C6	
G	102.4	70.2	82.2	68.8	75.3	61.4	Lahaye et al. (1989)
A	98.3	69.9	80.1	77.4	75.7	69.4	
G	102.3	70.1	82.2	68.6	75.2	61.3	Usov et al. (1980)
A	98.2	69.7	80.0	77.2	75.5	69.7	
G	102.07	69.99	81.93	68.53	75.10	61.20	Truus et al. (2006) (Sigma agarose)
A	98.05	69.56	79.77	77.04	75.25	69.07	
G	102.08	69.98	81.93	68.52	75.10	61.20	Truus et al. (2006) (LKB agarose)
A	98.06	69.55	79.77	77.04	75.25	69.06	
G	102.23	70.00	81.96	68.49	75.10	61.16	Sigma agarose (A0576) of present study
A	97.92	69.75	79.91	77.03	75.40	69.14	
G	102.05	69.76	81.70	68.23	74.94	60.92	Agarose from *G. dura* of present study
A	97.66	69.59	79.72	76.75	75.13	68.90	

[a] G, 1,3-β-D-galactose and A, 1,4-α-L-3,6-anhydrogalactose.

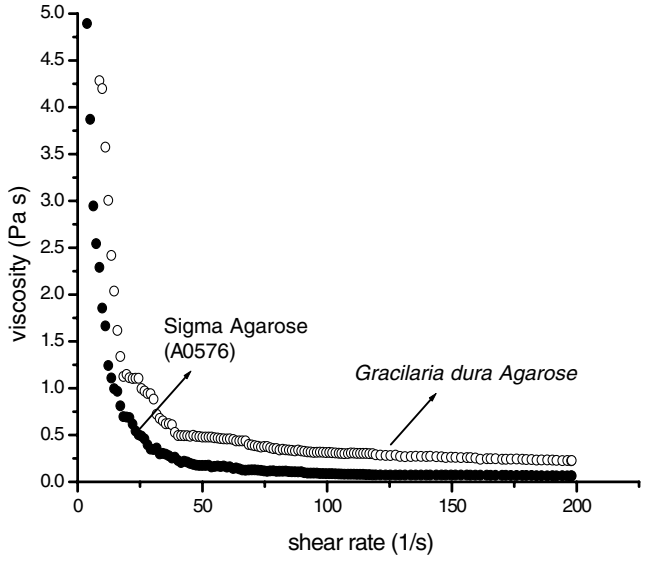

Fig. 5. Shear rate vs. dynamic viscosity of *Gracilaria dura* and Sigma (A0576) agarose gel samples.

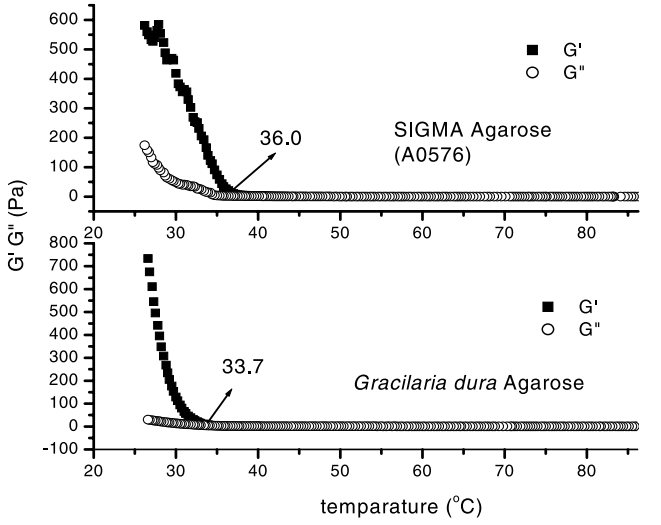

Fig. 6. Temperature dependence of G' and G'' of *Gracilaria dura* and Sigma (A0576) agarose gel samples at a strain value 0.05.

dependence is not the only factor of variations of the yield and quality of agars (Cote & Hanisak, 1986), but the environmental factors, such as seasonal variations (Lahaye & Yaphe, 1988) and extraction methods (Armisen & Galatas, 1987; Craigie & Leigh, 1978; Lemus, Bird, Kapraun, & Koehn, 1991) also influence the properties of agar as well.

The gel strength of native agar increased and the molecular weight decreased with increasing concentration of alkali in the pretreatment stage (Table 1). In other words, the weight average molecular weight of native agar was ca. 3-fold greater than that of the agarose polymer obtained with 10% alkali pre-treatment ($p < 0.05$). Similar trend was reported by (Murano et al. (1992)). The agarose used in the present investigation had the greatest gel strength (2200 g cm^{-1}, in 1.0% gel) amongst those reported from the same seaweed as well as from other *Gracilaria* species. Marinho-Soriano and Bourret (2005) reported

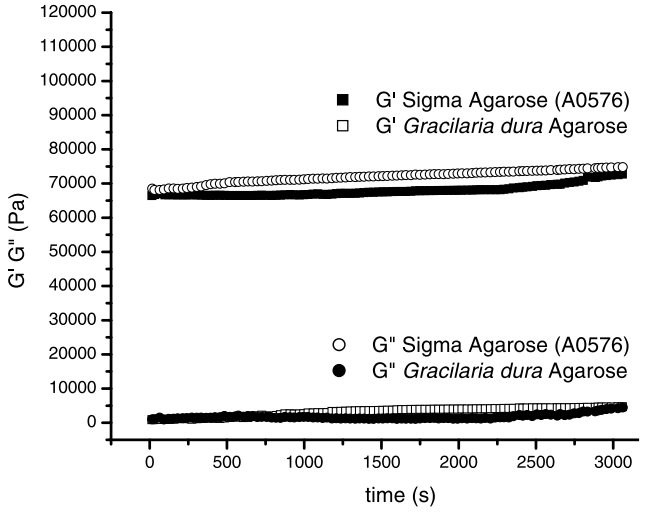

Fig. 7. Time dependence of G' and G'' of *Gracilaria dura* and Sigma agarose (A0576) gel samples.

600 g cm^{-1} as the gel strength for *G. dura* in 1.5% agar gel. Rochas and Lahaye (1989) used 0.75 M NaSCN for the measurement of [η]. In this investigation, 1.0 M NaCl was used for the measurement for preventing gelation at the measuring temperature 32 °C, and it was found that there was no difference in the mobility of the sols when measured in 0.75 M NaSCN, which was actually used by Rochas and Lahaye (1989). They reported that "same molecular weight was obtained when different solvents were used e.g. 0.1 M $NaNO_3$; 0.1, 0.5, or 0.75 M NaSCN, for which the conformational ordering and consequently the aggregation are completely different at room temperature". The weight average molecular weight of *G. dura* agarose prepared in this investigation was in good agreement with those of commercial agaroses [FMC, USA (Reference No. 291402 and 92364); Colab Laboratories, USA (A 37); IBF, France (FF 2743); Sigma, USA (VI) and Oxoid, England (LII)], as reported by the Rochas and Lahaye (1989).

This agarose was characterized by measuring the gel strength, viscosity, gelling temperature, metal ion contents, optical rotation, rheological properties, IR and ^{13}C NMR spectra. This was found to be of similar specifications when compared with Sigma (A0576) and Fluka agaroses. In the FTIR spectra of both the agarose polymers of this investigation and Sigma (A0576) no band in the region 845–850 cm^{-1} corresponding to C–O–S stretching was detected, indicating the absence of C_4-sulphate in the galactopyranose moiety (Melo, Feitosa, Freitas, & de Paula, 2002). The carbon resonances in the ^{13}C NMR and the CP-MAS spectra of both these agarose polymers differed marginally and showed no peak at ca. 59.0 ppm indicating absence of $-OCH_3$ group. It should be mentioned here that the source of Sigma agarose (A0576) is not known. In the CP-MAS spectra of these agaroses, five distinct peaks appeared, with a single peak appearing at ca. 100 ppm corresponding to the anomeric carbons of G and A moieties of the agarose repeating units (Fig. 1). In agar, these two carbon atoms appear as two distinct peaks ca. 100 ppm (Lahaye et al., 1989), possibly because of the presence of sulphate groups in agar resulting in anisotropy around these two carbons. The general agreement of the carbon resonances of the agarose of present investigation with those reported in the literature is presented in Table 4 (cf. Fig. 3).

Low sulphate and metal contents are desirable attributes of a superior quality agarose. The sulphate and metal contents of the agarose in the present investigation were measured and compared with those reported (Tables 1 and 3 respectively), showing excellent compatibility of this agarose with those available commercially.

The novelty of the improved method described herein lies in elimination of acids, organic solvents and chromatographic techniques from the entire process of preparing agarose, which were widely reported in the prior art (Siddhanta et al., 2005).

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